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(54) Title: COMPOSITIONS AND METHODS FOR ANALYTE DETECTION USING BIOLUMINESCENCE

(57) Abstract: Provided herein are systems and methods for the detection of an analyte or analytes in a sample. In particular, the present disclosure provides compositions, assays, and methods for detecting and/or quantifying a target analyte using a bioluminescent complex comprising substrates, peptides, and/or polypeptides capable of generating a bioluminescent signal that correlates to the presence, absence, or amount of the target analyte.

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COMPOSITIONS AND METHODS FOR ANALYTE DETECTION USING BIOLUMINESCENCE

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims priority to and the benefit of U.S. Provisional Patent Application No. 62/832,052, filed April 10, 2019, which is incorporated herein by reference in its entirety and for all purposes.

FIELD

[0002] Provided herein are systems and methods for the detection of an analyte or analytes in a sample. In particular, the present disclosure provides compositions, assays, and methods for detecting and/or quantifying a target analyte using a bioluminescent complex comprising substrates, peptides, and/or polypeptides capable of generating a bioluminescent signal that correlates to the presence, absence, or amount of the target analyte.

BACKGROUND

[0003] Biological processes rely on covalent and non-covalent interactions between molecules, macromolecules, and molecular complexes. In order to understand such processes, and to develop techniques and compounds to manipulate them for research and clinical and other practical applications, it is necessary to have tools available to detect and monitor these interactions and/or components involved in such interactions. The study of these interactions, particularly under physiological conditions (e.g., at normal expression levels for monitoring protein interactions), requires high sensitivity.

[0004] Creation of better assays for use in the field and in clinical settings is an ongoing area of urgent need. Speed, sensitivity, selectivity, robustness, simplicity, quantitative versus qualitative capabilities, and cost are all critical factors affecting the relevance of a diagnostic bioassays, and thus their utility to and adoption by the relevant community. Rapid diagnostic tests are not only relevant to clinical settings, but also can be applied to environmental, industrial, and direct to consumer contexts.

SUMMARY

[0005] Provided herein are compositions and formulations comprising a luminogenic substrate and a target analyte binding agent comprising a target analyte binding element and one

of a polypeptide component of a bioluminescent complex, or a peptide component of a bioluminescent complex.

[0006] In accordance with these embodiments, the polypeptide component of the target analyte binding agent comprises at least 60% sequence identity with SEQ ID NO: 5; at least 60% sequence identity with SEQ ID NO: 9; or at least 60% sequence identity with SEQ ID NO: 12.

[0007] In some embodiments, the peptide component of the target analyte binding agent comprises at least 60% sequence identity with SEQ ID NO: 10; at least 60% sequence identity with SEQ ID NO: 11; at least 60% sequence identity with SEQ ID NO: 13; or at least 60% sequence identity with SEQ ID NO: 14.

[0008] In some embodiments, the composition comprises a complementary peptide or polypeptide component of the bioluminescent complex, wherein the target analyte binding agent and the complementary peptide or polypeptide component of the bioluminescent complex form a bioluminescent analyte detection complex in the presence of a target analyte.

[0009] In some embodiments, the composition that comprises the luminogenic substrate and the target analyte binding agent are combined in a dried formulation, and the complementary peptide or polypeptide component of the bioluminescent complex comprises a liquid formulation, wherein the liquid formulation is added to the dried formulation and forms the bioluminescent analyte detection complex in the presence of the target analyte upon rehydration.

[0010] In some embodiments, the composition comprising the luminogenic substrate, the target analyte binding agent, and the complementary peptide or polypeptide component of the bioluminescent complex are combined in a dried formulation, wherein the dried formulation forms the bioluminescent analyte detection complex in the presence of the target analyte upon rehydration.

[0011] In some embodiments, the complementary peptide or polypeptide component comprises a second target analyte binding element that forms the bioluminescent analyte detection complex in the presence of the target analyte.

[0012] In some embodiments, the polypeptide component of the target analyte binding agent comprises at least 60% sequence identity with SEQ ID NO: 6, and wherein the complementary peptide or polypeptide component of the bioluminescent complex comprises at least 60% sequence identity with SEQ ID NO: 10.

[0013] In some embodiments, the polypeptide component of the target analyte binding agent comprises at least 60% sequence identity with SEQ ID NO: 6, and wherein the complementary peptide or polypeptide component of the bioluminescent complex comprises at least 60% sequence identity with SEQ ID NO: 14.

[0014] Embodiments of the present disclosure also include a composition comprising a dried formulation comprising (a) a first target analyte binding agent comprising a first target analyte binding element and a polypeptide component having at least 60% sequence identity with SEQ ID NO: 9, and (b) a second target analyte binding agent comprising a second target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 10.

[0015] In some embodiments, the dried formulation further comprises a luminogenic substrate.

[0016] In some embodiments, the composition further comprises a liquid formulation comprising the target analyte.

[0017] Embodiments of the present disclosure also include a composition comprising a dried formulation comprising (a) a first target analyte binding agent comprising a first target analyte binding element and a polypeptide component having at least 60% sequence identity with SEQ ID NO: 12, and (b) a second target analyte binding agent comprising a second target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 14.

[0018] In some embodiments, the dried formulation further comprises a luminogenic substrate.

[0019] In some embodiments, the composition further comprises a liquid formulation comprising the target analyte.

[0020] Embodiments of the present disclosure also include a composition comprising a dried formulation comprising (a) a first target analyte binding agent comprising a first target analyte binding element and a peptide component having at least 60% sequence identity with SEQ ID NO: 13, (b) a second target analyte binding agent comprising a second target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 15, and (c) a complementary polypeptide component having at least 60% sequence identity with SEQ ID NO: 12.

[0021] In some embodiments, the dried formulation further comprises a luminogenic substrate.

[0022] In some embodiments, the composition further comprises a liquid formulation comprising the target analyte.

[0023] Embodiments of the present disclosure also include a composition comprising (a) a dried formulation comprising a first target analyte binding agent comprising a target analyte binding element and a polypeptide component having at least 60% sequence identity with SEQ ID NO: 9, and (b) a liquid formulation comprising a second target analyte binding agent comprising a target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 10 or SEQ ID NO: 11.

[0024] Embodiments of the present disclosure also include a composition comprising (a) a dried formulation comprising a first target analyte binding agent comprising a target analyte binding element and a peptide component having at least 60% sequence identity with SEQ ID NO: 10 or SEQ ID NO: 11, and (b) a liquid formulation comprising a second target analyte binding agent comprising a target analyte binding element and a complementary polypeptide component having at least 60% sequence identity with SEQ ID NO: 9.

[0025] Embodiments of the present disclosure also include a composition comprising (a) a dried formulation comprising a first target analyte binding agent comprising a target analyte binding element and a polypeptide component having at least 60% sequence identity with SEQ ID NO: 12, and (b) a liquid formulation comprising a second target analyte binding agent comprising a target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 14.

[0026] In some embodiments, the dried formulation further comprises a luminogenic substrate.

[0027] In some embodiments, the liquid formulation further comprises a luminogenic substrate.

[0028] In some embodiments, the liquid formulation further comprises a sample comprising a target analyte, and wherein a bioluminescent analyte detection complex forms upon combining the dried formulation and the liquid formulation in the presence of the target analyte.

[0029] In some embodiments, the composition further comprises a second complementary peptide or polypeptide component of the bioluminescent complex, wherein the target analyte

binding agent, the first complementary peptide or polypeptide component of the bioluminescent complex, and the second complementary peptide or polypeptide component of the bioluminescent complex form a bioluminescent analyte detection complex in the presence of a target analyte.

[0030] In some embodiments, the composition comprising the target analyte binding agent comprises a dried formulation, and wherein the first complementary peptide or polypeptide component and the second complementary peptide or polypeptide of the bioluminescent complex comprise a liquid formulation; wherein the liquid formulation is added to the dried formulation and forms the bioluminescent analyte detection complex in the presence of the target analyte upon rehydration.

[0031] In some embodiments, the composition comprising the target analyte binding agent, and either the first or the second complementary peptide or polypeptide component are combined in a dried formulation, and wherein the first or the second complementary peptide or polypeptide component that is not present in the dried formulation comprises a liquid formulation; wherein the liquid formulation is added to the dried formulation and forms the bioluminescent analyte detection complex in the presence of the target analyte upon rehydration.

[0032] In some embodiments, the target analyte binding agent, the first complementary peptide or polypeptide component, and the second complementary peptide or polypeptide component are combined in a dried formulation that forms the bioluminescent analyte detection complex in the presence of the target analyte upon rehydration.

[0033] In some embodiments, the dried formulation further comprises a luminogenic substrate.

[0034] In some embodiments, the liquid formulation further comprises a luminogenic substrate.

[0035] In some embodiments, the liquid formulation further comprises a sample comprising a target analyte, and wherein a bioluminescent analyte detection complex forms upon combining the dried formulation and the liquid formulation in the presence of the target analyte.

[0036] In some embodiments, either the first or the second complementary peptide or polypeptide component comprises a second target analyte binding element that forms the bioluminescent analyte detection complex in the presence of the target analyte upon rehydration.

[0037] In some embodiments, the polypeptide component of the target analyte binding agent comprises at least 60% sequence identity with SEQ ID NO: 6, and wherein either the first or the second complementary peptide or polypeptide component of the bioluminescent complex comprises at least 60% sequence identity with either SEQ ID NO: 13 or SEQ ID NO: 15.

[0038] Embodiments of the present disclosure also include a composition comprising (a) a dried formulation comprising a first target analyte binding agent comprising a target analyte binding element and a polypeptide component having at least 60% sequence identity with SEQ ID NO: 6, and (b) a liquid formulation comprising a second target analyte binding agent comprising a target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 13 or SEQ ID NO: 15, and a second complementary peptide component having at least 60% sequence identity with SEQ ID NO: 13 or SEQ ID NO: 15.

[0039] Embodiments of the present disclosure also include (a) a dried formulation comprising a first target analyte binding agent comprising a target analyte binding element and a polypeptide component having at least 60% sequence identity with SEQ ID NO: 6, and a second target analyte binding agent comprising a target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 13 or SEQ ID NO: 15, and (b) a liquid formulation comprising a second complementary peptide component having at least 60% sequence identity with SEQ ID NO: 13 or SEQ ID NO: 15.

[0040] Embodiments of the present disclosure also include (a) a dried formulation comprising a first target analyte binding agent comprising a target analyte binding element and a polypeptide component having at least 60% sequence identity with SEQ ID NO: 6, and complementary peptide component having at least 60% sequence identity with SEQ ID NO: 13 or SEQ ID NO: 15, and (b) a liquid formulation comprising a second target analyte binding agent comprising a target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 13 or SEQ ID NO: 15.

[0041] Embodiments of the present disclosure also include (a) a dried formulation comprising a first target analyte binding agent comprising a target analyte binding element and a peptide component having at least 60% sequence identity with SEQ ID NO: 13, and a second target analyte binding agent comprising a target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 15, and (b) a liquid

formulation comprising a complementary polypeptide component having at least 60% sequence identity with SEQ ID NO: 6.

[0042] Embodiments of the present disclosure also include (a) a dried formulation comprising a complementary polypeptide component having at least 60% sequence identity with SEQ ID NO: 6, and (b) a liquid formulation comprising a first target analyte binding agent comprising a target analyte binding element and a peptide component having at least 60% sequence identity with SEQ ID NO: 13, and a second target analyte binding agent comprising a target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 15.

[0043] Embodiments of the present disclosure also include a composition comprising a dried formulation comprising a first target analyte binding agent comprising a target analyte binding element and a peptide component having at least 60% sequence identity with SEQ ID NO: 13, a second target analyte binding agent comprising a target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 15, and a complementary polypeptide component having at least 60% sequence identity with SEQ ID NO: 6.

[0044] In some embodiments, the dried formulation further comprises a luminogenic substrate.

[0045] In some embodiments, the liquid formulation further comprises a luminogenic substrate.

[0046] In some embodiments, the liquid formulation further comprises a sample comprising a target analyte, and wherein a bioluminescent analyte detection complex forms upon combining the dried formulation and the liquid formulation in the presence of the target analyte.

[0047] In some embodiments, a bioluminescent signal produced in the presence of the luminogenic substrate is substantially increased when the target analyte binding agent contacts one or more of the complementary peptide or polypeptide components of the bioluminescent complex, as compared to a bioluminescent signal produced by the target analyte binding agent and the luminogenic substrate alone.

[0048] In some embodiments, the target analyte is a target antibody.

[0049] In some embodiments, the target analyte binding agent comprises an element that binds non-specifically to antibodies.

[0050] In some embodiments, the target analyte binding agent comprises an element that binds specifically to an antibody.

[0051] In some embodiments, the target antibody is an antibody against a pathogen, toxin, or therapeutic biologic.

[0052] In some embodiments, a target analyte binding element is selected from the group consisting of an antibody, a polyclonal antibody, a monoclonal antibody, a recombinant antibody, an antibody fragment, protein A, an Ig binding domain of protein A, protein G, an Ig binding domain of protein G, protein A/G, an Ig binding domain of protein A/G, protein L, a Ig binding domain of protein L, protein M, an Ig binding domain of protein M, an oligonucleotide probe, a peptide nucleic acid, a DARPin, an aptamer, an affimer, a protein domain, and a purified protein.

[0053] In some embodiments, the luminogenic substrate is selected from coelenterazine, coelenterazine-h, coelenterazine-h-h, furimazine, JRW-0238, JRW-1404, JRW-1482, JRW, 1667, JRW-1743, JRW-1744, and other coelenterazine analogs or derivatives.

[0054] In some embodiments, the composition further comprises a polymer.

[0055] In some embodiments, the polymer is a naturally-occurring biopolymer. In some embodiments, the naturally-occurring biopolymer is selected from pullulan, trehalose, maltose, cellulose, dextran, and a combination of any thereof. In some embodiments, the naturally-occurring biopolymer is pullulan.

[0056] In some embodiments, the polymer is a cyclic saccharide polymer or a derivative thereof. In some embodiments, the polymer is hydroxypropyl β-cyclodextrin.

[0057] In some embodiments, the polymer is a synthetic polymer. In some embodiments, the synthetic polymer is selected from polystyrene, poly(meth)acrylate, and a combination of any thereof. In some embodiments, the synthetic polymer is a block copolymer comprising at least one poly(propylene oxide) block and at least one poly(ethylene oxide) block. In some embodiments, the synthetic polymer is poloxamer 188.

[0058] In some embodiments, the composition further comprises a substance to reduce autoluminescence.

[0059] In some embodiments, the substance to reduce autoluminescence is ATT (6-Aza-2-thiothymine), a derivative or analog of ATT, a thionucleoside, thiourea, and the like.

[0060] In some embodiments, the composition further comprises a buffer, a surfactant, a reducing agent, a salt, a radical scavenger, a chelating agent, a protein, or any combination thereof. In some embodiments, the is surfactant selected from polysorbate 20, polysorbate 40, and polysorbate 80.

[0061] In some embodiments, the composition is used in conjunction with an analyte detection platform to detect an analyte in a sample.

[0062] In some embodiments, sample is selected from blood, serum, plasma, urine, stool, cerebral spinal fluid, interstitial fluid, saliva, a tissue sample, a water sample, a soil sample, a plant sample, a food sample, a beverage sample, an oil, and an industrial fluid sample.

[0063] Embodiments of the present disclosure also include a method of detecting an analyte in a sample comprising combining any of the compositions described above with a sample comprising a target analyte.

[0064] In some embodiments, detecting the target analyte in the sample comprises detecting a bioluminescent signal generated from an analyte detection complex.

[0065] In some embodiments, the method further comprises quantifying a bioluminescent signal generated from the analyte detection complex.

[0066] In some embodiments, the bioluminescent signal generated from the analyte detection complex is proportional to the concentration of the analyte.

[0067] In some embodiments, one or more of the components of the composition exhibits enhanced stability within the composition compared to the component in solution alone.

[0068] Embodiments of the present disclosure also include systems and methods for the detection of an analyte or analytes in a sample. In particular, the present disclosure provides compositions, assays, and methods for detecting and/or quantifying a target analyte using a bioluminescent complex comprising substrates, peptides, and/or polypeptides capable of generating a bioluminescent signal that correlates to the presence, absence, or amount of the target analyte.

[0069] Embodiments of the present disclosure include a lateral flow detection system. In accordance with these embodiments, the system includes an analytical membrane that includes a detection region and a control region. In some embodiments, the detection region includes a first target analyte binding agent immobilized to the detection region, a conjugate pad comprising a second target analyte binding agent, and a sample pad. In some embodiments, the first target

analyte binding agent and the second target analyte binding agent form a bioluminescent analyte detection complex in the at least one detection region when a target analyte is detected in a sample.

[0070] In some embodiments, the first target analyte binding agent includes a target analyte binding element and is non-luminescent. In some embodiments, the second target analyte binding agent includes a target analyte binding element and a bioluminescent polypeptide. In some embodiments, the bioluminescent polypeptide has at least 60% sequence identity with SEQ ID NO: 5.

[0071] In some embodiments, the first target analyte binding agent includes a target analyte binding element and a polypeptide component of a bioluminescent complex, and the second target analyte binding agent includes a target analyte binding element and a peptide component of a bioluminescent complex. In some embodiments, a bioluminescent signal produced in the presence of a luminogenic substrate is substantially increased when the first target analyte binding agent contacts the second target analyte binding agent, as compared to a bioluminescent signal produced by the first target analyte binding agent and the luminogenic substrate alone.

[0072] In some embodiments, the first target analyte binding agent includes a target analyte binding element and a peptide component of a bioluminescent complex, and the second target analyte binding agent includes a target analyte binding element and a polypeptide component of a bioluminescent complex. In some embodiments, a bioluminescent signal produced in the presence of a luminogenic substrate is substantially increased when the first target analyte binding agent contacts the second target analyte binding agent, as compared to a bioluminescent signal produced by the first target analyte binding agent and the luminogenic substrate alone.

[0073] In some embodiments, the polypeptide component of a bioluminescent complex has at least 60% sequence identity with SEQ ID NO: 6. In some embodiments, the polypeptide component of a bioluminescent complex has at least 60% sequence identity with SEQ ID NO: 10. In some embodiments, the polypeptide component of a bioluminescent complex has at least 60% sequence identity with SEQ ID NO: 12. In some embodiments, the polypeptide component of a bioluminescent complex has at least 60% sequence identity with SEQ ID NO: 14.

[0074] In some embodiments, the first target analyte binding agent includes a target analyte binding element and a first peptide component of a tripartite bioluminescent complex, and the second target analyte binding agent includes a target analyte binding element and a second

peptide component of the tripartite bioluminescent complex. In some embodiments, a bioluminescent signal produced in the presence of a luminogenic substrate is substantially increased when the first target analyte binding agent contacts the second target analyte binding agent and a polypeptide component of the tripartite bioluminescent complex as compared to a bioluminescent signal produced by (i) the first target analyte binding agent, the second target analyte binding agent, and/or the polypeptide component and (ii) the luminogenic substrate alone.

[0075] In some embodiments, the first peptide component of a tripartite bioluminescent complex has at least 60% sequence identity with SEQ ID NO: 11. In some embodiments, the second first peptide component of a tripartite bioluminescent complex has at least 60% sequence identity with SEQ ID NO: 13. In some embodiments, the polypeptide component of a tripartite bioluminescent complex has at least 60% sequence identity with SEQ ID NO: 12.

[0076] In some embodiments, the target analyte is a target antibody. In some embodiments, the first target analyte binding element includes an agent that binds non-specifically to antibodies. In some embodiments, the second target analyte binding element comprises an agent that binds specifically to the target antibody. In some embodiments, the target antibody is an antibody against a pathogen, toxin, or therapeutic biologic.

[0077] In some embodiments, a target analyte binding element is selected from the group consisting of an antibody, a polyclonal antibody, a monoclonal antibody, a recombinant antibody, an antibody fragment, protein A, an Ig binding domain of protein A, protein G, an Ig binding domain of protein G, protein A/G, an Ig binding domain of protein A/G, protein L, a Ig binding domain of protein L, protein M, an Ig binding domain of protein M, an oligonucleotide probe, a peptide nucleic acid, a DARPin, an aptamer, an affimer, a protein domain, and a purified protein.

[0078] In some embodiments, the system further includes a luminogenic substrate. In some embodiments, the luminogenic substrate is selected from coelenterazine, coelenterazine-h, coelenterazine-h-h, furimazine, JRW-0238, JRW-1404, JRW-1482, JRW-1667, JRW-1743, JRW-1744, and other coelenterazine analogs or derivatives. In some embodiments, the luminogenic substrate is applied to the system as part of a composition that includes the luminogenic substrate and a polymer selected from pullulan, trehalose, maltose, cellulose, dextran, polystyrene, poly(meth)acrylate, and a combination of any thereof. In some

embodiments, the luminogenic substrate is applied to the system as part of a composition that includes the luminogenic substrate and a substance to reduce autoluminescence such as ATT (6-Aza-2-thiothymine), a derivative or analog of ATT, a thionucleoside, thiourea, and the like.

[0079] In some embodiments, the composition is applied to at least one of the sample pad, the conjugation pad, the detection region, and the control region.

[0080] In some embodiments, the analytical membrane includes a plurality of detection regions with each detection region comprising a distinct target analyte binding agent having distinct target analyte binding elements.

[0081] In some embodiments, the system further includes a device for detecting or quantifying bioluminescent signals from the analyte detection complex.

[0082] Embodiments of the present disclosure also include a conjugate pad comprising at least one target analyte binding agent. In accordance with these embodiments, the at least one target analyte binding agent includes a target analyte binding element and one of: a bioluminescent polypeptide comprising at least 60% sequence identity with SEQ ID NO: 5; a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 9; a peptide comprising at least 60% sequence identity with SEQ ID NO: 10; a peptide comprising at least 60% sequence identity with SEQ ID NO: 11; a peptide comprising at least 60% sequence identity with SEQ ID NO: 13; a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 12; a peptide comprising at least 60% sequence identity with SEQ ID NO: 14; or a fluorophore capable of being activated by energy transfer from an Oplophorus luciferase.

[0083] In some embodiments, the target analyte binding agent includes a target analyte binding element and one of: a bioluminescent polypeptide of SEQ ID NO: 5; a polypeptide of SEQ ID NO: 9; a peptide of SEQ ID NO: 10; a peptide of SEQ ID NO: 11; a peptide of SEQ ID NO: 13; a polypeptide of SEQ ID NO: 12; a peptide of SEQ ID NO: 14; or a fluorophore capable of being activated by energy transfer from an Oplophorus luciferase.

[0084] In some embodiments, the conjugate pad further includes a luminogenic substrate. In some embodiments, the luminogenic substrate is selected from coelenterazine, coelenterazine-h, coelenterazine-h-h, furimazine, JRW-0238, JRW-1404, JRW-1482, JRW-1667, JRW-1743, JRW-1744, and other coelenterazine analogs or derivatives. In some embodiments, the luminogenic substrate contained on or within the conjugate pad as part of a composition that includes the luminogenic substrate and a polymer selected from pullulan, trehalose, maltose,

cellulose, dextran, polystyrene, poly(meth)acrylate, and a combination of any thereof. In some embodiments, the luminogenic substrate is applied to the system as part of a composition that includes the luminogenic substrate and a substance to reduce autoluminescence such as ATT (6-Aza-2-thiothymine), a derivative or analog of ATT, a thionucleoside, thiourea, and the like.

[0085] Embodiments of the present disclosure also include an analytical membrane that includes a detection region and a control region. In accordance with these embodiments, the detection region includes at least one target analyte binding agent immobilized to the detection region.

[0086] In some embodiments, the at least one target analyte binding agent includes a target analyte binding element and one of: a bioluminescent polypeptide comprising at least 60% sequence identity with SEQ ID NO: 5; a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 9; a peptide comprising at least 60% sequence identity with SEQ ID NO: 10; a peptide comprising at least 60% sequence identity with SEQ ID NO: 11; a peptide comprising at least 60% sequence identity with SEQ ID NO: 13; a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 12; a peptide comprising at least 60% sequence identity with SEQ ID NO: 14; or a fluorophore capable of being activated by energy transfer from an Oplophorus luciferase.

[0087] In some embodiments, the target analyte binding agent includes a target analyte binding element and one of: a bioluminescent polypeptide of SEQ ID NO: 5; a polypeptide of SEQ ID NO: 9; a peptide of SEQ ID NO: 10; a peptide of SEQ ID NO: 11; a peptide of SEQ ID NO: 13; a polypeptide of SEQ ID NO: 12; a peptide of SEQ ID NO: 14; or a fluorophore capable of being activated by energy transfer from an Oplophorus luciferase.

[0088] In some embodiments, the analytical membrane further includes a plurality of detection regions with each detection region comprising a distinct target analyte binding agent having distinct target analyte binding elements. In some embodiments, the analytical membrane further includes a luminogenic substrate. In some embodiments, the luminogenic substrate is selected from coelenterazine, coelenterazine-h, coelenterazine-h-h, furimazine, JRW-0238, JRW-1404, JRW-1482, JRW-1667, JRW-1743, JRW-1744, and other coelenterazine analogs or derivatives.

[0089] In some embodiments, the luminogenic substrate is reversibly conjugated to the conjugate pad as part of a composition including the luminogenic substrate and a polymer

selected from pullulan, trehalose, maltose, cellulose, dextran, polystyrene, poly(meth)acrylate, and a combination of any thereof. In some embodiments, the luminogenic substrate is part of a composition that includes the luminogenic substrate and a substance that reduces autoluminescence such as ATT (6-Aza-2-thiothymine), a derivative or analog of ATT, a thionucleoside, thiourea, and the like.

[0090] Embodiments of the present disclosure also include a solid phase detection platform comprising a detection region. In accordance with these embodiments, the detection region includes at least one target analyte binding agent conjugated to the detection region. In some embodiments, the at least one target analyte binding agent includes a target analyte binding element and one of: a bioluminescent polypeptide comprising at least 60% sequence identity with SEQ ID NO: 5; a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 9; a peptide comprising at least 60% sequence identity with SEQ ID NO: 10; a peptide comprising at least 60% sequence identity with SEQ ID NO: 11; a peptide comprising at least 60% sequence identity with SEQ ID NO: 13; a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 12; a peptide comprising at least 60% sequence identity with SEQ ID NO: 14; or a fluorophore capable of being activated by energy transfer from an Oplophorus luciferase.

[0091] In some embodiments, the target analyte binding agent includes a target analyte binding element and one of: a bioluminescent polypeptide of SEQ ID NO: 5; a polypeptide of SEQ ID NO: 9; a peptide of SEQ ID NO: 10; a peptide of SEQ ID NO: 11; a peptide of SEQ ID NO: 13; a polypeptide of SEQ ID NO: 12; a peptide of SEQ ID NO: 14; or a fluorophore capable of being activated by energy transfer from an Oplophorus luciferase.

[0092] In some embodiments, the detection platform includes: a first target analyte binding agent comprising a target analyte binding element and a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 6 conjugated to the detection region; and a second target analyte binding agent comprising a target analyte binding element and a peptide comprising at least 60% sequence identity with SEQ ID NO: 10 applied to the detection region.

[0093] In some embodiments, the detection platform includes: a first target analyte binding agent comprising a target analyte binding element and a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 10 conjugated to the detection region; and a second target

analyte binding agent comprising a target analyte binding element and a peptide comprising at least 60% sequence identity with SEQ ID NO: 6 applied to the detection region.

[0094] In some embodiments, the detection platform includes: a first target analyte binding agent comprising a target analyte binding element and a peptide comprising at least 60% sequence identity with SEQ ID NO: 11 conjugated to the detection region; a second target analyte binding agent comprising a target analyte binding element and a peptide comprising at least 60% sequence identity with SEQ ID NO: 13 applied to the detection region; and a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 12 applied to the detection region.

[0095] In some embodiments, the detection platform includes: a first target analyte binding agent comprising a target analyte binding element and a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 6 conjugated to the detection region; and a second target analyte binding agent comprising a target analyte binding element and a polypeptide comprising at least 60% sequence identity with ID NO: 14 applied to the detection region.

[0096] In some embodiments, the detection platform includes: a first target analyte binding agent comprising a target analyte binding element and a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 14 conjugated to the detection region; and a second target analyte binding agent comprising a target analyte binding element and a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 6 applied to the detection region.

[0097] In some embodiments, the detection platform includes: a first target analyte binding agent comprising a target analyte binding element and a bioluminescent polypeptide at least 60% sequence identity with SEQ ID NO: 5 conjugated to the detection region; and a second target analyte binding agent comprising a target analyte binding element and a fluorophore capable of being activated by energy transfer from the bioluminescent polypeptide applied to the detection region.

[0098] In some embodiments, the detection platform includes: a first target analyte binding agent comprising a target analyte binding element and a bioluminescent polypeptide at least 60% sequence identity with SEQ ID NO: 5 applied to the detection region; and a second target analyte binding agent comprising a target analyte binding element and a fluorophore capable of being activated by energy transfer from the bioluminescent polypeptide conjugated to the detection region.

[0099] In some embodiments, the detection platform further includes a plurality of detection regions with each detection region comprising a distinct target analyte binding agent having distinct target analyte binding elements. In some embodiments, the detection platform further includes a control region. In some embodiments, the detection platform further includes a luminogenic substrate. In some embodiments, the luminogenic substrate is selected from coelenterazine, coelenterazine-h, coelenterazine-h-h, furimazine, JRW-0238, JRW-1404, JRW-1482, JRW-1667, JRW-1743, JRW-1744, and other coelenterazine analogs or derivatives. In some embodiments, the luminogenic substrate is reversibly conjugated to the conjugate pad as part of a composition comprising the luminogenic substrate and a polymer selected from pullulan, trehalose, maltose, cellulose, dextran, polystyrene, poly(meth)acrylate, and a combination of any thereof. In some embodiments, the luminogenic substrate is part of a composition comprising the luminogenic substrate and a substance that reduces autoluminescence such as ATT (6-Aza-2-thiothymine), a derivative or analog of ATT, a thionucleoside, thiourea, and the like.

[0100] Embodiments of the present disclosure also include a solution phase detection platform that includes at least one detection receptacle and a lyophilized tablet (lyocake). In accordance with these embodiments, the lyocake comprises a target analyte binding agent comprising a target analyte binding element and one of: a bioluminescent polypeptide comprising at least 60% sequence identity with SEQ ID NO: 5; a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 9; a peptide comprising at least 60% sequence identity with SEQ ID NO: 10; a peptide comprising at least 60% sequence identity with SEQ ID NO: 11; a peptide comprising at least 60% sequence identity with SEQ ID NO: 13; a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 12; a peptide comprising at least 60% sequence identity with SEQ ID NO: 14; or a fluorophore capable of being activated by energy transfer from an Oplophorus luciferase.

[0101] In some embodiments, the target analyte binding agent comprises a target analyte binding element and one of: a bioluminescent polypeptide of SEQ ID NO: 5; a polypeptide of SEQ ID NO: 9; a peptide of SEQ ID NO: 10; a peptide of SEQ ID NO: 11; a peptide of SEQ ID NO: 13; a polypeptide of SEQ ID NO: 12; a peptide of SEQ ID NO: 14; or a fluorophore capable of being activated by energy transfer from an Oplophorus luciferase.

[0102] In some embodiments, the lyocake comprises: a first target analyte binding agent comprising a target analyte binding element and a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 6; and a second target analyte binding agent comprising a target analyte binding element and a peptide comprising at least 60% sequence identity with SEQ ID NO: 10.

[0103] In some embodiments, the lyocake comprises: a first target analyte binding agent comprising a target analyte binding element and a peptide comprising at least 60% sequence identity with SEQ ID NO: 11; a second target analyte binding agent comprising a target analyte binding element and a peptide comprising at least 60% sequence identity with SEQ ID NO: 13; and a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 12.

[0104] In some embodiments, the lyocake comprises: a first target analyte binding agent comprising a target analyte binding element and a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 6; and a second target analyte binding agent comprising a target analyte binding element and a polypeptide comprising at least 60% sequence identity with ID NO: 14.

[0105] In some embodiments, the lyocake comprises: a first target analyte binding agent comprising a target analyte binding element and a bioluminescent polypeptide at least 60% sequence identity with SEQ ID NO: 5; and a second target analyte binding agent comprising a target analyte binding element and a fluorophore capable of being activated by energy transfer from the bioluminescent polypeptide.

[0106] In some embodiments, the detection platform comprises a 96-well microtiter plate comprising a plurality of detection receptacles, and at least two distinct target analyte binding agents comprising distinct target analyte binding elements.

[0107] In some embodiments, the lyocake comprises a luminogenic substrate. In some embodiments, the luminogenic substrate is selected from coelenterazine, coelenterazine-h, coelenterazine-h-h, furimazine, JRW-0238, JRW-1404, JRW-1482, JRW-1667, JRW-1743, JRW-1744, and other coelenterazine analogs or derivatives.

[0108] In some embodiments, the lyocake comprises a luminogenic substrate and a polymer selected from pullulan, trehalose, maltose, cellulose, dextran, polystyrene, poly(meth)acrylate, and a combination of any thereof.

[0109] In some embodiments, the lyocake comprises a luminogenic substrate and a substance to reduce autoluminescence such as ATT (6-Aza-2-thiothymine), a derivative or analog of ATT, a thionucleoside, thiourea, and the like.

[0110] In some embodiments, the detection platform further comprises at least one sample. In some embodiments, the sample is selected from blood, serum, plasma, urine, stool, cerebral spinal fluid, interstitial fluid, saliva, a tissue sample, a water sample, a soil sample, a plant sample, a food sample, a beverage sample, an oil, and an industrial fluid sample.

[0111] Embodiments of the present disclosure also include a method of detecting an analyte in a sample using the lateral flow assay systems described above. In accordance with these embodiments, the method includes applying a sample to the sample pad, facilitating flow of the sample from the sample pad to the conjugate pad, and then from the conjugate pad to the detection region and the control region on the analytical membrane. In some embodiments, the first target analyte binding agent, the second target analyte binding agent, and the target analyte form the analyte detection complex in the at least one detection region when the target analyte is detected in the sample.

[0112] In some embodiments, the sample is a sample from a subject selected from blood, serum, plasma, urine, stool, cerebral spinal fluid, interstitial fluid, tissue, and saliva. In some embodiments, the sample is selected from a water sample, a soil sample, a plant sample, a food sample, a beverage sample, an oil, and an industrial fluid sample. In some embodiments, detecting the target analyte in the sample comprises detecting a bioluminescent signal generated from the analyte detection complex.

[0113] In some embodiments, the method further comprises quantifying a bioluminescent signal generated from the analyte detection complex. In some embodiments, the method further comprises diagnosing a subject from which the sample was obtained as having or not having a disease based on the detection of the analyte.

[0114] Embodiments of the present disclosure also include a method of detecting an analyte in a sample using the solid phase detection platform described above. In accordance with these embodiments, the method includes exposing a sample to the detection region and control region. In some embodiments, the at least one target analyte binding agent and the at least one target analyte form an analyte detection complex in the at least one detection region when the target analyte is detected in the sample.

[0115] In some embodiments, the sample is a sample from a subject selected from blood, serum, plasma, urine, stool, cerebral spinal fluid, interstitial fluid, tissue, and saliva. In some embodiments, the sample is selected from a water sample, a soil sample, a plant sample, a food sample, a beverage sample, an oil, and an industrial fluid sample. In some embodiments, detecting the target analyte in the sample comprises detecting a bioluminescent signal generated from the analyte detection complex.

[0116] In some embodiments, the method further comprises quantifying a bioluminescent signal generated from the analyte detection complex. In some embodiments, the method further comprises diagnosing a subject from which the sample was obtained as having or not having a disease based on the detection of the analyte.

[0117] Embodiments of the present disclosure also include a method of producing a substrate for use in a bioluminescent assay. In accordance with these embodiments, the method includes applying a solution onto a substrate. In some embodiments, the solution contains at least one target analyte binding agent comprising a target analyte binding element and one of a polypeptide component of a bioluminescent complex or a peptide component of a bioluminescent complex. In some embodiments, the method includes drying the substrate containing the solution.

[0118] In some embodiments, the solution further includes a complementary peptide or polypeptide component of the bioluminescent complex. In some embodiments, the target analyte binding agent and the complementary peptide or polypeptide component of the bioluminescent complex form a bioluminescent analyte detection complex in the presence of a target analyte.

[0119] In some embodiments, the solution comprises a protein buffer and at least one excipient. In some embodiments, the solution comprises a luminogenic substrate.

[0120] In some embodiments, the substrate comprising the dried solution is W-903 paper, FTA paper, FTA Elute paper, FTA DMPK paper, Ahlstrom A-226 paper, M-TFN paper, FTA paper, FP705 paper, Bode DNA collection paper, nitrocellulose paper, nylon paper, cellulose paper, Dacron paper, cotton paper, and polyester papers, or combinations thereof. In some embodiments, the substrate is a mesh comprising plastic, nylon, metal, or combinations thereof.

[0121] In some embodiments, drying the substrate containing the solution comprises drying at a temperature from about 30°C to 40°C for a period of time between about 30 mins and 2 hours.

In some embodiments, drying the substrate containing the solution comprises lyophilizing and/or freezing the substrate.

[0122] In some embodiments, the method further comprises drying the at least one target analyte binding agent and/or the complementary peptide or polypeptide component of the bioluminescent complex onto a first substrate, and drying the luminogenic substrate onto a second substrate.

[0123] In accordance with these embodiments, a bioluminescent signal is generated upon exposure of the substrate containing the solution to the target analyte, and in some embodiments, the bioluminescent signal is proportional to the concentration of the target analyte.

[0124] In some embodiments, the at least one target analyte binding agent and/or the complementary peptide or polypeptide component of the bioluminescent complex exhibit(s) enhanced stability when dried on the substrate.

[0125] Embodiments of the present disclosure include a composition comprising a luminogenic substrate, a target analyte binding agent comprising a target analyte binding element and a polypeptide component of a bioluminescent complex, and a complementary polypeptide component of the bioluminescent complex. In accordance with these embodiments, the target analyte binding agent and the complementary polypeptide component of the bioluminescent complex are capable of forming a bioluminescent analyte detection complex in the presence of a target analyte.

[0126] In some embodiments, the composition further comprises a second target analyte binding agent comprising a second target analyte binding element and a second polypeptide component of a bioluminescent complex.

[0127] In some embodiments, the first and second target analyte binding agents bind separate portions of the same target analyte.

[0128] In some embodiments, the first and second polypeptide components of the bioluminescent complex bind the complementary polypeptide component of the bioluminescent complex to form a bioluminescent analyte detection complex in the presence of the target analyte.

[0129] In some embodiments, the first and the second polypeptide components are linked to a modified dehalogenase capable of forming a covalent bond with a haloalkane substrate.

[0130] In some embodiments, the first and the second target analyte binding elements comprise a haloalkane substrate.

[0131] In some embodiments, the first or second polypeptide components of the first and second target analyte binding agents comprise: at least 60% sequence identity with SEQ ID NO: 10; at least 60% sequence identity with SEQ ID NO: 11; at least 60% sequence identity with SEQ ID NO: 13; or at least 60% sequence identity with SEQ ID NO: 15.

[0132] In some embodiments, the complementary polypeptide component comprises: at least 60% sequence identity with SEQ ID NO: 6; at least 60% sequence identity with SEQ ID NO: 9; or at least 60% sequence identity with SEQ ID NO: 12.

[0133] In some embodiments, the target analyte binding element is selected from the group consisting of an antibody, a polyclonal antibody, a monoclonal antibody, a recombinant antibody, an antibody fragment, protein A, an Ig binding domain of protein A, protein G, an Ig binding domain of protein G, protein A/G, an Ig binding domain of protein A/G, protein L, a Ig binding domain of protein L, protein M, an Ig binding domain of protein M, an oligonucleotide probe, a peptide nucleic acid, a DARPin, an aptamer, an affimer, a protein domain, and a purified protein.

[0134] In some embodiments, the target analyte is an antibody, and wherein the target analyte binding element of the first target analyte binding agent comprises antigen recognized by the antibody, and wherein the target analyte binding element of the second target analyte binding agent comprises an Fc binding region.

[0135] In some embodiments, the first and/or second target analyte binding agents further comprise a fluorophore coupled to the first and/or second polypeptide components of the bioluminescent complex.

[0136] In some embodiments, one or more components of the composition is in the form of a lyophilized tablet (lyocake) capable of forming a bioluminescent complex when reconstituted in a solution to detect and/or quantify the target analyte.

[0137] In some embodiments, the composition comprises a solution-phase detection platform capable of detecting and/or quantifying the target analyte.

[0138] In some embodiments, the polypeptide components and the luminogenic substrate are in the form of a lyophilized tablet (lyocake) capable of forming a bioluminescent complex when reconstituted in a solution to detect and/or quantify the target analyte.

[0139] Embodiments of the present disclosure also includes a method of detecting an analyte in a sample comprising combining any of the compositions described above with a sample comprising a target analyte.

[0140] In some embodiments, detecting the target analyte in the sample comprises detecting a bioluminescent signal generated from an analyte detection complex.

[0141] In some embodiments, the method further comprises quantifying a bioluminescent signal generated from the analyte detection complex.

[0142] In some embodiments, the bioluminescent signal generated from the analyte detection complex is proportional to the concentration of the analyte.

BRIEF DESCRIPTION OF THE DRAWINGS

[0143] FIG. 1 shows a representative schematic diagram of a lateral flow assay for detecting and/or quantifying a target analyte(s) in a sample based on bioluminescent complex formation, according to one embodiment of the present disclosure.

[0144] FIG. 2 shows a representative schematic diagram of a solid phase detection platform for detecting and/or quantifying target analytes in a sample based on bioluminescent complex formation, according to one embodiment of the present disclosure.

[0145] FIG. 3 shows representative images demonstrating that components of the bioluminescent complexes produce detectable bioluminescence after being applied to a solid support substrate (e.g., membrane), dried, and stored at room temperature.

[0146] FIG. 4 shows representative images demonstrating that components of the bioluminescent complexes produce detectable bioluminescence after being applied to membrane and paper-based solid support substrates.

[0147] FIG. 5 shows a representative assay schematic (left) and a representative graph (right) demonstrating the ability of components of the bioluminescent complexes to be used as reporters on target analyte binding agents for target analyte detection.

[0148] FIG. 6 shows a representative depiction of an assay platform using components of the bioluminescent complexes as reporters on target analyte binding agents for target analyte detection.

[0149] FIGS. 7A-7E show representative stability tests of an assay platform using components of the bioluminescent complexes as reporters on target analyte binding agents for target analyte detection, according to one embodiment of the present disclosure (FIG. 7A at 4°C;

FIG. 7B at 25°C; FIG. 7C at 37°C; FIG. 7D at 37°C with NanoLuc added; and FIG. 7E at 4°C and 37°C with HiBiT added).

[0150] FIGS. 8A-8B show representative tests of storage conditions of an assay platform using components of the bioluminescent complexes as reporters on target analyte binding agents for target analyte detection, according to one embodiment of the present disclosure (FIG. 8A at 4°C and 25°C; FIG. 8B at 4°C and 25°C with a sucrose-based protein buffer).

[0151] FIGS. 9A-9C show representative images from a solid phase assay platform (FIG. 9A) in which a bioluminescence signal was produced in complex sampling environments (whole blood in FIG. 9B and serum in FIG. 9C) indicating target analyte detection.

[0152] FIG. 10A-10B shows that RLU signal derived from Whatman 903 paper spots after rehydration with an assay buffer can be measured either quantitatively (FIG. 10A) or qualitatively (FIG. 10B).

[0153] FIGS. 11A-11B show representative graphs demonstrating the ability of a high affinity dipeptide, Pep263, to form bioluminescent complexes (Pep263 is a peptide comprising the β9 and β10 stands of the NanoTrip complex; see, e.g., U.S. Pat. Appln. Serial No. 16/439,565 (PCT/US2019/036844), which is herein incorporated by reference in its entirety).

[0154] FIG. 12 shows representative results of a solid phase assay demonstrating qualitative assessment of bioluminescence from paper punches placed into a standard microtiter plate using a standard camera from an iPhone (e.g., iPhone 6S) or from an imager (e.g., LAS4000).

[0155] FIG. 13 shows quantitative analysis of the same solid phase assay depicted in FIG. 12, but luminescence was detected using a luminometer on day 3 of storage at 25°C.

[0156] FIG. 14 shows a quantitative time course of the same solid phase assay as depicted in FIGS. 12-13, demonstrating stability of all the proteins in the experimental conditions at all temps tested over the time frame.

[0157] FIG. 15 shows representative RLU signal kinetic results collected on day 0 of an accelerated stability study performed under two buffer conditions at 25°C and 60°C.

[0158] FIG. 16 shows time-course results for an accelerated stability study of the proteins placed using the conjugation buffer conditions defined in FIG. 15.

[0159] FIG. 17 shows a comparison of the impact of buffer conditions on luminescence from NanoLuc dried onto a nitrocellulose membrane.

[0160] FIG. 18 shows the effects of membrane blocking and sucrose pre-treatment on lateral flow assays performed in a running buffer of 20X SSC, 1% BSA, pH 7.0, and 10μM N205 (Live Cell Substrate; LCS).

[0161] FIG. 19 shows the effects of membrane blocking and sucrose pre-treatment on lateral flow assays performed in a running buffer of 0.01 M PBS, 1% BSA, pH 7.0, and 10μM Permeable Cell Substrate (PCS).

[0162] FIG. 20 shows the effects of membrane blocking and sucrose pre-treatment on lateral flow assays performed in a running buffer of 5x LCS dilution buffer + 5x LCS – diluted to 1X in PBS.

[0163] FIG. 21 shows effects of membrane properties on bioluminescent reagent absorption and capillary action in a lateral flow assay.

[0164] FIGS. 22A-22B show bioluminescent signal from NanoBiT/HiBiT complementation on nitrocellulose (left) and Whatman grade 541 (right) papers (FIG. 22A), and a compilation image from a corresponding movie taken across total exposure time (FIG. 22B).

[0165] FIG. 23 shows bioluminescent signal from NanoBiT/HiBiT complementation on Whatman 903 paper, with a spike of additional substrate and liquid at 20 minutes.

[0166] FIG. 24 shows bioluminescent signal from NanoBiT/HiBiT complementation on Whatman 903 paper.

[0167] FIGS. 25A-25C show bioluminescent signal resulting from reconstitution with a dipeptide of LgTrip and substrate in Whatman 903 paper, which was prepared with BSA (FIG. 25B) or without BSA (FIG. 25A); FIG. 25C shows maximum RLU signals obtained for each concentration tested in FIG. 25B.

[0168] FIGS. 26A-26B show bioluminescent signal resulting from reconstitution with a dipeptide of LgTrip and substrate from a lyocake (FIG. 26A), along with a titration of the dipeptide; FIG. 26B shows maximum RLU signals obtained for each concentration tested in FIG. 26A.

[0169] FIG. 27 shows bioluminescent signal in three different solid phase materials (Whatman 903, Ahlstrom 237, and Ahlstrom 6613H) resulting from reconstitution with a dipeptide added to dried LgTrip and substrate, or NanoLuc added to dried LgTrip and substrate.

[0170] FIG. 28 shows bioluminescent signal generated from Whatman 903 spots containing Lg/Trip/substrate and stored under ambient conditions over 25 days; spots were exposed to 1 nM dipeptide in PBS.

[0171] FIGS. 29A-29C show bioluminescent signal (RLU) for NanoLuc (FIG. 29A), LgBiT (FIG. 29B), and LgTrip (FIG. 29C) that were dried in Whatman 903 papers with various protein buffer formulations and reconstituted with furimazine.

[0172] FIGS. 30A-30C show bioluminescent signal (B_{max}) for NanoLuc (FIG. 30A), LgBiT (FIG. 30B), and LgTrip (FIG. 30C) that were dried in Whatman 903 papers with various protein buffer formulations and reconstituted with furimazine, as shown in FIG. 29.

[0173] FIGS. 31A-31B show bioluminescent background levels for LgBiT (FIG. 31A) and LgTrip (FIG. 31B) that were dried in Whatman 903 papers with various protein buffer formulations and reconstituted with furimazine, as shown in FIG. 29.

[0174] FIGS. 32A-32F show bioluminescent signal (RLU signal kinetics) after reconstitution with furimazine in FIGS. 32A-32C; B_{max} in FIGS. 32D-32F) for NanoLuc (FIGS. 32A and 32D), LgBiT (FIGS. 32B and 32E), and LgTrip (FIGS. 32C and 32F) that were dried in Whatman 903 papers with various protein buffer formulations and reconstituted with furimazine after 6 days of storage at 60°C.

[0175] FIG. 33 includes representative embodiments of all-in-one lyophilized cakes ("lyocakes") or tablets containing all necessary reagents to perform an analyte detection test supporting several types of assay formats including cuvettes, test tubes, large volumes in bottles, snap test type assays, etc.

[0176] FIG. 34 shows bioluminescent signal from substrate movement across a lateral flow strip containing NanoLuc from a compilation image corresponding to a movie taken across total exposure time.

[0177] FIG. 35 shows bioluminescent signal from NanoLuc movement across a lateral flow strip from a compilation image corresponding to a movie taken across total exposure time.

[0178] FIG. 36 shows various tracers generated by tethering fumonisin B1 to a peptide tag (e.g., comprising SEQ ID NO: 10) via a biotin/streptavidin linkage, via a HaloTag linkage, or directly (e.g., via sulfo-SE labeling described in, for example, U.S. Patent Appln. Serial No. 16/698,143 (PCT/US2019/063652), herein incorporated by reference), which can be used in competitive binding assays in accordance with the materials and methods described herein.

[0179] FIG. 37 shows an exemplary competitive binding assay in which varying concentrations of unlabeled fumonisin B1 disrupts the bioluminescent complex and results in decreased luminescence and the ability to detect/quantify the amount of fumonisin B1 in a sample.

[0180] FIGS. 38A-38B show bioluminescent signal resulting from a lyophilized cake containing LgBiT and substrate when reconstituted with a dipeptide in PBS (FIG. 38A); FIG. 38B shows maximum RLU signals obtained for each concentration tested in FIG. 38A.

[0181] FIG. 39 shows the bioluminescent signal resulting from reconstitution of LgBiT or LgTrip 3546 that was lyophilized directly into a standard 96-well plate with or without substrate; reconstitution was performed with dipeptide in PBS with or without substrate.

[0182] FIGS. 40A-40C show the bioluminescent signal resulting from the complementation of LgBiT-protein G, SmBiT-TNFα, and substrate in Whatman 903 paper spots (FIGS. 40A-40B) and in a lyocake format (FIG. 40C) after reconstitution with varying concentrations of the target analyte Remicade in PBS.

[0183] FIGS. 41A-41C show the bioluminescent signal resulting from the complementation of LgTrip, SmTrip9-protein G, HiBiT-TNFα, and substrate in Whatman 903 paper spots (FIG. 41A) and in a lyocake format (FIG. 41B-41C) after reconstitution with varying concentrations of the target analyte Remicade in PBS.

[0184] FIGS. 42A-42E show the bioluminescent signal resulting from the complementation of bioluminescent complexes dried down in a form that does not include a substrate (FIGS. 42B-42C: mesh-based lyocakes; FIGS. 42D-42E: mesh-based film); the substrate is added separately to generate the bioluminescent signal in the presence of the analyte.

[0185] FIG. 43 shows lyophilized cake formations and colorimetric pHs of four different furimazine substrate formulations.

[0186] FIG. 44 shows the kinetic activity performance of various furimazine (Fz) substrate formulations in the presence of purified NanoLuc (Nluc) enzyme.

[0187] FIG. 45 shows the activity performance of a furimazine substrate formulation that had been stored at 60°C for the indicated time in days.

[0188] FIGS. 46A-46B show thermal stability over time in days of various furimazine substrate formulations maintained at ambient temperature (FIG. 46A) or 60°C (FIG. 46B) as

analyzed by HPLC for absolute furimazine concentration remaining after reconstitution in PBS, pH 7.0 containing 0.01% BSA.

[0189] FIG. 47 shows the amount of furimazine remaining for various furimazine substrate formulations after 12 days of reconstitution in water as analyzed by HPLC indicating liquid stability.

[0190] FIG. 48 shows a schematic representation of the homogenous tripartite immunoassay for the analyte interleukin-6 (IL-6).

[0191] FIG. 49 shows an example of an SDS-PAGE gel of antibody labeling with tripartite-HaloTag fusion proteins. Variants of SmTrip9 or SmTrip10 were fused to HaloTag and expressed, purified, and used to label mouse anti-human IL-6 antibodies.

[0192] FIGS. 50A-50B show the signal kinetics of a solution-based homogeneous tripartite IL-6 immunoassay with and without IL-6 (raw RLUs in FIG. 50A, and fold response in FIG. 50B).

[0193] FIGS. 51A-51B show the dose response curve of recombinant human IL-6 for the solution-based homogeneous IL-6 tripartite immunoassay (log graph in FIG. 51A; linear graph in FIG. 51B).

[0194] FIGS. 52A-52C show the lyophilized cake product (FIG. 52A; #1 and #2) and IL-6 immunoassay performance and shelf-stability of various formulated, single reagent lyophilized cakes without furimazine (Fz; FIG. 52B) and with furimazine (Fz; FIG. 52C) after reconstitution following storage at ambient temperature for the indicating time in days.

[0195] FIGS. 53A-53B show cake appearance (FIG. 53A) and performance (FIG. 53B) and shelf-stability of a formulated, lyophilized single-reagent IL-6 tripartite immunoassays stored for 90 days at ambient storage.

[0196] FIG. 54 shows the signal kinetics of a single reagent, lyophilized tripartite IL-6 immunoassay post-reconstitution.

[0197] FIG. 55 shows the compatibility of a lyophilized single reagent IL-6 immunoassay with complex human matrices.

[0198] FIGS. 56A-56B show a lyophilized single-reagent, IL-6 tripartite immunoassay in a pre-filled 96-well microtiter plate (FIG. 56A) and a rhIL-6 dose response curve using the lyophilized, single reagent, IL-6 tripartite immunoassay assay plate following reconstitution (FIG. 56B).

[0199] FIGS. 57A-57B show the assay performance of the solution-based IL-6 tripartite immunoassay in single formulation excipients (FIG. 57A) and in various formulated solutions (FIG. 57B).

[0200] FIG. 58 shows a schematic representation of the homogenous tripartite immunoassay for the model analyte cardiac troponin I.

[0201] FIGS. 59A-59B show dose response curves for the solution-based, homogeneous cardiac troponin I tripartite immunoassay using recombinant human cardiac tropoinin I in raw RLUs (FIG. 59A) and signal over background (FIG. 59B).

[0202] FIG. 60 shows the assay performance in raw RLUs of the single-reagent, formulated lyophilized troponin cardiac I tripartite immunoassay after reconstitution with 0.01% BSA in PBS or 10% normal pooled human serum diluted in general serum diluent.

[0203] FIGS. 61A-61B show raw RLU results of the solution-based, homogeneous IL-6 tripartite immunoassay background signals in the presence of human sera when using assay buffers 0.01% BSA in PBS (FIG. 61A) and in general serum diluent (FIG. 61B).

[0204] FIGS. 62A-62B show the raw Bmax RLU results of the solution-based, homogeneous IL-6 tripartite immunoassay in the presence of 50 ng/ml of rhIL-6 in the presence of human sera when using assay buffers 0.01% BSA in PBS (FIG. 62A) and in general serum diluent (FIG. 62B).

[0205] FIGS. 63A-63D show the signal to background results of the solution-based, homogeneous IL-6 tripartite immunoassay in the presence or absence of 50 ng/ml rhIL-6 with increasing amounts of normal pooled human serum (FIGS. 63A and 63C) or normal pooled human plasma (FIGS. 63B and 63D) when run in either 0.01% BSA in PBS or General Serum Diluent as assay buffer and NanoGlo (Promega Cat #N113) (FIGS. 63C and 63D) or Live Cell (Promega Cat # N205) substrates (FIGS. 63A and 63B).

[0206] FIG. 64 shows the signal-to-background results of the solution-based, homogeneous IL-6 tripartite immunoassay in the presence or absence of 50 ng/ml rhIL-6 with increasing amounts of normal, pooled human sera and pooled human sera that has been depleted of endogenous IgG when using general serum diluent as assay buffer.

[0207] FIGS. 65A-65C show the results of background RLU (FIG. 65A), Bmax RLU (FIG. 65B), and resulting signal over background (FIG. 65C) for the solution-based, homogeneous IL-6 tripartite immunoassay in the presence or absence of 50 ng/ml rhIL-6 with increasing amounts

of human blood chemistry panel components provided in the VeriChem matrix plus chemistry reference kit.

[0208] FIGS. 66A-66C show the results of background RLU (FIG. 66A), Bmax RLU (FIG. 66B), and resulting signal over background (FIG. 66C) for the solution-based, homogeneous IL-6 tripartite immunoassay in the presence or absence of 50 ng/ml rhIL-6 with increasing amounts of pooled normal human urine and NanoGlo (Promega Cat # N113) or Live Cell (Promega Cat# N205) substrates.

[0209] FIGS. 67A-67C show the raw RLU activity assay response of reconstituted lyophilized formulated furimazine tested with purified NanoLuc enzyme (Nluc) (FIG. 67A), formulated LgTrip polypeptide (SEQ ID NO: 12) tested with purified di-peptide (SEQ ID NO: 14) (FIG. 67B), and formulated furimazine and LgTrip polypeptide (SEQ ID NO: 12) tested with purified di-peptide (SEQ ID NO: 14) combined analyzing the thermal stability of the lyophilized vials (FIG. 67C).

[0210] FIG. 68 shows a schematic representation of a homogenous tripartite immunoassay for three anti-TNFα biologics: Remicade, Enbrel, and Humira.

[0211] FIGS. 69A-69C show the assay performance in raw RLUs of the solution-based, homogenous tripartite (LgTrip 3546 + SmTrip9 pep521 + SmTrip10) immunoassays quantitating the anti-TNFα biologics Remicade, Humira, and Enbrel.

[0212] FIGS. 70A-70B show the kinetic assay performance displayed as raw RLUs of reconstituted formulated, lyophilized single-reagent immunoassays for detection of Remicade using NanoTrip (tripartite-NanoLuc; FIG. 70A) and NanoBiT (FIG. 70B).

[0213] FIG. 71 shows the thermal stability at ambient temperatures of the single-reagent, lyophilized NanoBiT ("Bits") and NanoTrip ("Trips;" tripartite NanoLuc) immunoassay systems for the detection of Remicade. Lyocakes were reconstituted at the time points indicated in the absence or presence of 100nM Remicade, and the resulting raw RLU were analyzed.

[0214] FIGS. 72A-72D show representative results using the NanoBiT system to detect Remicade in which the formulated components are separated into two separate cakes prior to use in the assay: (FIG. 72A) an image of two separate, lyophilized components with one containing LgBiT-TNFα fusion protein and furimazine (yellow), and the other containing the SmBiT-protein G fusion protein (white); (FIG. 72B) an image after manually combining the two lyophilized components in FIG. 72A; (FIG. 72C) an image of the reconstituted lyophilized

components; and (D) kinetic bioluminescence RLU signals resulting in the presence of increasing amounts of Remicade.

[0215] FIG. 73 shows the resulting kinetic bioluminescence RLU signal resulting in the presence of increasing amounts of Remicade using the dual-lyophilized NanoTrip immunoassay system, whereby the TNFα + furimazine and protein G fusion proteins were formulated, lyophilized separately, and then combined prior to reconstitution.

[0216] FIG. 74 shows a schematic representation of the homogenous, NanoTrip (tripartite NanoLuc), cell-based immunoassay system for detection of anti-EGFR biologics (e.g., panitumumab).

[0217] FIG. 75 shows a panitumumab dose response curve using the homogenous, cell-based NanoTrip immunoassay system for anti-EGFR biologics.

[0218] FIG. 76 shows a panitumumab dose response curve using the homogeneous, cell-based NanoTrip immunoassay system for anti-EGFR biologics testing different variants of SmTrip9 (SEQ ID NO: 13) fused to protein G.

[0219] FIGS. 77A-77B show a Remicade dose response curve using the homogeneous, solution-based NanoTrip immunoassay system for anti-TNFα biologics testing different variants of SmTrip9 (SEQ ID NO: 13) fused to protein G (FIG. 77A), and a Remicade dose response curve using the lyophilized NanoTrip immunoassay system for anti-TNFα biologics (FIG. 77B).

[0220] FIG. 78 shows a schematic representation of the tripartite IL-6 immunoassay system using antibodies directly labeled with reactive peptides (e.g., SEQ ID NO: 18).

[0221] FIGS. 79A-79C show denaturing SDS-PAGE gel analysis of directly-labeled antibody conjugates.

[0222] FIGS. 80 shows the raw RLU output from IL-6 titration in the presence of anti-IL-6 antibody pairs directly labeled with reactive peptides HW-0984 (SEQ ID NO: 20), HW-1010 (SEQ ID NO: 24), and HW-0977 (SEQ ID NO: 18).

[0223] FIG. 81 shows the raw RLU output from IL-6 titration in the presence of anti-IL-6 antibody pairs directly labeled with reactive peptides HW-0984 (SEQ ID NO: 20) and HW-1053 (SEQ ID NO: 19).

[0224] FIG. 82 shows the raw RLU output from IL-6 titration in the presence of anti-IL-6 antibody pairs labeled with reactive peptides HW-1042 (SEQ ID NO: 20), HW-1050 (SEQ ID

NO: 27), HW-1052 (SEQ ID NO: 25), HW-1043 (SEQ ID NO: 24) and HW-1055 (SEQ ID NO: 25).

[0225] FIG. 83 shows the raw RLU output from IL-6 titration in the presence of individual anti-IL-6 antibodies directly labeled with reactive peptides HW-0977 (SEQ ID NO: 18), HW-0984 (SEQ ID NO: 20), HW-1010 (SEQ ID NO: 24), HW-1042 (SEQ ID NO: 20), HW-1050 (SEQ ID NO: 27), HW-1052 (SEQ ID NO: 25), HW-1053 (SEQ ID NO: 19), HW-1043 (SEQ ID NO: 24), and HW-1055 (SEQ ID NO: 25).

[0226] FIG. 84 shows the raw RLU output from IL-6 titration in the presence of LgTrip 5146 (SEQ ID NO: 451) and anti-IL-6 antibody pairs labeled with reactive peptides HW-1050 (SEQ ID NO: 27), HW-1043 (SEQ ID NO: 24), and HW-0977 (SEQ ID NO: 18).

[0227] FIG. 85 shows a schematic representation of the tripartite IL-6 immunoassay model using antibodies directly labeled with reactive peptides containing fluorophores, enabling BRET between the luciferase and labeled antibodies.

[0228] FIG. 86 shows IL-6 induced BRET between the complemented tripartite luciferase and fluorophores on the labeled anti-IL-6 antibodies.

[0229] FIGS. 87A-87C show the luminescence derived from luminogenic substrates N113 Fz (FIG. 87A), JRW-1404 (FIG. 87B), and JRW-1482 (FIG. 87C) in complex matrices.

DETAILED DESCRIPTION

[0230] Embodiments of the present disclosure provide systems and methods for the detection of an analyte or analytes in a sample. In particular, the present disclosure provides compositions, assays, and methods for detecting and/or quantifying a target analyte using a bioluminescent complex comprising substrates, peptides, and/or polypeptides capable of generating a bioluminescent signal that correlates to the presence, absence, or amount of the target analyte.

[0231] Most rapid diagnostic bioassays are based on immunological principles. Some embodiments of the present disclosure combine immunoassay-based concepts with the advantages of bioluminescence, which include a large linear range and extremely low background, among other advantages. However, despite these advantages, point-of-care bioluminescence-based immunoassays are not yet commercially available. Some reasons for this may be that many currently available luciferases have low signal, which inherently limits their usefulness in immunoassays. Additionally, when a bioluminescent signal output is configured to be conditional (e.g., through complementation or bioluminescence resonance energy transfer

(BRET)), the signal can be reduced even further. Many currently available luciferases also have a low tolerance or sensitivity to certain assay conditions, such as high temperatures, non-optimal buffer compositions, and complex sample matrices, thus requiring specialized chemistries to be compatible with point-of-care devices.

[0232] Embodiments of the present disclosure also address the need for "all-in-one" assay formats for analyte detection, which until the present application, have not been developed or described in the prior art. For example, Tenda, K. *et al.* (*Angew. Chem. Int. Ed.* 57, 15369 – 15373 (2018)) discloses paper devices where the substrate and bioluminescent components are dried onto separate sections of the paper, rather than being included together in a single-format system. Additionally, Yu, Q. et al. (*Science* 361, 1122–1126 (2018)) discloses that, although the bioluminescent components can be dried together, the substrate is separately mixed with the analyte-of-interest and subsequently added to the paper rather than drying the substrate and the bioluminescent components in a single format system. As described further herein, embodiments of the present disclosure provide methods, compositions, and systems that include all the necessary components of a bioluminescent detection complex (excluding the analyte-of-interest) in a single-format (e.g., "all-in-one") system. This contrasts with currently available systems, which include at least one of the necessary bioluminescent components in a separate format/solution. Thus, embodiments of the present disclosure provide surprising and unexpected advantages over currently available bioluminescent analyte detection systems.

[0233] To address the need for bioluminescent-based point-of-care immunoassay platforms that are not necessarily limited to the use of typical immunoassay reagents, embodiments of the present disclosure include the use of the NanoLuc® bioluminescent platform, including compositions and methods for the assembly of a bioluminescent complex from two or more peptide and/or polypeptide components. In some embodiments, the peptide and/or polypeptide components are not fragments of a preexisting protein (e.g., are not complementary subsequences of a known polypeptide sequence), but confer bioluminescent activity via structural complementation (See, e.g., WO/2014/151736 (Intl. App. No. PCT/US2014/026354) and U.S. Pat. Appln. Serial No. 16/439,565 (PCT/US2019/036844), herein incorporated by reference in their entireties), as described further herein. In some embodiments, peptide and/or polypeptide components are non-luminescent in the absence of complementation and/or complementation enhances bioluminescence of a peptide or polypeptide component. In some

embodiments, target analyte binding agents are labeled with the various components of the bioluminescent complexes described herein without comprising the ability of the binding agents to bind their target analytes. Components of the bioluminescent complexes of the present disclosure are configured to be compatible with currently available point-of-care devices and systems such as lateral flow devices, paper-based spot tests, dip stick tests, lab-on-a-chip, microfluidic devices, pre-filled 96-well microtiter plates, and the like.

[0234] For example, embodiments of the present disclosure incorporate NanoLuc®-based technologies (e.g., NanoBiT, NanoTrip, Nano-Glo (e.g., NANOGLO Live Cell Substrate or NANOGLO LCS (Promega Cat. Nos. N205 and N113)), NanoBRET, etc.) into target analyte detection assays that can be embedded in a solid phase assay or device, including plastics, matrices, and membranes of various composition, and/or used in other assay formats such as lyophilized cakes or tablets for solution phase assays, all of which function reliably even in complex sampling environments (e.g., blood components, food matrix, soil samples, stool, urine, water, and other human and animal biological samples). In some embodiments, NanoLuc®-based reporter systems are incorporated into lateral flow assay (LFA) technology, paper spot tests, and similar devices. LFAs are a commonly used point-of-care technology used to measure a variety of target analytes including, but not limited to, antibodies, bacterial and viral antigens, metabolites, proteins, and the like. As demonstrated in FIG. 1, LFAs can be combined with NanoLuc®-based reporter technology to provide a multiplexed viral infection detection assay to detect anti-viral antibodies at the point of care. The only currently available, approved emergency use immunoassay to detect Zika exposure is a traditional plate based, multi-step sandwich ELISA to detect the presence of anti-Zika IgM in blood samples. In contrast to this system, the multiplexed capability of a NanoLuc®-based bioluminescent reporter platform allows for the rapid detection of multiple antibodies in a sample, whether the antibodies recognize multiple different epitopes of the same virus, or whether they recognize multiple different epitopes on more than one virus. The ability to detect and identify viral infections quickly and sensitively with bioluminescence will aid treatment decisions. In addition to antibodies and antigens, the small size of the component peptides of the bioluminescent complexes described herein allow for the detection of many other target analytes using alternative binding agents and materials, such as, but not limited to, DARPins, aptamers, oligonucleotide probes, peptide nucleic acids (PNAs), and locked nucleic assays (LNAs).

[0235] Section headings as used in this section and the entire disclosure herein are merely for organizational purposes and are not intended to be limiting.

1. Definitions

[0236] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. In case of conflict, the present document, including definitions, will control. Preferred methods and materials are described below, although methods and materials similar or equivalent to those described herein can be used in practice or testing of the present disclosure. All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety. The materials, methods, and examples disclosed herein are illustrative only and not intended to be limiting.

[0237] The terms "comprise(s)," "include(s)," "having," "has," "can," "contain(s)," and variants thereof, as used herein, are intended to be open-ended transitional phrases, terms, or words that do not preclude the possibility of additional acts or structures. The singular forms "a," "and" and "the" include plural references unless the context clearly dictates otherwise. Many embodiments herein are described using open "comprising" language. Such embodiments encompass multiple closed "consisting of" and/or "consisting essentially of" embodiments, which may alternatively be claimed or described using such language. The present disclosure also contemplates other embodiments "comprising," "consisting of" and "consisting essentially of," the embodiments or elements presented herein, whether explicitly set forth or not.

[0238] For the recitation of numeric ranges herein, each intervening number there between with the same degree of precision is explicitly contemplated. For example, for the range of 6-9, the numbers 7 and 8 are contemplated in addition to 6 and 9, and for the range 6.0-7.0, the number 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, and 7.0 are explicitly contemplated.

[0239] "Bioluminescence" refers to production and emission of light by a chemical reaction catalyzed by, or enabled by, an enzyme, protein, protein complex, or other biomolecule (e.g., bioluminescent complex). In typical embodiments, a substrate for a bioluminescent entity (e.g., bioluminescent protein or bioluminescent complex) is converted into an unstable form by the bioluminescent entity; the substrate subsequently emits light.

[0240] "Complementary" refers to the characteristic of two or more structural elements (e.g., peptide, polypeptide, nucleic acid, small molecule, etc.) of being able to hybridize, dimerize, or

otherwise form a complex with each other. For example, a "complementary peptide and polypeptide" are capable of coming together to form a complex. Complementary elements may require assistance to form a complex (e.g., from interaction elements), for example, to place the elements in the proper conformation for complementarity, to co-localize complementary elements, to lower interaction energy for complementation, etc.

[0241] "Complex" refers to an assemblage or aggregate of molecules (e.g., peptides, polypeptides, etc.) in direct and/or indirect contact with one another. In one aspect, "contact," or more particularly, "direct contact" means two or more molecules are close enough so that attractive noncovalent interactions, such as Van der Waal forces, hydrogen bonding, ionic and hydrophobic interactions, and the like, dominate the interaction of the molecules. In such an aspect, a complex of molecules (e.g., a peptide and polypeptide) is formed under assay conditions such that the complex is thermodynamically favored (e.g., compared to a non-aggregated, or non-complexed, state of its component molecules). As used herein the term "complex," unless described as otherwise, refers to the assemblage of two or more molecules (e.g., peptides, polypeptides or a combination thereof).

[0242] "Derivative" of an antibody as used herein may refer to an antibody having one or more modifications to its amino acid sequence when compared to a genuine or parent antibody and exhibit a modified domain structure. The derivative may still be able to adopt the typical domain configuration found in native antibodies, as well as an amino acid sequence, which is able to bind to targets (antigens) with specificity. Typical examples of antibody derivatives are antibodies coupled to other polypeptides, rearranged antibody domains, or fragments of antibodies. The derivative may also comprise at least one further compound, such as a protein domain linked by covalent or non-covalent bonds. The linkage can be based on genetic fusion according to the methods known in the art. The additional domain present in the fusion protein comprising the antibody may preferably be linked by a flexible linker, advantageously a peptide linker, wherein said peptide linker comprises plural, hydrophilic, peptide-bonded amino acids of a length sufficient to span the distance between the C-terminal end of the further protein domain and the N-terminal end of the antibody or vice versa. The antibody may be linked to an effector molecule having a conformation suitable for biological activity or selective binding to a solid support, a biologically active substance (*e.g.*, a cytokine or growth hormone), a chemical agent, a peptide, a protein, or a drug, for example.

[0243] "Fragment" refers to a peptide or polypeptide that results from dissection or "fragmentation" of a larger whole entity (e.g., protein, polypeptide, enzyme, etc.), or a peptide or polypeptide prepared to have the same sequence as such. Therefore, a fragment is a subsequence of the whole entity (e.g., protein, polypeptide, enzyme, etc.) from which it is made and/or designed. A peptide or polypeptide that is not a subsequence of a preexisting whole protein is not a fragment (e.g., not a fragment of a preexisting protein). A peptide or polypeptide that is "not a fragment of a preexisting bioluminescent protein" is an amino acid chain that is not a subsequence of a protein (e.g., natural or synthetic) that: (1) was in physical existence prior to design and/or synthesis of the peptide or polypeptide, and (2) exhibits substantial bioluminescent activity.

[0244] As used herein, the term "antibody fragment" refers to a portion of a full-length antibody, including at least a portion of the antigen binding region or a variable region. Antibody fragments include, but are not limited to, Fab, Fab', $F(ab')_2$, Fv, scFv, Fd, variable light chain, variable heavy chain, diabodies, and other antibody fragments that retain at least a portion of the variable region of an intact antibody. See, e.g., Hudson et al. (2003) Nat. Med. 9:129-134; herein incorporated by reference in its entirety. In certain embodiments, antibody fragments are produced by enzymatic or chemical cleavage of intact antibodies (e.g., papain digestion and pepsin digestion of antibody) produced by recombinant DNA techniques, or chemical polypeptide synthesis. For example, a "Fab" fragment comprises one light chain and the C_{H1} and variable region of one heavy chain. The heavy chain of a Fab molecule cannot form a disulfide bond with another heavy chain molecule. A "Fab'" fragment comprises one light chain and one heavy chain that comprises additional constant region, extending between the C_{H1} and C_{H2} domains. An interchain disulfide bond can be formed between two heavy chains of a Fab' fragment to form a "$F(ab')_2$" molecule. An "Fv" fragment comprises the variable regions from both the heavy and light chains, but lacks the constant regions. A single-chain Fv (scFv) fragment comprises heavy and light chain variable regions connected by a flexible linker to form a single polypeptide chain with an antigen-binding region. Exemplary single chain antibodies are discussed in detail in WO 88/01649 and U.S. Pat. Nos. 4,946,778 and 5,260,203; herein incorporated by reference in their entireties. In certain instances, a single variable region (e.g., a heavy chain variable region or a light chain variable region) may have the ability to recognize and bind antigen. Other antibody fragments will be understood by skilled artisans.

[0245] "Isolated polynucleotide" as used herein may mean a polynucleotide (*e.g.*, of genomic, cDNA, or synthetic origin, or a combination thereof) that, by virtue of its origin, the isolated polynucleotide is not associated with all or a portion of a polynucleotide with which the "isolated polynucleotide" is found in nature; is operably linked to a polynucleotide that it is not linked to in nature; or does not occur in nature as part of a larger sequence.

[0246] "Non-luminescent" refers to an entity (e.g., peptide, polypeptide, complex, protein, etc.) that exhibits the characteristic of not emitting a detectable amount of light in the visible spectrum (e.g., in the presence of a substrate). For example, an entity may be referred to as non-luminescent if it does not exhibit detectable luminescence in a given assay. As used herein, the term "non-luminescent" is synonymous with the term "substantially non-luminescent. For example, a non-luminescent polypeptide is substantially non-luminescent, exhibiting, for example, a 10-fold or more (e.g., 100-fold, 200-fold, 500-fold, $1x10^3$-fold, $1x10^4$-fold, $1x10^5$-fold, $1x10^6$-fold, $1x10^7$-fold, etc.) reduction in luminescence compared to a complex of the polypeptide with its non-luminescent complement peptide. In some embodiments, an entity is "non-luminescent" if any light emission is sufficiently minimal so as not to create interfering background for a particular assay.

[0247] "Non-luminescent peptide" and "non-luminescent polypeptide" refer to peptides and polypeptides that exhibit substantially no luminescence (e.g., in the presence of a substrate), or an amount that is beneath the noise, or a 10-fold or more (e.g., 100-fold, 200-fold, 500-fold, $1x10^3$-fold, $1x10^4$-fold, $1x10^5$-fold, $1x10^6$-fold, $1x10^7$-fold, etc.) when compared to a significant signal (e.g., luminescent complex) under standard conditions (e.g., physiological conditions, assay conditions, etc.) and with typical instrumentation (e.g., luminometer, etc.). In some embodiments, such non-luminescent peptides and polypeptides assemble, according to the criteria described herein, to form a bioluminescent complex. As used herein, a "non-luminescent element" is a non-luminescent peptide or non-luminescent polypeptide. The term "bioluminescent complex" refers to the assembled complex of two or more non-luminescent peptides and/or non-luminescent polypeptides. The bioluminescent complex catalyzes or enables the conversion of a substrate for the bioluminescent complex into an unstable form; the substrate subsequently emits light. When uncomplexed, two non-luminescent elements that form a bioluminescent complex may be referred to as a "non-luminescent pair." If a bioluminescent complex is formed by three or more non-luminescent peptides and/or non-luminescent

polypeptides, the uncomplexed constituents of the bioluminescent complex may be referred to as a "non-luminescent group."

[0248] "Peptide" and "polypeptide" as used herein, and unless otherwise specified, refer to polymer compounds of two or more amino acids joined through the main chain by peptide amide bonds (--C(O)NH--). The term "peptide" typically refers to short amino acid polymers (e.g., chains having fewer than 25 amino acids), whereas the term "polypeptide" typically refers to longer amino acid polymers (e.g., chains having more than 25 amino acids).

[0249] "Preexisting protein" refers to an amino acid sequence that was in physical existence prior to a certain event or date. A "peptide that is not a fragment of a preexisting protein" is a short amino acid chain that is not a fragment or sub-sequence of a protein (e.g., synthetic or naturally-occurring) that was in physical existence prior to the design and/or synthesis of the peptide.

[0250] "Sample," "test sample," "specimen," "sample from a subject," and "patient sample" as used herein may be used interchangeable and may be a sample of blood, such as whole blood, tissue, urine, serum, plasma, amniotic fluid, cerebrospinal fluid, placental cells or tissue, endothelial cells, leukocytes, or monocytes. The sample can be used directly as obtained from a patient or can be pre-treated, such as by filtration, distillation, extraction, concentration, centrifugation, inactivation of interfering components, addition of reagents, and the like, to modify the character of the sample in some manner as discussed herein or otherwise as is known in the art.

[0251] "Sequence identity" refers to the degree two polymer sequences (e.g., peptide, polypeptide, nucleic acid, etc.) have the same sequential composition of monomer subunits. The term "sequence similarity" refers to the degree with which two polymer sequences (e.g., peptide, polypeptide, nucleic acid, etc.) have similar polymer sequences. For example, similar amino acids are those that share the same biophysical characteristics and can be grouped into the families, e.g., acidic (e.g., aspartate, glutamate), basic (e.g., lysine, arginine, histidine), non-polar (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan) and uncharged polar (e.g., glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine). The "percent sequence identity" (or "percent sequence similarity") is calculated by: (1) comparing two optimally aligned sequences over a window of comparison (e.g., the length of the longer sequence, the length of the shorter sequence, a specified window), (2) determining the number of

positions containing identical (or similar) monomers (e.g., same amino acids occurs in both sequences, similar amino acid occurs in both sequences) to yield the number of matched positions, (3) dividing the number of matched positions by the total number of positions in the comparison window (e.g., the length of the longer sequence, the length of the shorter sequence, a specified window), and (4) multiplying the result by 100 to yield the percent sequence identity or percent sequence similarity. For example, if peptides A and B are both 20 amino acids in length and have identical amino acids at all but 1 position, then peptide A and peptide B have 95% sequence identity. If the amino acids at the non-identical position shared the same biophysical characteristics (e.g., both were acidic), then peptide A and peptide B would have 100% sequence similarity. As another example, if peptide C is 20 amino acids in length and peptide D is 15 amino acids in length, and 14 out of 15 amino acids in peptide D are identical to those of a portion of peptide C, then peptides C and D have 70% sequence identity, but peptide D has 93.3% sequence identity to an optimal comparison window of peptide C. For the purpose of calculating "percent sequence identity" (or "percent sequence similarity") herein, any gaps in aligned sequences are treated as mismatches at that position.

[0252] "Subject" and "patient" as used herein interchangeably refers to any vertebrate, including, but not limited to, a mammal and a human. In some embodiments, the subject may be a human or a non-human. The subject or patient may be undergoing forms of treatment. "Mammal" as used herein refers to any member of the class Mammalia, including, without limitation, humans and nonhuman primates such as chimpanzees and other apes and monkey species; farm animals such as cattle, sheep, pigs, goats, llamas, camels, and horses; domestic mammals such as dogs and cats; laboratory animals including rodents such as mice, rats, rabbits, guinea pigs, and the like. The term does not denote a particular age or sex. Thus, adult and newborn subjects, as well as fetuses, whether male or female, are intended to be included within the scope of this term.

[0253] "Subsequence" refers to peptide or polypeptide that has 100% sequence identify with another, larger peptide or polypeptide. The subsequence is a perfect sequence match for a portion of the larger amino acid chain.

[0254] "Substantially" as used herein means that the recited characteristic, parameter, and/or value need not be achieved exactly, but that deviations or variations, including for example, tolerances, measurement error, measurement accuracy limitations and other factors known to

skill in the art, may occur in amounts that do not preclude the effect the characteristic was intended to provide. A characteristic or feature that is substantially absent (e.g., substantially non-luminescent) may be one that is within the noise, beneath background, below the detection capabilities of the assay being used, or a small fraction (e.g., <1%, <0.1%, <0.01%, <0.001%, <0.00001%, <0.000001%, <0.0000001%) of the significant characteristic (e.g., luminescent intensity of a bioluminescent protein or bioluminescent complex).

[0255] "Variant" is used herein to describe a peptide or polypeptide that differs in amino acid sequence by the insertion, deletion, or conservative substitution of amino acids, but retain at least one biological activity. "SNP" refers to a variant that is a single nucleotide polymorphism. Representative examples of "biological activity" include the ability to be bound by a specific antibody or to promote an immune response. Variant is also used herein to describe a protein with an amino acid sequence that is substantially identical to a referenced protein with an amino acid sequence that retains at least one biological activity. A conservative substitution of an amino acid (*e.g.*, replacing an amino acid with a different amino acid of similar properties, such as hydrophilicity, degree, and distribution of charged regions) is recognized in the art as typically involving a minor change. These minor changes can be identified, in part, by considering the hydropathic index of amino acids, as understood in the art. The hydropathic index of an amino acid is based on a consideration of its hydrophobicity and charge. It is known in the art that amino acids of similar hydropathic indexes can be substituted and still retain protein function. In one aspect, amino acids having hydropathic indexes of ±2 are substituted. The hydrophilicity of amino acids can also be used to reveal substitutions that would result in proteins retaining biological function. A consideration of the hydrophilicity of amino acids in the context of a peptide permits calculation of the greatest local average hydrophilicity of that peptide, a useful measure that has been reported to correlate well with antigenicity and immunogenicity. Substitution of amino acids having similar hydrophilicity values can result in peptides retaining biological activity, for example immunogenicity, as is understood in the art. Substitutions may be performed with amino acids having hydrophilicity values within ±2 of each other. Both the hydrophobicity index and the hydrophilicity value of amino acids are influenced by the particular side chain of that amino acid. Consistent with that observation, amino acid substitutions that are compatible with biological function are understood to depend on the relative similarity of the

amino acids, and particularly the side chains of those amino acids, as revealed by the hydrophobicity, hydrophilicity, charge, size, and other properties.

[0256] "Target analyte" or "analyte" as used herein refers to a substance in a sample that can be detected, quantified, measured, tested, and/or monitored, often as part of a method of evaluating a process or condition (e.g., diagnostic or prognostic assay). Target analytes can include, but are not limited to, a protein, a peptide, a polypeptide, an enzyme, a cofactor, a nucleotide, a polynucleotide, DNA, RNA, a small molecule compound, an antibody, and any variation, combination, and derivative thereof.

[0257] "Target analyte binding agent" as used herein refers to an agent capable of binding to a target analyte. In some embodiments, target analyte binding agents include agents that can bind multiple substances, such as a target analyte and a solid phase support. In some embodiments, target analyte binding agents include agents that bind both a target analyte (e.g., via a target analyte binding element) and a distinct peptide/polypeptide to form a target analyte detection complex (e.g., to generate a bioluminescent signal). In some embodiments, target analyte binding agents can include target analyte binding elements capable of binding a group or class of analytes (e.g., protein L binding to antibodies); and in other embodiments, target analyte binding agents can include target analyte binding elements capable of binding a specific analyte (e.g., an antigen binding a monoclonal antibody). A target analyte binding agent may be an antibody, antibody fragment, a receptor domain that binds a target ligand, proteins or protein domains that bind to immunoglobulins (e.g., protein A, protein G, protein A/G, protein L, protein M), a binding domain of a proteins that bind to immunoglobulins (e.g., protein A, protein G, protein A/G, protein L, protein M), oligonucleotide probe, peptide nucleic acid, DARPin, aptamer, affimer, a purified protein, or a protein domain (either the analyte itself or a protein that binds to the analyte), and analyte binding domain(s) of proteins etc. Table A provides a lists of exemplary binding moieties that could be used singly or in various combinations in methods, systems, and assays (e.g., immunoassays) herein.

[0258] **Table 1: Exemplary target analyte binding agents.**

Binding Moiety A	Binding Moiety B
Protein A	Protein A
Ig Binding domain of protein A	Ig binding domain of protein A
Protein G	Protein G
Ig Binding domain of protein G	Ig binding domain of protein G
Protein L	Protein L

Ig Binding domain of protein L	Ig binding domain of protein L
Protein M	Protein M
Ig Binding domain of protein M	Ig binding domain of protein M
polyclonal antibody against analyte X	polyclonal antibody: same antibody or second polyclonal antibody recognizing same target analyte X
monoclonal antibody	monoclonal antibody recognizing different epitope on same target analyte X
recombinant antibody	recombinant antibody recognizing different epitope on same target analyte X
scFv	scFv recognizing different epitope on same target analyte X
variable light chain (V_L) of antibody (monoclonal, recombinant, polyclonal) recognizing target analyte X	variable heavy chain (V_H) of same antibody (monoclonal, recombinant, polyclonal) recognizing target analyte X
protein (e.g. receptor) binding domain 1 that binds to analyte X	protein (e.g. receptor) binding domain 2 that binds to analyte X
(Fab) fragment	(Fab) fragment from different antibody recognizing different epitope to same target analyte X
Fab' fragment	Fab' from different antibody recognizing different epitope to same target analyte X
Fv fragment	Fv from different antibody recognizing different epitope to same target analyte X
F(ab')2 fragment	F(ab')2 from different antibody recognizing different epitope to same target analyte X
oligonucleotide probe	oligonucleotide probe to same DNA or RNA target but recognizing non-overlapping sequence
DARPin	DARPin recognizing non-overlapping domain of same target
peptide nucleic acid	peptide nucleic acid recognizing same DNA or RNA target but non-overlapping sequence
aptamer	aptamer binding to same target analyte X but recognizing non-overlapping sequence or epitope
affimer	aptamer binding to same target analyte X but recognizing different epitope

[0259] Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. For example, any nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those that are well known and commonly used in the art. The meaning and scope of the terms should be clear; in the event, however of any latent ambiguity, definitions provided herein take precedent

over any dictionary or extrinsic definition. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

2. Bioluminescence

[0260] The present disclosure includes materials and methods related to bioluminescent polypeptides, bioluminescent complexes and components thereof, and bioluminescence resonance energy transfer (BRET).

[0261] In some embodiments, provided herein are solid phase and/or lateral flow assays, devices, and systems incorporating bioluminescent polypeptides and/or bioluminescent complexes (of non-luminescent peptide(s) and/or non-luminescent polypeptide components) based on (e.g., structurally, functionally, etc.) the luciferase of *Oplophorus gracilirostris*, the NanoLuc® luciferase (Promega Corporation; U.S. Pat. No. 8,557,970; U.S. Pat. No. 8,669, 103; herein incorporated by reference in their entireties), the NanoBiT (U.S. Pat. No. 9,797,889; herein incorporated by reference in its entirety), or NanoTrip (U.S. Pat. Appln. Serial No. 16/439,565; and U.S. Prov. Appln. Serial No. 62/941,255; both of which are herein incorporated by reference in their entireties). As described below, in some embodiments, the compositions, assays, devices, methods, and systems herein incorporate commercially available NanoLuc®-based technologies (e.g., NanoLuc® luciferase, NanoBRET, NanoBiT, NanoTrip, NanoGlo, etc.), but in other embodiments, various combinations, variations, or derivations from the commercially available NanoLuc®-based technologies are employed.

a. NanoLuc

[0262] PCT Appln. No. PCT/US2010/033449, U.S. Patent No. 8,557,970, PCT Appln. No. PCT/2011/059018, and U.S. Patent No. 8,669,103 (each of which is herein incorporated by reference in their entirety and for all purposes) describe compositions and methods comprising bioluminescent polypeptides. Such polypeptides find use in embodiments herein and can be used in conjunction with the compositions, assays, devices, systems, and methods described herein.

[0263] In some embodiments, compositions, assays, devices, systems, and methods provided herein comprise a bioluminescent polypeptide of SEQ ID NO: 5, or having at least 60% (e.g., 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 100%, or ranges therebetween) sequence identity with SEQ ID NO: 5.

[0264] In some embodiments, any of the aforementioned bioluminescent polypeptides are linked (e.g., fused, chemically linked, etc.) to a binding element or other component of the assays and systems described herein.

[0265] In some embodiments, any of the aforementioned bioluminescent polypeptides, or fusions or conjugates thereof (e.g., with a binding element, etc.), are immobilized to a portion of a device described herein (e.g., a detection or control region of a lateral flow assay, a solid phase detection element, etc.).

b. NanoBiT

[0266] PCT Appln. No. PCT/US14/26354 and U.S. Patent No. 9,797,889 (each of which is herein incorporated by reference in their entirety and for all purposes) describe compositions and methods for the assembly of bioluminescent complexes; such complexes, and the peptide and polypeptide components thereof, find use in embodiments herein and can be used in conjunction with the assays and methods described herein.

[0267] In some embodiments, provided herein are non-luminescent (NL) polypeptides having at least 60% (e.g., 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 100%, or ranges therebetween) sequence identity with SEQ ID NO: 9, but less than 100% (e.g., <99%, <98%, <97%, <96%, <95%, <94%, <93%, <92%, <91%, <90%) sequence identity with SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 6.

[0268] In some embodiments, provided herein are non-luminescent (NL) peptides having at least 60% (e.g., 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 100%, or ranges therebetween) sequence identity with SEQ ID NO: 10, but less than 100% (e.g., <99%, <98%, <97%, <96%, <95%, <94%, <93%, <92%, <91%, <90%) sequence identity with SEQ ID NO: 1, SEQ ID NO: 4, and SEQ ID NO: 8.

[0269] In some embodiments, provided herein are NL peptides having at least 60% (e.g., 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 100%, or ranges therebetween) sequence identity with SEQ ID NO: 11, but less than 100% (e.g., <99%, <98%, <97%, <96%, <95%, <94%, <93%, <92%, <91%, <90%) sequence identity with SEQ ID NO: 1, SEQ ID NO: 4, and SEQ ID NO: 8.

[0270] In some embodiments, any of the aforementioned NL peptides or NL polypeptides are linked (e.g., fused, chemically linked, etc.) to a binding element or other component of the composition, assays, devices, methods, and systems described herein.

[0271] In some embodiments, any of the aforementioned NL peptides or NL polypeptides, or fusions or conjugates thereof (e.g., with a binding element, etc.), are immobilized to a portion of a device described herein (e.g., a detection or control region of a lateral flow assay, a solid phase detection element, etc.).

[0272] In some embodiments, provided herein is a lateral flow detection system comprising: an analytical membrane comprising a detection region and a control region, wherein the detection region comprises a first target analyte binding agent immobilized to the detection region; a conjugate pad comprising a second target analyte binding agent; and a sample pad; wherein the first target analyte binding agent comprises a first target analyte binding element and a first NanoBiT-based NL peptide or NL polypeptide component (as described above), and wherein the second target analyte binding agent comprises a second target analyte binding element and a complementary NanoBiT-based NL peptide or NL polypeptide component (as described above). In some embodiments, the first target analyte binding agent and the second target analyte binding agent form an analyte detection complex in the at least one detection region when a target analyte is detected in a sample. In some embodiments, a bioluminescent signal produced in the presence of a luminogenic substrate is substantially increased when the first target analyte binding agent contacts the second target analyte binding agent, as compared to a bioluminescent signal produced by the second target analyte binding agent or the first target analyte binding agent and the luminogenic substrate alone.

[0273] In some embodiments, provided herein is solid-phase detection system comprising: an solid phase substrate comprising a first target analyte binding agent and a second target analyte binding agent; wherein the first target analyte binding agent comprises a first target analyte binding element and a first NanoBiT-based NL peptide or NL polypeptide component (as described above), and wherein the second target analyte binding agent comprises a second target analyte binding element and a complementary NanoBiT-based NL peptide or NL polypeptide component (as described above). In some embodiments, the first target analyte binding agent and the second target analyte binding agent form an analyte detection complex in the solid-phase substrate when a target analyte is detected in a sample. In some embodiments, a bioluminescent signal produced in the presence of a luminogenic substrate is substantially increased when the first target analyte binding agent contacts the second target analyte binding agent, as compared to

a bioluminescent signal produced by the second target analyte binding agent or the first target analyte binding agent and the luminogenic substrate alone.

c. NanoTrip

[0274] U.S. Pat. Appln. Serial No. 16/439,565 (PCT/US2019/036844) and U.S. Prov. Appln. Serial No. 62/941,255 (both of which are herein incorporated by reference in their entireties and for all purposes) describes compositions, systems, and methods for the assembly of bioluminescent complexes. Such complexes, and the peptides and polypeptide components thereof, find use in embodiments herein and can be used in conjunction with the assays and methods described herein.

[0275] In some embodiments, provided herein are non-luminescent (NL) polypeptides having at least 60% (e.g., 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 100%, or ranges therebetween) sequence identity with SEQ ID NO: 12, but less than 100% (e.g., <99%, <98%, <97%, <96%, <95%, <94%, <93%, <92%, <91%, <90%) sequence identity with SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 6, and SEQ ID NO: 9.

[0276] In some embodiments, provided herein are non-luminescent (NL) peptides having at least 60% (e.g., 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 100%, or ranges therebetween) sequence identity with SEQ ID NO: 11, but less than 100% (e.g., <99%, <98%, <97%, <96%, <95%, <94%, <93%, <92%, <91%, <90%) sequence identity with SEQ ID NO: 1, SEQ ID NO: 4, and SEQ ID NO: 8.

[0277] In some embodiments, provided herein are NL peptides having at least 60% (e.g., 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 100%, or ranges therebetween) sequence identity with SEQ ID NO: 13, but less than 100% (e.g., <99%, <98%, <97%, <96%, <95%, <94%, <93%, <92%, <91%, <90%) sequence identity with SEQ ID NO: 1, SEQ ID NO: 3, and SEQ ID NO: 7.

[0278] In some embodiments, provided herein are NL peptides having at least 60% (e.g., 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 100%, or ranges therebetween) sequence identity with SEQ ID NO: 14, but less than 100% (e.g., <99%, <98%, <97%, <96%, <95%, <94%, <93%, <92%, <91%, <90%) sequence identity with SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 7, and SEQ ID NO: 8.

[0279] In some embodiments, any of the aforementioned NanoTrip-based NL peptide or NL polypeptides are linked (e.g., fused, chemically linked, etc.) to a binding element or other component of the compositions, methods, devices, assays, and systems described herein.

[0280] In some embodiments, any of the aforementioned NanoTrip-based NL peptide or NL polypeptides, or fusions or conjugates thereof (e.g., with a binding element, etc.), are immobilized to a portion of a device described herein (e.g., a detection or control region of a lateral flow assay, a solid phase detection element, etc.).

[0281] In some embodiments, provided herein is a lateral flow detection system comprising: an analytical membrane comprising a detection region and a control region, wherein the detection region comprises a first target analyte binding agent immobilized to the detection region; a conjugate pad comprising a second target analyte binding agent; and a sample pad; wherein the first target analyte binding agent comprises a first target analyte binding element and a first NanoTrip-based NL peptide (as described above), and wherein the second target analyte binding agent comprises a second target analyte binding element and a complementary NanoTrip-based NL peptide (as described above). In some embodiments, the first target analyte binding agent and the second target analyte binding agent form an analyte detection complex in the at least one detection region in the presence of a NanoTrip-based NL polypeptide component (as described above) when a target analyte is detected in a sample. In some embodiments, a bioluminescent signal produced in the presence of a luminogenic substrate is substantially increased when the first target analyte binding agent contacts the second target analyte binding agent in the presence of a NanoTrip-based NL polypeptide component, as compared to a bioluminescent signal produced by the second target analyte binding agent or the first target analyte binding agent and the luminogenic substrate alone.

[0282] In some embodiments, provided herein is a solid-phase detection system comprising: a solid phase (e.g., paper substrate, etc.) comprising a first target analyte binding agent and a second target analyte binding agent, wherein the first target analyte binding agent comprises a first target analyte binding element and a first NanoTrip-based NL peptide (as described above), and wherein the second target analyte binding agent comprises a second target analyte binding element and a complementary, second NL NanoTrip-based peptide (as described above). In some embodiments, the first target analyte binding agent and the second target analyte binding agent form an analyte detection complex in the presence of a NanoTrip-based NL polypeptide

(as described above) when a target analyte is detected in a sample. In some embodiments, a bioluminescent signal produced in the presence of a luminogenic substrate is substantially increased when the first target analyte binding agent contacts the second target analyte binding agent and a NanoTrip-based NL polypeptide, as compared to a bioluminescent signal produced by the second target analyte binding agent or the first target analyte binding agent and the luminogenic substrate alone.

d. NanoBRET

[0283] As disclosed in PCT Appln. No. PCT/US13/74765 and U.S. Patent Appln. Ser. No. 15/263,416 (herein incorporated by reference in their entireties and for all purposes) describe bioluminescence resonance energy transfer (BRET) compositions, systems, and methods (e.g., incorporating NanoLuc®-based technologies); such compositions, systems and methods, and the bioluminescent polypeptide and fluorophore-conjugated components thereof, find use in embodiments herein and can be used in conjunction with the compositions, systems, devices, assays, and methods described herein.

[0284] In some embodiments, any of the NanoLuc®-based, NanoBiT-based, and/or NanoTrip-based (described in sections a-c, above) peptides, polypeptide, complexes, fusions, and conjugates may find use in BRET-based applications with the compositions, assays, methods, devices, and systems described herein. For example, in certain embodiments, a first target analyte binding agent comprises a first target analyte binding element and a NanoLuc®-based, NanoBiT-based, and/or NanoTrip-based polypeptide, peptide, or complex, and a second target analyte binding agent comprises a second target analyte binding element and a fluorophore (e.g., fluorescent protein, small molecule fluorophore, etc.), wherein the emission spectrum of the NanoLuc®-based, NanoBiT-based, and/or NanoTrip-based polypeptide, peptide, or complex overlaps the excitation spectrum of the fluorophore. In some embodiments, the NanoLuc®-based, NanoBiT-based, and/or NanoTrip-based polypeptide, peptide, or complex can be prepared in lyophilized form, which can include, or not include, the luminogenic substrate (e.g., furimazine).

[0285] In some embodiments, a target analyte binding agent comprises a target analyte binding element and a fluorophore capable of being activated by energy transfer from a bioluminescent polypeptide.

[0286] As used herein, the term "energy acceptor" refers to any small molecule (e.g., chromophore), macromolecule (e.g., autofluorescent protein, phycobiliproteins, nanoparticle, surface, etc.), or molecular complex that produces a readily detectable signal in response to energy absorption (e.g., resonance energy transfer). In certain embodiments, an energy acceptor is a fluorophore or other detectable chromophore. Suitable fluorophores include, but are not limited to: xanthene derivatives (e.g., fluorescein, rhodamine, Oregon green, eosin, Texas red, etc.), cyanine derivatives (e.g., cyanine, indocarbocyanine, oxacarbocyanine, thiacarbocyanine, merocyanine, etc.), naphthalene derivatives (e.g., dansyl and prodan derivatives), oxadiazole derivatives (e.g., pyridyloxazole, nitrobenzoxadiazole, benzoxadiazole, etc.), pyrene derivatives (e.g., cascade blue), oxazine derivatives (e.g., Nile red, Nile blue, cresyl violet, oxazine 170, etc.), acridine derivatives (e.g., proflavin, acridine orange, acridine yellow, etc.), arylmethine derivatives (e.g., auramine, crystal violet, malachite green, etc.), tetrapyrrole derivatives (e.g., porphin, phtalocyanine, bilirubin, etc.), CF dye (Biotium), BODIPY (Invitrogen), ALEXA FLuoR (Invitrogen), DYLIGHT FLUOR (Thermo Scientific, Pierce), ATTO and TRACY (Sigma Aldrich), FluoProbes (Interchim), DY and MEGASTOKES (Dyomics), SULFO CY dyes (CYANDYE, LLC), SETAU AND SQUARE DYES (SETA BioMedicals), QUASAR and CAL FLUOR dyes (Biosearch Technologies), SURELIGHT DYES (APC, RPE, PerCP, Phycobilisomes)(Columbia Biosciences), APC, APCXL, RPE, BPE (Phyco-Biotech), autofluorescent proteins (e.g., YFP, RFP, mCherry, mKate), quantum dot nanocrystals, etc. In some embodiments, a fluorophore is a rhodamine analog (e.g., carboxy rhodamine analog), such as those described in U.S. Pat. App. Ser. No. 13/682,589, herein incorporated by reference in its entirety.

e. HALOTAG

[0287] Some embodiments herein comprise a capture protein capable of forming a covalent bond with a capture ligand. The capture protein may be linked to a first element (e.g., a peptide component of a bioluminescent complex) and the capture ligand to a second element (e.g., a target analyte binding element (e.g., an antibody or antigen binding protein)) and the formation of a covalent bond links the first and second elements to each other. In some embodiments, linking the first and second elements creates a target analyte binding agent. In some embodiments, two or more target analyte binding agents so formed can bind to a complementary polypeptide component (e.g., LgTrip) and form a bioluminescent complex in the presence of an

analyte (e.g., a target antigen recognized by the target analyte binding element) (See e.g., FIGS. 48 and 58). In some embodiments, the capture ligand is a haloalkane (aka "alkyl halide"). In some embodiments, the capture ligand is a chloroalkane. In some embodiments, the capture ligand is -A-X. In some embodiments, X is Cl. In some embodiments, -A-X is $-(CH_2)_6Cl$. When the capture ligand is a haloalkane, the capture protein is typically a dehalogenase enzyme modified to form covalent bonds with its substrate (See, e.g., U.S. Patent No. 7,425,436; U.S. Patent No. 7,429,472; U.S. Patent No. 7,867,726; U.S. Patent No. 7,888,086; U.S. Patent No. 7,935,803; U.S. Patent No. RE42,931; U.S. Patent No. 8,168,405; U.S. Patent No. 8,202,700; U.S. Patent No. 8,257,939; herein incorporated by reference in their entireties).

[0288] One such modified dehalogenase is the commercially-available HALOTAG protein (SEQ ID NO: 720). In some embodiments, a capture protein comprises a polypeptide with at least 70% sequence identity (e.g., 75% identity, 80% identity, 85% identity, 90% identity, 95% identity, 98% identity, 99% identity) with SEQ ID NO.: 720. Some embodiment comprise a fusion protein of the capture protein (e.g., HALOTAG) and another peptide/polypeptide element (e.g., a binding moiety, a peptide/polypeptide component of a bioluminescent complex, etc.). In some embodiments, a capture ligand is a haloalkane comprising a halogen (e.g., Cl, Br, F, I, etc.) covalently attached to the end of an alkyl chain (e.g., $(CH_2)_{4\text{-}24}$). In some embodiments, the other end of the alkyl chain is attached to a linker or to another element (e.g., a peptide, analyte, etc.). A linker may comprise an alkyl chain or substituted alkyl chain (e.g., C=O, NH, S, O, carbamate, ethylene etc.) such as those disclosed in U.S. Pat. Appln. No. 14/207,959, herein incorporated by reference.

3. Compositions and Formulations

[0289] Embodiments of the present disclosure include compositions and formulations comprising one or more of the peptide and/or polypeptide components of the bioluminescent complexes provided herein. In accordance with these embodiments, compositions and formulations of the present disclosure can include a luminogenic substrate and/or various other components. The compositions and methods provided herein can be used to formulate shelf-stable liquid formulations (e.g., used in a solution phase assay format) and shelf-stable dried formulations (e.g., used in a solid phase assay format) capable of producing a luminescent signal in the presence of an analyte-of-interest, even after storage for prolonged time periods. As described further below, the compositions and formulations of the present disclosure can include

one or more components of NanoLuc, NanoBiT, NanoTrip, and NanoBRET as well as the various luminogenic substrates described herein (e.g., furimazine).

[0290] In contrast to many currently available fluorescent and colorimetric assays, the compositions and formulations of the present disclosure provide means for conducting bioassays in which one or more of the peptide and/or polypeptide components of the bioluminescent complexes exist in a stable, dried formulation that is capable of being reconstituted in a solution containing, for example, a complementary peptide/polypeptide and/or a luminogenic substrate, such that the bioluminescent complex is formed in the presence of the analyte-of-interest. In some embodiments, the compositions and formulations of the present disclosure provide the means for conducting robust solid phase bioassays in which the bioluminescent signal produced is quantitative and proportional to the concentration of the analyte-of-interest.

[0291] In some embodiments, the compositions and formulations of the present disclosure include a luminogenic substrate and a target analyte binding agent that includes a target analyte binding element and a polypeptide component of a bioluminescent complex or a peptide component of a bioluminescent complex. In some embodiments, the polypeptide component of the target analyte binding agent comprises at least 60% sequence identity with SEQ ID NO: 6, at least 60% sequence identity with SEQ ID NO: 9, or at least 60% sequence identity with SEQ ID NO: 12. In some embodiments, the polypeptide component of the target analyte binding agent comprises at least 70% sequence identity with SEQ ID NO: 6, at least 70% sequence identity with SEQ ID NO: 9, or at least 70% sequence identity with SEQ ID NO: 12. In some embodiments, the polypeptide component of the target analyte binding agent comprises at least 80% sequence identity with SEQ ID NO: 6, at least 80% sequence identity with SEQ ID NO: 9, or at least 80% sequence identity with SEQ ID NO: 12. In some embodiments, the polypeptide component of the target analyte binding agent comprises at least 85% sequence identity with SEQ ID NO: 6, at least 85% sequence identity with SEQ ID NO: 9, or at least 85% sequence identity with SEQ ID NO: 12. In some embodiments, the polypeptide component of the target analyte binding agent comprises at least 90% sequence identity with SEQ ID NO: 6, at least 90% sequence identity with SEQ ID NO: 9, or at least 90% sequence identity with SEQ ID NO: 12. In some embodiments, the polypeptide component of the target analyte binding agent comprises at least 95% sequence identity with SEQ ID NO: 6, at least 95% sequence identity with SEQ ID NO: 9, or at least 95% sequence identity with SEQ ID NO: 12.

[0292] In other embodiments, the peptide component of the target analyte binding agent comprises at least 60% sequence identity with SEQ ID NO: 10, at least 60% sequence identity with SEQ ID NO: 11, at least 60% sequence identity with SEQ ID NO: 13, or at least 60% sequence identity with SEQ ID NO: 14. In some embodiments, the peptide component of the target analyte binding agent comprises at least 70% sequence identity with SEQ ID NO: 10, at least 70% sequence identity with SEQ ID NO: 11, at least 70% sequence identity with SEQ ID NO: 13, or at least 70% sequence identity with SEQ ID NO: 14. In some embodiments, the peptide component of the target analyte binding agent comprises at least 80% sequence identity with SEQ ID NO: 10, at least 80% sequence identity with SEQ ID NO: 11, at least 80% sequence identity with SEQ ID NO: 13, or at least 80% sequence identity with SEQ ID NO: 14. In some embodiments, the peptide component of the target analyte binding agent comprises at least 85% sequence identity with SEQ ID NO: 10, at least 85% sequence identity with SEQ ID NO: 11, at least 85% sequence identity with SEQ ID NO: 13, or at least 85% sequence identity with SEQ ID NO: 14. In some embodiments, the peptide component of the target analyte binding agent comprises at least 90% sequence identity with SEQ ID NO: 10, at least 90% sequence identity with SEQ ID NO: 11, at least 90% sequence identity with SEQ ID NO: 13, or at least 90% sequence identity with SEQ ID NO: 14. In some embodiments, the peptide component of the target analyte binding agent comprises at least 95% sequence identity with SEQ ID NO: 10, at least 95% sequence identity with SEQ ID NO: 11, at least 95% sequence identity with SEQ ID NO: 13, or at least 95% sequence identity with SEQ ID NO: 14.

[0293] In some embodiments, the composition or formulation comprises a complementary peptide or polypeptide component of the bioluminescent complex. In accordance with these embodiments, the target analyte binding agent and the complementary peptide or polypeptide component of the bioluminescent complex can form a bioluminescent analyte detection complex in the presence of a target analyte. In some embodiments, the composition that comprises the luminogenic substrate and the target analyte binding agent can be combined in a dried formulation, and the complementary peptide or polypeptide component of the bioluminescent complex can be formulated as a liquid formulation. In some embodiments, the liquid formulation is added to the dried formulation and forms the bioluminescent analyte detection complex in the presence of the target analyte upon rehydration. In other embodiments, the composition or formulation comprising the luminogenic substrate, the target analyte binding agent, and the

complementary peptide or polypeptide component of the bioluminescent complex are combined in a dried formulation, wherein the dried formulation forms the bioluminescent analyte detection complex in the presence of the target analyte upon rehydration.

[0294] In some embodiments, the complementary peptide or polypeptide component comprises a second target analyte binding element that forms the bioluminescent analyte detection complex in the presence of the target analyte. In some embodiments, the polypeptide component of the target analyte binding agent comprises at least 60% sequence identity with SEQ ID NO: 6, and wherein the complementary peptide or polypeptide component of the bioluminescent complex comprises at least 60% sequence identity with SEQ ID NO: 10. In some embodiments, the polypeptide component of the target analyte binding agent comprises at least 60% sequence identity with SEQ ID NO: 6, and wherein the complementary peptide or polypeptide component of the bioluminescent complex comprises at least 60% sequence identity with SEQ ID NO: 14.

[0295] Embodiments of the present disclosure also include a composition or formulation comprising a dried formulation that includes a first target analyte binding agent comprising a first target analyte binding element and a polypeptide component having at least 60% sequence identity with SEQ ID NO: 9, and a second target analyte binding agent comprising a second target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 10. In some embodiments, the dried formulation further comprises a luminogenic substrate. In some embodiments, the composition further comprises a liquid formulation comprising the target analyte.

[0296] Embodiments of the present disclosure also include a composition comprising a dried formulation that includes a first target analyte binding agent comprising a first target analyte binding element and a polypeptide component having at least 60% sequence identity with SEQ ID NO: 12, and a second target analyte binding agent comprising a second target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 14. In some embodiments, the dried formulation further comprises a luminogenic substrate. In some embodiments, the composition further comprises a liquid formulation comprising the target analyte.

[0297] Embodiments of the present disclosure also include a composition comprising a dried formulation that includes a first target analyte binding agent comprising a first target analyte

binding element and a peptide component having at least 60% sequence identity with SEQ ID NO: 13, a second target analyte binding agent comprising a second target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 15, and a complementary polypeptide component having at least 60% sequence identity with SEQ ID NO: 12. In some embodiments, the dried formulation further comprises a luminogenic substrate. In some embodiments, the composition further comprises a liquid formulation comprising the target analyte.

[0298] Embodiments of the present disclosure also include a composition that includes a dried formulation comprising a first target analyte binding agent comprising a target analyte binding element and a polypeptide component having at least 60% sequence identity with SEQ ID NO: 9, and a liquid formulation comprising a second target analyte binding agent comprising a target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 10 or SEQ ID NO: 11.

[0299] Embodiments of the present disclosure also include a composition that includes a dried formulation comprising a first target analyte binding agent comprising a target analyte binding element and a peptide component having at least 60% sequence identity with SEQ ID NO: 10 or SEQ ID NO: 11, and a liquid formulation that contains a second target analyte binding agent comprising a target analyte binding element and a complementary polypeptide component having at least 60% sequence identity with SEQ ID NO: 9.

[0300] Embodiments of the present disclosure also include a composition that includes a dried formulation comprising a first target analyte binding agent comprising a target analyte binding element and a polypeptide component having at least 60% sequence identity with SEQ ID NO: 12, and a liquid formulation comprising a second target analyte binding agent comprising a target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 14. In some embodiments, the dried formulation further comprises a luminogenic substrate. In some embodiments, the liquid formulation further comprises a luminogenic substrate. In some embodiments, the liquid formulation further includes a sample comprising a target analyte. In accordance with these embodiments, a bioluminescent analyte detection complex forms upon combining the dried formulation and the liquid formulation in the presence of the target analyte.

[0301] In some embodiments, the composition further comprises a second complementary peptide or polypeptide component of the bioluminescent complex. In accordance with these embodiments, the target analyte binding agent, the first complementary peptide or polypeptide component of the bioluminescent complex, and the second complementary peptide or polypeptide component of the bioluminescent complex form a bioluminescent analyte detection complex in the presence of a target analyte.

[0302] In some embodiments, the composition comprising the target analyte binding agent are produced as a dried formulation. In some embodiments, the first complementary peptide or polypeptide component and the second complementary peptide or polypeptide of the bioluminescent complex are produced as a liquid formulation. In accordance with these embodiments, the liquid formulation can be added to the dried formulation, which facilitates the formation of the bioluminescent analyte detection complex in the presence of the target analyte upon rehydration.

[0303] In some embodiments, the composition comprising the target analyte binding agent, and either the first or the second complementary peptide or polypeptide component are combined in a dried formulation, and the first or the second complementary peptide or polypeptide component that is not present in the dried formulation are produced as a liquid formulation. The liquid formulation can be added to the dried formulation, which facilitates the formation of the bioluminescent analyte detection complex in the presence of the target analyte upon rehydration.

[0304] In some embodiments, the target analyte binding agent, the first complementary peptide or polypeptide component, and the second complementary peptide or polypeptide component are combined in a dried formulation that forms the bioluminescent analyte detection complex in the presence of the target analyte upon rehydration. In some embodiments, the dried formulation further comprises a luminogenic substrate. In some embodiments, the liquid formulation further comprises a luminogenic substrate. In some embodiments, the liquid formulation further comprises a sample comprising a target analyte, wherein a bioluminescent analyte detection complex forms upon combining the dried formulation and the liquid formulation in the presence of the target analyte.

[0305] In some embodiments, either the first or the second complementary peptide or polypeptide component comprises a second target analyte binding element that forms the bioluminescent analyte detection complex in the presence of the target analyte upon rehydration.

[0306] In some embodiments, the polypeptide component of the target analyte binding agent comprises at least 60% sequence identity with SEQ ID NO: 12, and wherein either the first or the second complementary peptide or polypeptide component of the bioluminescent complex comprises at least 60% sequence identity with either SEQ ID NO: 13 or SEQ ID NO: 15.

[0307] Embodiments of the present disclosure also include a composition that includes a dried formulation comprising a first target analyte binding agent comprising a target analyte binding element and a polypeptide component having at least 60% sequence identity with SEQ ID NO: 12, and a liquid formulation comprising a second target analyte binding agent comprising a target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 13 or SEQ ID NO: 15, and further including a second complementary peptide component having at least 60% sequence identity with SEQ ID NO: 13 or SEQ ID NO: 15.

[0308] Embodiments of the present disclosure also include a dried formulation comprising a first target analyte binding agent comprising a target analyte binding element and a polypeptide component having at least 60% sequence identity with SEQ ID NO: 12, and a second target analyte binding agent comprising a target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 13 or SEQ ID NO: 15, and further including a liquid formulation comprising a second complementary peptide component having at least 60% sequence identity with SEQ ID NO: 13 or SEQ ID NO: 15.

[0309] Embodiments of the present disclosure also include a dried formulation comprising a first target analyte binding agent comprising a target analyte binding element and a polypeptide component having at least 60% sequence identity with SEQ ID NO: 12, and complementary peptide component having at least 60% sequence identity with SEQ ID NO: 13 or SEQ ID NO: 15, and a liquid formulation comprising a second target analyte binding agent comprising a target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 13 or SEQ ID NO: 15.

[0310] Embodiments of the present disclosure also include a dried formulation comprising a first target analyte binding agent comprising a target analyte binding element and a peptide component having at least 60% sequence identity with SEQ ID NO: 13, and a second target analyte binding agent comprising a target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 15, and further including a

liquid formulation comprising a complementary polypeptide component having at least 60% sequence identity with SEQ ID NO: 12.

[0311] Embodiments of the present disclosure also include a dried formulation comprising a complementary polypeptide component having at least 60% sequence identity with SEQ ID NO: 12, and a liquid formulation comprising a first target analyte binding agent comprising a target analyte binding element and a peptide component having at least 60% sequence identity with SEQ ID NO: 13, and a second target analyte binding agent comprising a target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 15.

[0312] Embodiments of the present disclosure also include a composition comprising a dried formulation comprising a first target analyte binding agent comprising a target analyte binding element and a peptide component having at least 60% sequence identity with SEQ ID NO: 13, a second target analyte binding agent comprising a target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 15, and a complementary polypeptide component having at least 60% sequence identity with SEQ ID NO: 12. In some embodiments, the dried formulation further comprises a luminogenic substrate. In some embodiments, the liquid formulation further comprises a luminogenic substrate. In some embodiments, the liquid formulation further comprises a sample comprising a target analyte, and wherein a bioluminescent analyte detection complex forms upon combining the dried formulation and the liquid formulation in the presence of the target analyte.

[0313] In some embodiments, a bioluminescent signal produced in the presence of the luminogenic substrate is substantially increased when the target analyte binding agent contacts one or more of the complementary peptide or polypeptide components of the bioluminescent complex, as compared to a bioluminescent signal produced by the target analyte binding agent and the luminogenic substrate alone.

[0314] In some embodiments, the target analyte is a target antibody. In some embodiments, the target analyte binding agent comprises an element that binds non-specifically to antibodies. In some embodiments, the target analyte binding agent comprises an element that binds specifically to an antibody. In some embodiments, the target antibody is an antibody against a pathogen, toxin, or therapeutic biologic.

[0315] In some embodiments, a target analyte binding element is selected from the group consisting of an antibody, a polyclonal antibody, a monoclonal antibody, a recombinant antibody, an antibody fragment, protein A, an Ig binding domain of protein A, protein G, an Ig binding domain of protein G, protein A/G, an Ig binding domain of protein A/G, protein L, a Ig binding domain of protein L, protein M, an Ig binding domain of protein M, an oligonucleotide probe, a peptide nucleic acid, a DARPin, an aptamer, an affimer, a protein domain, and a purified protein.

[0316] In some embodiments, the luminogenic substrate is selected from coelenterazine, coelenterazine-h, coelenterazine-h-h, furimazine, JRW-0238, JRW-1404, JRW-1482, JRW-1667, JRW-1743, JRW-1744, and other coelenterazine analogs or derivatives. In some embodiments, the coelenterazine analogs or derivatives are pro-luminogenic substrates such as those disclosed in U.S. Patent No. 9,487,520, herein incorporated by reference. In some embodiments, the coelenterazine analogs or derivatives are Enduazine (Promega Corporation) and Vivazine (Promega Corporation).

[0317] In some embodiments, the composition further comprises a polymer. In some embodiments, the polymer is a naturally-occurring biopolymer. In some embodiments, the naturally-occurring biopolymer is selected from pullulan, trehalose, maltose, cellulose, dextran, and a combination of any thereof. In some embodiments, the naturally-occurring biopolymer is pullulan. In some embodiments, the polymer is a cyclic saccharide polymer or a derivative thereof. In some embodiments, the polymer is hydroxypropyl β-cyclodextrin.

[0318] In some embodiments, the polymer is a synthetic polymer. In some embodiments, the synthetic polymer is selected from polystyrene, poly(meth)acrylate, and a combination of any thereof. In some embodiments, the synthetic polymer is a block copolymer comprising at least one poly(propylene oxide) block and at least one poly(ethylene oxide) block. In some embodiments, the synthetic polymer is poloxamer 188.

[0319] In some embodiments, the composition further comprises a buffer, a surfactant, a reducing agent, a salt, a radical scavenger, a chelating agent, a protein, or any combination thereof. In some embodiments, the is surfactant selected from polysorbate 20, polysorbate 40, and polysorbate 80.

[0320] In some embodiments, the composition further comprises a substance that reduces autoluminescence. In some embodiments, the substance is ATT (6-Aza-2-thiothymine), a

derivative or analog of ATT, a thionucleoside, thiourea, and the like. In some embodiments, the substance is a thionucleoside disclosed in U.S. Patent No. 9,676,997, herein incorporated by reference. In some embodiments, the substance is thiourea, which use for reducing autoluminescence is disclosed in U.S. Patent Nos. 7,118,878; 7,078,181; and 7,108,996, herein incorporated by reference.

[0321] In some embodiments, the composition is used in conjunction with an analyte detection platform to detect an analyte in a sample. In some embodiments, sample is selected from blood, serum, plasma, urine, stool, cerebral spinal fluid, interstitial fluid, saliva, a tissue sample, a water sample, a soil sample, a plant sample, a food sample, a beverage sample, an oil, and an industrial fluid sample.

[0322] Embodiments of the present disclosure also include a method of detecting an analyte in a sample comprising combining any of the compositions described above with a sample comprising a target analyte. In some embodiments, detecting the target analyte in the sample comprises detecting a bioluminescent signal generated from an analyte detection complex. In some embodiments, the method further comprises quantifying a bioluminescent signal generated from the analyte detection complex. In some embodiments, the bioluminescent signal generated from the analyte detection complex is proportional to the concentration of the analyte. In some embodiments, one or more of the components of the composition exhibits enhanced stability within the composition compared to the component in solution alone.

[0323] The various embodiments of the compositions and formulations described above demonstrate enhanced stability, as demonstrated in the Examples and FIGS. For example, when produced as a dried formulation such as a lyocake, when dried onto a substrate or matrix (e.g., Whatman 903, Ahlstrom 237, and Ahlstrom 6613H; wells of a 96-well plate, nylon mesh), or when dried in various protein buffer formulations, with or without the luminogenic substrate, the compositions and formulations of the present disclosure exhibit enhanced stability when stored for a prolonged period of time. As provided herein, the compositions and formulations of the present disclosure are able to generate a luminescent signal in the presence of a target analyte after storage for extended periods of time. In some embodiments, the compositions and formulations of the present disclosure exhibit enhanced stability as compared to compositions and formulations that contain the same or similar components of a bioluminescent complex (e.g., complementary peptides/polypeptides, luminogenic substrates), but which are formulated

without one or more of the other components of the formulation, and/or are not formulated according to the methods described herein.

[0324] In some embodiments, the compositions and formulations of the present disclosure exhibit enhanced stability for at least about 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 2 weeks, 3 weeks, 4 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 12 months, and up to 1 year. In some embodiments, the compositions and formulations of the present disclosure exhibit enhanced stability at temperatures ranging from about 0°C to 65°C, from about 4°C to 65°C, from about 10°C to 65°C, from about 15°C to 65°C, from about 15°C to 65°C, from about 20°C to 65°C, from about 25°C to 65°C, from about 30°C to 65°C, from about 35°C to 65°C, from about 37°C to 65°C, from about 40°C to 65°C, from about 45°C to 65°C, from about 50°C to 65°C, from about 55°C to 65°C, from about 60°C to 65°C, from about 4°C to 55°C, from about 10°C to 50°C, from about 15°C to 45°C, and from about 20°C to 40°C.

4. Detection Assays and Systems

[0325] Embodiments of the present disclosure include compositions, systems, assays, and methods for detecting one or more analytes in a sample. In accordance with these embodiments, described below are exemplary assays and devices for use with various embodiments herein. The following devices and assays should not be viewed as limiting the full scope of the systems, assays, and methods described herein.

a. Lateral Flow Assays

[0326] In certain embodiments, the present disclosure provides compositions and materials for conducting a lateral flow assay (e.g., a lateral flow immunoassay). Lateral flow assays are based on the principles of immunochromatography and can be used to detect, quantify, test, measure, and monitor a wide array of analytes, such as, but not limited to, analytes pertaining to monitoring ovulation, detecting/diagnosing infectious diseases/organisms, analyzing drugs of abuse, detecting/quantifying analytes important to human physiology, security screening, veterinary testing, agriculture applications, environmental testing, product quality evaluation, etc.

[0327] As shown in FIG. 1, embodiments of the present disclosure include lateral flow detection systems (100) for detecting and/or quantifying a target analyte based on bioluminescent complex formation. In some embodiments, lateral flow assay systems of the present disclosure

include an analytical membrane (105) that is divided into one or more detection regions (110) and one or more control regions (115). The detection region or regions can include a target analyte binding agent immobilized to a portion of the detection region such that it is not displaced when facilitating lateral flow across the analytical membrane. Lateral flow assay systems of the present disclosure can also include a conjugate pad (120) within which a target analyte binding agent is contained. In some embodiments, a target analyte binding agent is contained within the conjugate pad but flows from the conjugate pad and across the analytical membrane towards the detection and control regions when lateral flow occurs. Lateral flow assay systems of the present disclosure can also include a sample pad (125) that is positioned at one distal end of the lateral flow assay system (e.g., opposite an absorbent pad). A sample that contains (or may contain) a target analyte is applied to the sample pad. In some embodiments, a lateral flow assay system also comprises a wicking pad (130) at an end of the device distal to the sample pad. The wicking pad generates capillary flow of the sample from the sample pad through the conjugate pad, analytical membrane, detection region, and control region.

[0328] In accordance with these embodiments, upon addition of a sample to the sample pad, the facilitation of lateral flow causes a target analyte within the sample to contact a first target analyte binding agent within the conjugate pad; subsequently, lateral flow causes the target analyte and the first target analyte binding agent to contact a second target analyte binding agent immobilized to a detection region of the analytical membrane. The presence and/or quantity of the target analyte is then determined based on detection of the analyte in the detection region (e.g., in the presence of a luminogenic substrate for the bioluminescent complex) and/or in comparison to the control.

[0329] In some embodiments, the above lateral flow systems make use of one or more NanoLuc®-based technologies (e.g., NanoBiT, NanoTrip, NanoBRET, etc.) for detection of the bound target analyte.

[0330] In an exemplary embodiment, as shown in FIG. 1, a target analyte is an antibody generated in a subject in response to being exposed to an infectious disease/organism. The first target analyte binding agent includes a both a target analyte binding element that binds the antibody (e.g., a non-specific antibody binding agent (e.g., protein L)) and a first peptide or polypeptide capable of interacting with a distinct peptide or polypeptide to generate a bioluminescent signal (e.g., a NanoBiT non-luminescent peptide or polypeptide or variant

thereof (e.g., one of SEQ ID NOs: 9-11 or 12/14)). The second target analyte binding agent can include a target analyte binding element that binds the antibody, such as an epitope of an antigen recognized by the antibody, and a second peptide or polypeptide capable of interacting with a the first peptide or polypeptide to generate a bioluminescent signal (e.g., a NanoBiT non-luminescent peptide or polypeptide or variant thereof (e.g., one of SEQ ID NOs: 9-11 or 12/14)). Once the bioluminescent complex firms at the detection region, the bioluminescent signal can be detected and/or quantified (e.g., in the presence of a luminogenic substrate for the bioluminescent complex), thus indicating the presence/quantity of the antibody in the sample.

[0331] As shown in FIG. 1, lateral flow assays of the present disclosure can be configured to test for multiple different analytes such as antibodies generated to distinct diseases/microorganisms, in a single sample from a subject (e.g., multiplexing). In accordance with these embodiments, the analytical membrane can include a plurality of detection regions with each detection region comprising a distinct target analyte binding agent having distinct target analyte binding elements (e.g., distinct disease antigens).

[0332] In an alternative lateral flow embodiment to the one depicted in FIG. 1, a target analyte is an antibody generated in a subject in response to being exposed to an infectious disease/organism. The first target analyte binding agent that includes a both a target analyte binding element that binds the antibody (e.g., an epitope of an antigen recognized by the antibody) and a bioluminescent polypeptide (e.g., NanoLuc or a variant thereof (e.g., SEQ ID NO: 5, SEQ ID NO: 6)). The second target analyte binding agent can include a target analyte binding element that binds the antibody, such as a non-specific antibody binding agent (e.g., protein L). Detection of bioluminescence in the detection region (e.g., in the presence of a luminogenic substrate for the bioluminescent complex) then indicates that both target analyte binding agents bound to the target analyte, and therefore the target analyte was present in the sample.

[0333] In another exemplary alternative embodiment, a target analyte is an antibody generated in a subject in response to being exposed to an infectious disease/organism. The first target analyte binding agent includes a both a target analyte binding element that binds the antibody (e.g., a non-specific antibody binding agent (e.g., protein L), a target-specific (e.g., antibody) binding agent) and a first non-luminescent (NL) peptide tag (e.g., SEQ ID NO: 13 or 11 or variants thereof) capable of interacting with a second non-luminescent (NL) peptide (e.g., SEQ

ID NO: 11 or 13 or variants thereof) and a non-luminescent (NL) polypeptide (e.g., SEQ ID NO: 12 or variants thereof) to generate a bioluminescent signal. The second target analyte binding agent includes a target analyte binding element that binds the antibody (e.g., a target-specific (e.g., antibody) binding agent, a non-specific antibody binding agent (e.g., protein L)) and a second NL peptide tag (e.g., SEQ ID NO: 11 or 13 or variants thereof). Formation of the bioluminescent complex in the presence of the NL polypeptide component (e.g., SEQ ID NO: 12 or variants thereof) and a luminogenic substrate in the detection region indicates the presence of the target analyte in the sample. The bioluminescent signal is detected and/or quantified to detect/quantity the antibody in the sample.

[0334] Additional alternatives to the exemplary embodiments set forth above are contemplated. For example, alternative binding agents, target analytes, detectable elements, order of the various components (e.g., non-specific binding agent/target-specific binding agent, target-specific binding agent/non-specific binding agent, target-specific binding agent/target-specific binding agent, etc.) are described herein and embodiments incorporating various combinations of the components are within the scope herein.

[0335] In some embodiments, a target analyte is not an antibody, but is instead a small molecule, peptide, protein, carbohydrate, lipid, etc. In some embodiments, the lateral flow assays and systems described above are configured (e.g., using one or more NanoLuc®-based technologies (e.g., NanoBiT, NanoTrip, NanoBRET, etc.)) for the detection of any such target analytes.

b. Solid Phase Assays

[0336] Embodiments of the present disclosure include compositions, assays, systems, devices, and methods for detecting one or more analytes in a sample. In accordance with these embodiments, the present disclosure provides compositions and materials for conducting a solid phase assay (e.g., a solid phase platform for conducting an immunoassay). Solid phase detection platforms are generally the simplest form of an immunoassay and can be used to detect, quantify, test, measure, and monitor a wide array of analytes such as, but not limited to, analytes pertaining to monitoring ovulation, detecting/diagnosing infectious diseases/organisms, analyzing drugs of abuse, detecting/quantifying analytes important to human physiology, veterinary testing, security screening, agriculture applications, environmental testing, and product quality evaluation. In contrast to lateral flow assays, solid phase detection platforms do

not involve facilitating the flow of assay reagents across a membrane, but instead typically include a solid support to which components of the assay are attached or contained within (e.g., dipstick test or spot test).

[0337] As shown in FIG. 2, embodiments of the present disclosure include solid phase detection platforms (200) for detecting and/or quantifying a target analyte based on bioluminescent complex formation. In some embodiments, solid phase detection platforms of the present disclosure include one or more detection regions (205) and one or more control regions (210) to which a sample is applied. In some embodiments, the detection region or regions includes a target analyte binding agent within and/or conjugated to a portion of the detection region. Solid phase detection platforms of the present disclosure can also include a solid support (215) to which the detection regions and the control regions are attached and demarcated from each other, and which allow for a sample to be applied to the detection and control regions (e.g., dipstick test).

[0338] In accordance with these embodiments, a sample or a portion of a sample is applied to the detection and control regions of the solid phase assay platform such that a target analyte contacts a target analyte binding agent (220) conjugated to and/or within the detection region under conditions such that the binding event and/or the immobilization of the target analyte on the solid phase is detectable (e.g., a bioluminescent entity is immobilized, a bioluminescent complex is formed), thereby indicating the presence of the analyte in the sample.

[0339] In some embodiments, the solid phase assay platform includes a first target analyte binding agent (e.g., a target-specific binding agent (e.g., target-specific antibody, antigen for the target antibody, etc.)) immobilized on the solid phase. A second target analyte binding agent (e.g., a target-specific binding agent (e.g., target-specific antibody, antigen for the target antibody, etc.), a non-specific binding agent (e.g., protein L)) linked to a bioluminescent polypeptide (e.g., SEQ ID NO: 5 or variants thereof) is added to the solid phase with the sample (e.g., concurrently, sequentially, etc.). If both target analyte binding agent bind to the target analyte, the bioluminescent polypeptide becomes immobilized on the solid phase. Detection/quantification of bioluminescence on the solid phase (e.g., after a wash step) indicates the presence/amount of target analyte in the sample. In some cases, the first target analyte binding agent is conjugated to the detection region, and the second target analyte binding agent (attached to the bioluminescent polypeptide) is applied to the detection region with or without

the sample. In some cases, the second target analyte binding agent is conjugated to the detection region, and the first target analyte binding agent (attached to the bioluminescent polypeptide) is applied to the detection region with or without the sample. In accordance with these embodiments, immobilization of bioluminescence at the detection region can be detected and/or quantified when in the presence of a luminogenic substrate (as described further below), thus indicating the presence (or absence) of the antibody in the sample.

[0340] In alternative embodiments, a solid phase assay platform utilizes a binary complementation approach, in which a bioluminescent complex is formed upon binding of two non-luminescent (NL) peptide/polypeptide components (e.g., NanoBiT system), to detect a target analyte. Multiple configurations of solid phase assays and systems utilizing a binary complementation approach are within the scope herein. For example, an exemplary system can include (i) a first target analyte binding agent linked to a first NL peptide or NL polypeptide (e.g., SEQ ID NOs: 9 or 10 or variants thereof) capable of interacting with high affinity with a second distinct NL polypeptide or NL peptide (e.g., SEQ ID NOs: 10 or 9 or variants thereof) to generate a bioluminescent signal, and (ii) a second target analyte binding agent linked to the complementary NL polypeptide or NL peptide, wherein the second target analyte binding agent is immobilized to the solid phase. Upon binding of the target analyte binding agents to the target analyte, a bioluminescent complex is formed on the solid phase and the bioluminescent signal is detectable/quantifiable, when in the presence of a luminogenic substrate (as described further below).

[0341] In other embodiments, a solid phase assay platform utilizes a tripartite complementation approach, in which a bioluminescent complex is formed upon binding of two non-luminescent (NL) peptide components and a non-luminescent (NL) polypeptide component (e.g., NanoTrip system), to detect a target analyte. In some embodiments, the solid phase assay platform includes: (i) a first target analyte binding agent comprising both a target analyte binding element (e.g., general or specific) and a NL peptide (e.g., SEQ ID NOs: 11 or 13) capable of forming a tripartite bioluminescent complex (e.g., NanoTrip complex), (ii) a second target analyte binding agent comprising both a target analyte binding element (e.g., specific) and a NL peptide (e.g., SEQ ID NOs: 11 or 13) capable of forming a tripartite bioluminescent complex (e.g., NanoTrip complex), (iii) a NL polypeptide component of the tripartite bioluminescent complex (e.g., NanoTrip complex), and (iv) a luminogenic substrate. In some cases, the first

target analyte binding agent is conjugated to the detection region, and the second target analyte binding agent is applied to the detection region with or without the sample. In some cases, the second target analyte binding agent is conjugated to the detection region, and the first target analyte binding agent is applied to the detection region with or without the sample. Once the bioluminescent complex forms at the detection region, the bioluminescent signal is detected and/or quantified, thus indicating the presence (or absence) of the antibody in the sample.

[0342] In other embodiments, the solid phase assay platform includes (i) a first target analyte binding agent comprising a target analyte binding element and a NanoLuc®-based peptide or polypeptide, (ii) target analyte binding agent comprising a target analyte binding element and a fluorophore, and (iii) optionally the additional peptide/polypeptide components to form a bioluminescent complex (e.g., in embodiments in which the NanoLuc®-based peptide or polypeptide is not a bioluminescent polypeptide, e.g. non-luminescent), wherein upon binding of the first and second target analyte binding agents to a target analyte in a sample, in the presence of any additional components necessary for bioluminescence (e.g., luminogenic substrate, complementary components, etc.), emission from the NanoLuc®-based components (e.g., NanoLuc® protein or bioluminescent complex) excites the fluorophore (e.g., via BRET). In some cases, the first target analyte binding agent is conjugated to the detection region, and the second target analyte binding agent is applied to the detection region with or without the sample. In some cases, the second target analyte binding agent is conjugated to the detection region, and the first target analyte binding agent is applied to the detection region with or without the sample.

[0343] As shown in FIG. 2, solid phase platforms of the present disclosure can be configured to test for multiple different analytes, such as antibodies generated to distinct diseases/microorganisms, in a single sample from a subject (e.g., multiplexing). In accordance with these embodiments, the solid phase platforms can include a plurality of detection regions with each detection region comprising a distinct target analyte binding agent having distinct target analyte binding elements (e.g., distinct disease antigens).

[0344] In some embodiments, the solid phase platforms of the present disclosure can include a plurality of detection regions such as one or more wells of a microtiter plate, for example. In such embodiments, one or more distinct target analyte binding agents can be conjugated (e.g., coated) to wells of the microtiter plate along one or more of the other detection reagents required

to carry out a particular bioluminescent assay (e.g., a second target analyte binding agent, a luminogenic substrate, assay buffer, etc.). In some embodiments, one or more of the other detection reagents (reagents not conjugated to the microtiter plate) required to carry out the assay can be added to the wells of the microtiter plate in the form of a lyophilized cake (lyocake) or tablet and reconstituted as part of the bioluminescent assay.

c. Solution Phase Assays

[0345] Embodiments of the present disclosure include compositions, assays, systems, devices, and methods for detecting one or more analytes in a sample. In accordance with these embodiments, the present disclosure provides compositions and materials for conducting a solution phase assay (e.g., a liquid-based format for conducting an immunoassay within a solution). Solution phase detection platforms can be used to detect, quantify, test, measure, and monitor a wide array of analytes such as, but not limited to, analytes pertaining to monitoring ovulation, detecting/diagnosing infectious diseases/organisms, analyzing drugs of abuse, detecting/quantifying analytes important to human physiology, veterinary testing, security screening, agriculture applications, environmental testing, and product quality evaluation. In contrast to lateral flow assays and solid phase detection platforms, solution phase detection platforms typically include a receptacle for the solution/liquid in which reactions involving the detection reagents take place, instead of conjugating one or more of the detection reagents to a solid support or membrane to facilitate detection.

[0346] For example, as shown in FIG. 33, embodiments of solution phase platforms of the present disclosure can include one or more components of the bioluminescent complexes in a tablet or lyophilized cake that can be reconstituted in a solution (e.g., buffered solution) to facilitate analyte detection. In some embodiments, the tablet or lyocake can include all the reagents necessary to carry out a reaction to detect an analyte. Such lyocakes or tablets are compatible with many different assay formats, including but not limited to, cuvettes, wells of microtiter plates (e.g., 96-well microtiter plate), test tubes, large volume bottles, SNAP assays, and the like.

[0347] In some embodiments, the solution phase assay platform includes a lyocake or tablet comprising one or more of a first target analyte binding agent (e.g., a target-specific binding agent (e.g., target-specific antibody, antigen for the target antibody, etc.)), a second target analyte binding agent (e.g., a target-specific binding agent (e.g., target-specific antibody, antigen for the

target antibody, etc.), and a non-specific binding agent (e.g., protein L)) linked to a bioluminescent polypeptide (e.g., SEQ ID NO: 5 and variants thereof). Detection/quantification of bioluminescence in the solution indicates the presence/amount of target analyte in the sample.

[0348] In some embodiments, a solution phase assay platform utilizes a binary complementation approach, in which a bioluminescent complex is formed upon binding of two non-luminescent (NL) peptide/polypeptide components (e.g., NanoBiT system), to detect a target analyte. Multiple configurations of solution phase assays and systems utilizing a binary complementation approach are within the scope herein. For example, an exemplary system can include (i) a first target analyte binding agent linked to a first NL peptide or NL polypeptide (e.g., SEQ ID NOs: 9 or 10 or variants thereof) capable of interacting with high affinity with a second distinct NL polypeptide or NL peptide (e.g., SEQ ID NOs: 10 or 9 or variants thereof) to generate a bioluminescent signal, and (ii) a second target analyte binding agent linked to the complementary NL polypeptide or NL peptide. Upon binding of the target analyte binding agents to the target analyte, a bioluminescent complex is formed in the solution and the bioluminescent signal is detectable/quantifiable, when in the presence of a luminogenic substrate (as described further below).

[0349] In other embodiments, a solution phase assay platform utilizes a tripartite complementation approach, in which a bioluminescent complex is formed upon binding of two non-luminescent (NL) peptide components and a non-luminescent (NL) polypeptide component (e.g., NanoTrip system), to detect a target analyte. In some embodiments, the solution phase assay platform includes: (i) a first target analyte binding agent comprising both a target analyte binding element (e.g., general or specific) and a NL peptide (e.g., SEQ ID NOs: 11 or 13) capable of forming a tripartite bioluminescent complex (e.g., NanoTrip complex), (ii) a second target analyte binding agent comprising both a target analyte binding element (e.g., specific) and a NL peptide (e.g., SEQ ID NOs: 11 or 13) capable of forming a tripartite bioluminescent complex (e.g., NanoTrip complex), (iii) a NL polypeptide component of the tripartite bioluminescent complex (e.g., NanoTrip complex), and (iv) a luminogenic substrate. Once the bioluminescent complex forms in the solution, the bioluminescent signal is detected and/or quantified, thus indicating the presence (or absence) of the antibody in the sample.

[0350] In other embodiments, the solution phase assay platform includes (i) a first target analyte binding agent comprising a target analyte binding element and a NanoLuc®-based

peptide or polypeptide, (ii) target analyte binding agent comprising a target analyte binding element and a fluorophore, and (iii) optionally the additional peptide/polypeptide components to form a bioluminescent complex (e.g., in embodiments in which the NanoLuc®-based peptide or polypeptide is not a bioluminescent polypeptide, e.g., non-luminescent), wherein upon binding of the first and second target analyte binding agents to a target analyte in a sample, in the presence of any additional components necessary for bioluminescence (e.g., luminogenic substrate, complementary components, etc.), emission from the NanoLuc®-based components (e.g., NanoLuc® protein or bioluminescent complex) excites the fluorophore (e.g., via BRET).

[0351] Solution phase platforms of the present disclosure can be configured to test for multiple different analytes (e.g., multiplexing), such as antibodies generated to distinct diseases/microorganisms in a single sample from a subject. In some embodiments, one or more of the detection reagents required to carry out a bioluminescent reaction to detect/quantify an analyte are present in one or more receptacles of a particular assay platform being used (e.g., individual wells of a 96-well plate), for example, as a lyocake or tablet that is to be reconstituted in a buffered solution. In other embodiments, one or more types of a sample solution are already present in the receptacles, and one or more lyocakes or tables are added to the receptacles and rehydrated to facilitate a bioluminescent reaction. In accordance with these embodiments, the solution phase platforms can include a plurality of receptacles comprising a distinct target analyte binding agent having distinct target analyte binding elements (e.g., distinct disease antigens).

d. Other Assays

[0352] Embodiments of the present disclosure include compositions, assays, systems, devices, and methods for detecting one or more analytes in a sample using other assay platforms known in the art. For example, target analytes can be detected and/or measured using the bioluminescent polypeptides and/or complexes described herein in the context of a microfluidic and/or chip-based assay. Because microfluidic systems integrate a wide variety of operations for manipulating fluids, such as chemical or biological samples, these systems are applicable to many different areas, such as biological and medical diagnostics. One type of microfluidic device is a microfluidic chip. Microfluidic chips may include micro-scale features (or micro-features), such as channels, valves, pumps, and/or reservoirs for storing fluids, for routing fluids to and from various locations on the chip, and/or for reacting fluidic reagents.

[0353] Microfluidic chips, or labs-on-a-chip (LOC), configured with bioluminescent polypeptides and/or complexes that include peptides and polypeptides capable of generating a bioluminescent signal in the presence of the target analyte offer increased flexibility for automation, integration, miniaturization, and multiplexing. For example, pathogen detection based on microfluidic chips use reaction chambers that are usually on the micro- or nano-scale, which allows devices to be miniaturized and portable; this is particularly advantageous for point-of-care testing. LOC technology allows for the integration of sample preparation, amplification, and signal detection, which reduces the time need to generate results. The high throughput and low consumption of sample and reagents make the technology flexible and relatively cost effective. Nucleic acid-based microfluidic pathogen detection for the detection of bacteria, viruses, and fungi that eliminates the need for PCR or real-time PCR for amplification is a distinct advantage of the bioluminescent complexes of the present disclosure.

5. Assay Compositions, Components, and Methods of Manufacturing

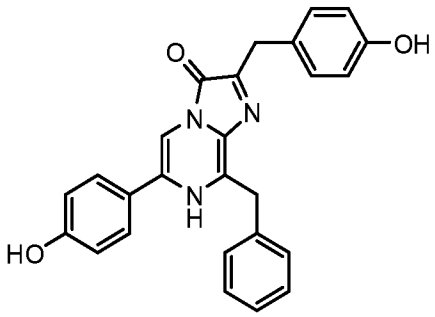
[0354] Embodiments of the present disclosure also include methods of manufacturing an assay platform for use with bioluminescent peptides and polypeptides for target analyte detection. Although assay platforms may vary depending on various factors, such as the analyte being detected, the complexity of the sampling environment, and the diagnostic parameters, the compositions, materials and methods of the present disclosure can be applied to most currently available assay platforms, such as solid phase assays, lateral flow assays, and microfluidic-based assays.

a. Luminogenic Substrates

[0355] In some embodiments, methods of manufacturing assay platforms of the present disclosure include application of a luminogenic substrate. Luminogenic substrates, such as coelenterazine, and analogs and derivatives thereof, can decompose during storage thereby resulting in loss of the substrate before addition to or use in a biological assay. Such decomposition can be the result of instability of the luminogenic substrate in solution over time in a temperature-dependent manner. This decomposition results in waste of the luminogenic substrate and reduced sensitivity and reproducibility of luminescent measurements derived from biological assays that employed the decomposed luminogenic substrate.

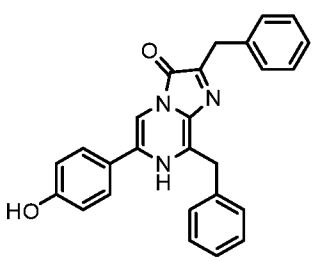
[0356] Provided herein are compositions that include a luminogenic substrate, such as coelenterazine or an analog or derivative thereof. Exemplary coelenterazine analogs include coelenterazine-h, coelenterazine-h-h, furimazine, JRW-0238, JRW-1404, JRW-1482, JRW-1667, JRW-1743, and JRW-1744.

[0357] In some embodiments, the substrate is coelenterazine, which has the following structure:

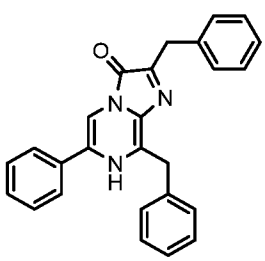


coelenterazine

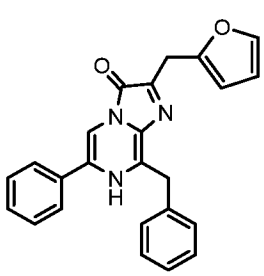
Exemplary coelenterazine analogs include coelenterazine-h (2-deoxycoelenterazine or 2,8-dibenzyl-6-(4-hydroxyphenyl)imidazo[1,2-a]pyrazin-3(7H)-one), coelenterazine-h-h (dideoxycoelenterazine or 2,8-dibenzyl-6-phenylimidazo[1,2-a]pyrazin-3(7H)-one), furimazine (8-benzyl-2-(furan-2-ylmethyl)-6-phenylimidazo[1,2-a]pyrazin-3(7H)-one), JRW-0238 (8-benzyl-2-(furan-2-ylmethyl)-6-(3-hydroxyphenyl)imidazo[1,2-a]pyrazin-3(7H)-one), JRW-1404 (8-benzyl-6-(2-fluoro-3-hydroxyphenyl)-2-(furan-2-ylmethyl)imidazo[1,2-a]pyrazin-3(7H)-one), JRW-1482 (. 6-(3-amino-2-fluorophenyl)-8-benzyl-2-(furan-2-ylmethyl)imidazo[1,2-a]pyrazin-3(7H)-one), JRW-1667 (6-(3-amino-2-fluorophenyl)-8-(2-fluorobenzyl)-2-(furan-2-ylmethyl)imidazo[1,2-a]pyrazin-3(7H)-one), JRW-1744 (6-(3-amino-2-fluorophenyl)-8-benzyl-2-(furan-2-ylmethyl)imidazo[1,2-α]pyrazin-3(7H)-one), and JRW-1743 (6-(3-amino-2-fluorophenyl)-8-(2-fluorobenzyl)-2-(furan-2-ylmethyl)imidazo[1,2-α]pyrazin-3(7H)-one), which have the following structures:



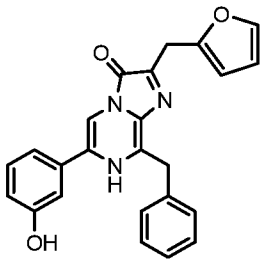
coelenterazine-h



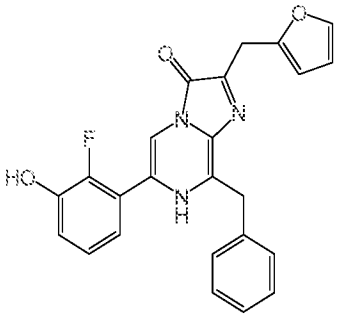
coelenterazine-hh



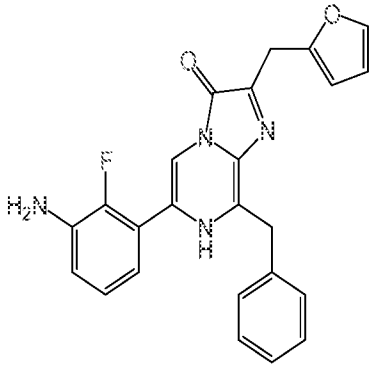
furimazine



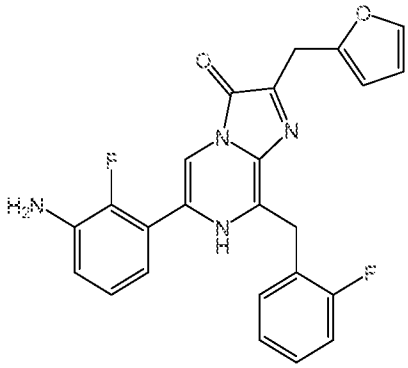
JRW-0238



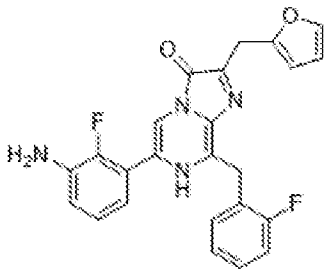
JRW-1404



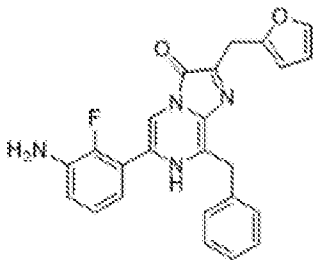
JRW-1482



JRW-1667



JRW-1743



JRW-1744

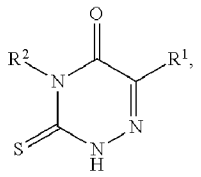
[0358] Additional exemplary coelenterazine analogs include coelenterazine-n, coelenterazine-f, coelenterazine-hcp, coelenterazine-cp, coelenterazine-c, coelenterazine-e, coelenterazine-fcp, coelenterazine-i, coelenterazine-icp, coelenterazine-v, 2-methyl coelenterazine, and the like. In some embodiments, the compound may be a coelenterazine analog described in WO 2003/040100; U.S. Pat. Pub. 2008/0248511 (e.g., paragraph [0086]); U.S. Pat. No. 8,669,103; WO 2012/061529; U.S. Pat. Pub. 2017/0233789; U.S. Pat. No. 9,924,073; U.S. Pat. Pub. 2018/0030059; U.S. Pat. No. 10,000,500; U.S. Pat. Pub. 2018/0155350; U.S. Pat. App. No. 16/399,410 (PCT/US2019/029975); U.S. Pat. App. No. 16/548,214 (PCT/US2019/047688); U.S. Pat. Pub. 2014/0227759; U.S. Pat. No. 9,840,730; U.S. Pat. No. 7,268,229; U.S. Pat. No. 7,537,912; U.S. Pat. No. 8,809,529; U.S. Pat. No. 9,139,836; U.S. Pat. No. 10,077,244; U.S. Pat. No. 9,487,520; U.S. Pat. No. 9,924,073; U.S. Pat. No. 9,938,564; U.S. Pat. No. 9,951,373; U.S. Pat. No. 10,280,447; U.S. Pat. No. 10,308,975; U.S. Pat. No. 10,428,075; the disclosures of which are incorporated by reference herein in their entireties. In some embodiments, coelenterazine analogs include pro-substrates such as, for example, those described in U.S. Pat. Pub. 2008/0248511; U.S. Pat. Pub. 2012/0707849; U.S. Pat. Pub. 2014/0099654; U.S. Pat. No. 9,487,520; U.S. Pat. No. 9,927,430; U.S. Pat. No. 10,316,070; herein incorporated by reference

in their entireties. In some embodiments, the compound is furimazine. In some embodiments, the compound is JRW-0238. In some embodiments, the compound is JRW-1743. In some embodiments, the compound is JRW-1744.

[0359] Provided herein are compositions that include a luminogenic substrate, such as coelenterazine or an analog or derivative thereof, and a polymer or a paper/fibrous substrate for the manufacture of bioluminescent target analyte detection platforms. Compositions that stabilize and/or enhance the reconstitution efficiency of luminogenic substrates such as coelenterazine or an analog or derivative thereof, are described in U.S. Pat. Appln. Serial No. 16/592,310 (PCT/US2019/054501); herein incorporated by reference in its entirety. In some embodiments, the composition stabilizes the compound against decomposition. In some embodiments, the composition stabilizes the compound against decomposition as compared to a composition that does not contain the polymer or paper/fibrous substrate. In some embodiments, the polymer or the paper/fibrous substrate reduces or suppresses the formation of one or more decomposition products from the compound. In some embodiments, the compositions enhance the reconstitution efficiency or reconstitution rate of the substrate.

[0360] Additionally, embodiments of the present disclosure include means for stabilizing (e.g., enhancing storage stability) the compositions described further herein. In some embodiments, enhancing the storage stability of the compositions provided herein includes methods and compositions for stabilizing a luminogenic substrate. The luminogenic substrate may be, but is not limited to, coelenterazine, coelenterazine-h, coelenterazine-h-h, furimazine, a derivative thereof, an analog thereof, or any combination thereof. The compositions may include the luminogenic substrate, a thionucleoside, and an organic solvent. The composition may not include or contain a luminogenic enzyme. As provided in U.S. Pat. No. 9,676,997, which is herein incorporated by reference, a thionucleoside may be a compound of formula (I) or a tautomer thereof,

(I)

[0361] 

[0362] wherein

[0363] R^1 is hydrogen, alkyl, substituted alkyl, alkyl-aryl, alkyl-heteroaryl, cycloalkyl, aryl, heteroaryl, carboxylic acid, ester, NR^aR^b, imine, hydroxyl, or oxo;

[0364] R^2 is hydrogen, NR^aR^b, imine, alkyl, or aryl; and

[0365] R^a and R^b are each independently hydrogen, alkyl, or aryl.

[0366] In some embodiments, the compound of formula (I) may be ATT (6-methyl-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2H)-one); 3-(4-Amino-5-oxo-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)propanoic acid; tetrahydro-2-methyl-3-thioxo-1,2,4-triazine-5,6-dione; 4-((2-furylmethylene)amino)-3-mercapto-6-methyl-1,2,4-triazin-5(4H)-one; 6-benzyl-3-sulfanyl-1,2,4-triazin-5-ol; 4-amino-3-mercapto-6-methyl-1,2,4-triazin-5(4H)-one; 3-(5-oxo-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)propanoic acid; (E)-6-methyl-4-((thiophen-2-ylmethylene)amino)-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2H)-one; (E)-6-methyl-4-((3-nitrobenzylidene)amino)-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2H)-one; (E)-4-((4-(diethylamino)benzylidene)amino)-6-methyl-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2H)-one; ATCA ethyl ester; TAK-0021,TAK-0020, TAK-0018, TAK-0009,TAK-0014, TAK-0007, TAK-0008, TAK-0003, and TAK-0004, as provided in U.S. Pat. No. 9,676,997 (incorporated herein by reference); 3-thioxo-6-(trifluoromethyl)-3,4-dihydro-1,2,4-triazin-5(2H)-one; 6-cyclopropyl-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2H)-one; 6-(hydroxymethyl)-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2H)-on; or any combinations thereof.

[0367] In some embodiments, a thionucleoside may stabilize the luminogenic substrate against decomposition over time, in the presence of light, in the absence of light, and/or at different temperatures. The thionucleoside may stabilize the luminogenic substrate against decomposition into one or more decomposition products over time, in the presence of light, in the absence of light, and/or at different temperatures. As such, inclusion of the thionucleoside in the compositions described further herein may stabilize the luminogenic substrate against decomposition by suppressing or reducing the formation of the one or more decomposition products as compared to a composition that does not include the thionucleoside. This, in turn, provides the capability of storing or incubating the luminogenic substrate for a period of time at a particular temperature, in the presence of light, and/or in the absence of light without significant decomposition of the luminogenic substrate before use of the luminogenic substrate in an assay. In accordance with these embodiments, the inclusion of a thionucleoside in the compositions described herein can enhance storage stability of the compositions. These embodiments also

relate to methods for stabilizing the luminogenic substrate. Such a method may stabilize the luminogenic substrate against decomposition and/or suppress or reduce the formation of the one or more decomposition products. The method may include contacting the luminogenic substrate with an effective amount of the thionucleoside (e.g., 225 mM) in the presence of the organic solvent. This contacting step may include forming the composition described above.

[0368] In some embodiments, one or more of the non-luminescent (NL) peptide/polypeptide components that form the bioluminescent complexes described above can be included with or without a luminogenic substrate as part of a composition, such as a lyophilized powder. These compositions can be applied directly, with or without other components, to a portion of a detection platform, or they can be reconstituted as part of a separate solution that is applied to the detection platform.

[0369] Coelenterazine and analogs and derivatives thereof may suffer from challenges associated with their reconstitution into buffer systems used in many assays such as the bioluminogenic methods described herein. For example, coelenterazines, or analogs or derivatives thereof, such as furimazine, may dissolve slowly and/or inconsistently in buffer solutions (e.g., due to the heterogeneous microcrystalline nature of the solid material). While dissolution in organic solvent prior to dilution with buffer may provide faster and more consistent results, coelenterazine compounds may suffer from instability in organic solutions on storage, including both thermal instability and photo-instability. In some embodiments, the composition further comprises a polymer. As further described herein, the presence of the polymer may stabilize the compound against decomposition, and the presence of the polymer may improve the solubility of the compound in water or in aqueous solutions.

[0370] The polymer may be a naturally-occurring biopolymer or a synthetic polymer. In some embodiments, the polymer is a naturally-occurring biopolymer. Suitable naturally-occurring biopolymers are carbohydrates, including disaccharides (e.g., trehalose and maltose), and polysaccharides (e.g., pullulan, dextran, and cellulose). Mixtures of naturally-occurring biopolymers may also be used. In some embodiments, the polymer is pullulan, which is a polysaccharide that includes maltotriose repeating units. Maltotriose is a trisaccharide that includes three glucose units that are linked via α-1,4 glycosidic bonds. The maltotriose units within the pullulan polymer are linked to each other via α-1,6 glycosidic bonds.

[0371] In some embodiments, the polymer is a synthetic polymer. A synthetic polymer may be a homopolymer, copolymer, or block copolymer (e.g., diblock copolymer, triblock copolymer, etc.). Non-limiting examples of suitable polymers include, but are not limited to polyamines, polyethers, polyamides, polyesters, polycarbamates, polyureas, polycarbonates, polystyrenes, polyimides, polysulfones, polyurethanes, polyacetylenes, polyethylenes, polyethyeneimines, polyisocyanates, polyacrylates, polymethacrylates, polyacrylonitriles, and polyarylates. Non-limiting examples of specific polymers include poly(caprolactone) (PCL), ethylene vinyl acetate polymer (EVA), poly(lactic acid) (PLA), poly(L-lactic acid) (PLLA), poly(glycolic acid) (PGA), poly(lactic acid-co-glycolic acid) (PLGA), poly(L-lactic acid-co-glycolic acid) (PLLGA), poly(D,L-lactide) (PDLA), poly(L-lactide) (PLLA), poly(D,L-lactide-co-caprolactone), poly(D,L-lactide-co-caprolactone-co-glycolide), poly(D,L-lactide-co-PEO-co-D,L-lactide), poly(D,L-lactide-co-PPO-co-D,L-lactide), polyalkyl cyanoacrylate, polyurethane, poly-L-lysine (PLL), hydroxypropyl methacrylate (HPMA), poly(ethylene glycol), poly-L-glutamic acid, poly(hydroxy acids), polyanhydrides, polyorthoesters, poly(ester amides), polyamides, poly(ester ethers), polycarbonates, polyalkylenes (e.g., polyethylene and polypropylene), polyalkylene glycols (e.g., poly(ethylene glycol) (PEG)), polyalkylene terephthalates (e.g., poly(ethylene terephthalate), etc.), polyvinyl alcohols (PVA), polyvinyl ethers, polyvinyl esters (e.g., poly(vinyl acetate), etc.), polyvinyl halides (e.g., poly(vinyl chloride) (PVC), etc.), polyvinylpyrrolidone, polysiloxanes, polystyrene (PS), polyurethanes, derivatized celluloses (e.g., alkyl celluloses, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitro celluloses, hydroxypropylcellulose, carboxymethylcellulose, etc.), polymers of acrylic acids ("polyacrylic acids") (e.g., poly(methyl(meth)acrylate) (PMMA), poly(ethyl(meth)acrylate), poly(butyl(meth)acrylate), poly(isobutyl(meth)acrylate), poly(hexyl(meth)acrylate), poly(isodecyl(meth)acrylate), poly(lauryl(meth)acrylate), poly(phenyl(meth)acrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), poly(octadecyl acrylate), polydioxanone and its copolymers (e.g., polyhydroxyalkanoates, polypropylene fumarate), polyoxymethylene, poloxamers, poly(ortho)esters, poly(butyric acid), poly(valeric acid), poly(lactide-co-caprolactone), trimethylene carbonate, and mixtures and copolymers thereof.

[0372] In some embodiments, the composition further comprises a paper substrate. As further described herein, the presence of the paper substrate may stabilize the compound against

decomposition, and the presence of the paper substrate may improve the solubility of the compound in aqueous solutions. Exemplary paper substrates include, but are not limited to, Whatman brand papers, (e.g., W-903 paper, FTA paper, FTA Elute paper, FTA DMPK paper, etc.), Ahlstrom papers (e.g., A-226 paper, etc.), M-TFN paper, FTA paper, FP705 paper, Bode DNA collection paper, nitrocellulose paper, nylon paper, cellulose paper, Dacron paper, cotton paper, and polyester papers, and combinations thereof.

[0373] In addition to the compound and the polymer and/or the paper substrate, the composition may include additional components such as buffers, surfactants, salts, proteins, or any combination thereof. For example, the composition may include a buffer such as a phosphate buffer, a borate buffer, an acetate buffer, or a citrate buffer, or other common buffers such as bicine, tricine, tris(hydroxymethyl)aminomethane (tris), *N*-[tris(hydroxymethyl)methyl]-3-aminopropanesulfonic acid (TAPS), 3-[*N*-tris(hydroxymethyl)methylamino]-2-hydroxypropanesulfonic acid (TAPSO), 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES), *N*-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid (TES), piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES), 2-(*N*-morpholino)ethanesulfonic acid (MES), or the like.

[0374] In some embodiments, the composition may include a surfactant. Exemplary surfactants include non-ionic surfactants, anionic surfactants, cationic surfactants, and zwitterionic surfactants. For example, the surfactant may be a non-ionic surfactant such as sorbitan 20.

[0375] In some embodiments, the composition may include a salt, such as sodium chloride, potassium chloride, magnesium chloride, or the like.

[0376] In some embodiments, the composition may include a protein. For example, the composition can include a carrier protein to prevent surface adsorption of luminogenic enzymes that may be added in downstream assays. In some embodiments, the protein may be bovine serum albumin (BSA).

[0377] In some embodiments, the composition may include a substance that reduces autoluminescence. In some embodiments, the substance is ATT (6-Aza-2-thiothymine), a derivative or analog of ATT, a thionucleoside, thiourea, and the like. In some embodiments, the substance is a thionucleoside disclosed in U.S. Patent No. 9,676,997, herein incorporated by reference. In some embodiments, the substance is thiourea, which use for reducing

autoluminescence is disclosed in U.S. Patent Nos. 7,118,878; 7,078,181; and 7,108,996, herein incorporated by reference.

[0378] The composition may be in the form of a lyophilized powder. Such a composition can be prepared by drying a mixture of the components of the composition. For example, the composition can be prepared by dissolving the compound in a solvent (e.g., an organic solvent) to form a first solution, adding the polymer to the first solution to form a second solution, and then drying the second solution to provide the composition. In some embodiments, the drying step may comprise lyophilization. This may provide the composition in the form of a powder. In some embodiments, the drying step may comprise air-drying. This may provide the composition in the form of a malleable disk.

[0379] In some embodiments (e.g., those in which the composition includes a polymer rather than a paper substrate), the composition is in the form of a solution. When the composition is a solution, the composition may have a pH of about 5.5 to about 8.0, e.g., about 6.5 to about 7.5. In some embodiments, the composition has a pH of about 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, or 8.0.

b. Lateral Flow Components

[0380] In some embodiments, the present disclosure provides methods of manufacturing a lateral flow assay platform that includes a conjugate pad, an analytical membrane, a sample pad, and other components necessary for facilitating lateral flow across a membrane (e.g., an absorbent pad). For example, a conjugate pad can include at least one target analyte binding agent reversibly conjugated to the conjugate pad, such that the target analyte binding agent is able to be transferred from the conjugate pad to the analytical membrane when lateral flow is applied, whereupon the target analyte binding agent can bind a target analyte and form a bioluminescent complex. In some embodiments, the target analyte binding agent includes a target analyte binding element to facilitate binding to the target analyte, as well as a bioluminescent polypeptide or component of a bioluminescent complex, such as a bioluminescent polypeptide of SEQ ID NO: 5 (NanoLuc and variants thereof), a non-luminescent (NL) polypeptide of SEQ ID NO: 9 (LgBiT), an NL peptide of SEQ ID NO: 10 (SmBiT), an NL peptide of SEQ ID NO: 11 (HiBiT), an NL polypeptide of SEQ ID NO: 12 (LgTrip-3546), an NL peptide of SEQ ID NO: 13 (SmTrip), an NL peptide of SEQ ID NO: 14 (β9/β10 dipeptide), or variants thereof. In some embodiments, target analyte binding agent

comprises a fluorophore capable of being activated by energy transfer (e.g., from a bioluminescent polypeptide or component of a bioluminescent complex).

[0381] In some embodiments, the conjugate pad comprises a first target analyte binding agent. In some embodiments, the first target analyte binding agent comprises a first target analyte binding element and a first bioluminescent polypeptide or a first component of a bioluminescent complex (e.g., NL peptide or NL polypeptide). In some embodiments, the target analyte binding agent is stored on or within the conjugate pad such that it remains with the conjugate pad until being displaced by lateral from through the device.

[0382] In some embodiments, the conjugate pad comprises a luminogenic substrate, such as coelenterazine, coelenterazine-h, coelenterazine-h-h, furimazine, JRW-0238, , JRW-1404, JRW-1482, JRW-1667, JRW-1743, JRW-1744, other coelenterazine analogs or derivatives, a pro-substrate, and/or other substrates (e.g., coelenterazine analog or derivative) described herein. In some embodiments, the luminogenic substrate is reversibly conjugated to the conjugate pad. In some embodiments, the luminogenic substrate is dried on or within the conjugate pad. In some embodiments, the luminogenic substrate is part of a composition comprising the luminogenic substrate and a polymer selected from pullulan, trehalose, maltose, cellulose, dextran, polystyrene, poly(meth)acrylate, and a combination of any thereof (e.g., described in greater detail above and/or in U.S. Prov. Appln. Serial No. 62/740,622. In some embodiment, the luminogenic substrate is applied as part of a composition or solution, such as a protein buffer. In some embodiment, the protein buffer includes 20mM Na_3PO_4; 5% w/v BSA; 0.25% v/v Tween 20; 10% w/v sucrose. In some embodiments, luminogenic substrate is added to the protein buffer and dried for 1 hour at 37°C onto a substrate or matrix (e.g., filter paper or membrane). In other embodiments, the luminogenic substrate is applied as a separate reagent as part of an assay method or system.

[0383] In some embodiments, the assay platform includes an analytical membrane comprising a detection region and a control region to facilitate the detection of the bioluminescent complex indicating target analyte detection. The detection region can include at least one target analyte binding agent immobilized to the detection region such that it will not be displaced by the application of lateral flow across the membrane. In some embodiments, the analytical membrane includes at least one target analyte binding agent. In some embodiments, the target analyte

binding agent comprises a target analyte binding element and a bioluminescent polypeptide or a first component of a bioluminescent complex (e.g., NL peptide or NL polypeptide).

[0384] In some embodiments, the analytical membrane includes a plurality of detection regions with each detection region comprising a distinct target analyte binding agent comprising distinct target analyte binding elements (e.g., multiplexing capability).

[0385] In some embodiments, the analytical membrane comprises a luminogenic substrate, such as coelenterazine, coelenterazine-h, coelenterazine-h-h, furimazine, JRW-0238, JRW-1404, JRW-1482, JRW-1667, JRW-1743, JRW-1744, other coelenterazine analogs or derivatives, a pro-substrate, or other substrates (e.g., coelenterazine analog or derivative) described herein. In some embodiments, the luminogenic substrate is reversibly conjugated to and/or contained on/within the analytical membrane, for example, as part of a composition comprising the luminogenic substrate and a polymer selected from pullulan, trehalose, maltose, cellulose, dextran, polystyrene, poly(meth)acrylate, and a combination of any thereof. In some embodiment, the luminogenic substrate is applied as part of a composition or solution, such as a protein buffer. In some embodiment, the protein buffer includes 20mM Na_3PO_4; 5% w/v BSA; 0.25% v/v Tween 20; 10% w/v sucrose. In some embodiments, the protein buffer includes 20mM Na_3PO_4; 5% w/v BSA; 0.25% v/v Tween 20; 5% w/v pullulan. In some embodiments, the protein buffer includes 20mM Na_3PO_4; 1-5% w/v BSA; 0.25% v/v Tween 20. In some embodiments, the protein buffer includes 20mM Na_3PO_4; 1-5% w/v Prionex; 0.25% v/v Tween 20. In some embodiments, the protein buffer includes 20mM Na_3PO_4; 1-5% w/v BSA, 5 mM ATT. In some embodiments, the protein buffer includes 20mM Na_3PO_4; 1-5% v/v Prionex, 5 mM ATT. In some embodiments, the protein buffer includes 20mM Na_3PO_4; 1-5% w/v BSA, 5 mM ATT, 5 mM ascorbate. In some embodiments, the protein buffer includes 20mM Na_3PO_4; 1-5% w/v Prionex, 5 mM ATT, 5 mM ascorbate. In some embodiments, the protein buffer includes 20mM Na_3PO_4; 1-5% w/v BSA, 5 mM ATT, 5 mM ascorbate. In some embodiments, the protein buffer includes; 1-5% w/v BSA, 5 mM ATT, 5 mM ascorbate. In some embodiments, luminogenic substrate is added to the protein buffer and dried for 1 hour at 37 °C onto a substrate or matrix (e.g., filter paper or membrane). In other embodiments, the luminogenic substrate is applied as a separate reagent as part of an assay method or system.

c. Solid Phase Components

[0386] In some embodiments, the present disclosure provides methods of manufacturing a solid phase detection platform (e.g., dipstick assay or spot test) that includes a detection region and a control region. In some embodiments, the detection region comprises at least one target analyte binding agent conjugated to the detection region. In some embodiments, the detection region comprises at least one target analyte binding agent that is not conjugated to the detection region. Such a non-conjugated binding agent may be added to the detection region (e.g., with the sample or as part of a detection reagent) or may reside on or within the detection region, without conjugation. In some embodiments, the non-conjugated binding agent comprises a target analyte binding element and bioluminescent polypeptide or component of a bioluminescent complex, such as a bioluminescent polypeptide of SEQ ID NO: 5 (NanoLuc and variants thereof), a non-luminescent (NL) polypeptide of SEQ ID NO: 9 (LgBiT), an NL peptide of SEQ ID NO: 10 (SmBiT), an NL peptide of SEQ ID NO: 11 (HiBiT), an NL polypeptide of SEQ ID NO: 12 (LgTrip-3546), an NL peptide of SEQ ID NO: 13 (SmTrip), an NL peptide of SEQ ID NO: 14 (β9/β10 dipeptide), or variants thereof.

[0387] In some embodiments, the solid phase detection platform includes a plurality of detection regions with each detection region comprising a distinct target analyte binding agent comprising distinct target analyte binding elements (e.g., multiplexing capability). In some embodiments, one or more distinct target analyte binding agents can be conjugated (e.g., coated) to wells of a microtiter plate, along one or more of the other detection reagents required to carry out a particular assay (e.g., a second target analyte binding agent, a luminogenic substrate, assay buffer, etc.). In other embodiments, the detection reagents can be applied as a separate reagent as part of an assay method or system (e.g., as part of a lyocake or tablet and reconstituted as part of the assay).

[0388] The detection platform can also include a luminogenic substrate, such as coelenterazine, coelenterazine-h, coelenterazine-h-h, furimazine, JRW-0238, JRW-1404, JRW-1482, JRW-1667, JRW-1743, JRW-1744, other coelenterazine analogs or derivatives, a pro-substrate, or other substrates (e.g., coelenterazine analog or derivative) described herein. In some embodiments, the luminogenic substrate is reversibly conjugated to the detection region. In some embodiments, the luminogenic substrate is stably stored on or within the detection region. In some embodiments, the luminogenic substrate is part of a composition comprising the

luminogenic substrate and a polymer selected from pullulan, trehalose, maltose, cellulose, dextran, polystyrene, poly(meth)acrylate, and a combination of any thereof. In some embodiments, the luminogenic substrate is applied as part of a composition or solution, such as a protein buffer, detection reagent, or with the sample. In some embodiments, the protein buffer includes 20mM Na_3PO_4; 5% w/v BSA; 0.25% v/v Tween 20; 10% w/v sucrose. In some embodiments, luminogenic substrate is added to the protein buffer and dried for 1 hour at 37°C onto a substrate or matrix (e.g., filter paper, membrane, individual wells of a microtiter plate). In other embodiments, the luminogenic substrate is applied as a separate reagent as part of an assay method or system (e.g., as part of a lyocake or tablet and reconstituted as part of the assay).

[0389] Embodiments of the present disclosure also include methods for producing a substrate or matrix for use in a bioluminescent assay. In accordance with these embodiments, the method includes generating a solution or liquid formulation containing at least one target analyte binding agent comprising a target analyte binding element and one of a polypeptide component of a bioluminescent complex or a peptide component of a bioluminescent complex. In some embodiments, the solution includes a protein buffer and at least one excipient, including but not limited to, a surfactant, a reducing agent, a salt, a radical scavenger, a chelating agent, a protein, or any combination thereof. In some embodiment, the solution includes a complementary peptide or polypeptide component of the bioluminescent complex, such that the target analyte binding agent and the complementary peptide or polypeptide component of the bioluminescent complex form a bioluminescent analyte detection complex in the presence of a target analyte. In some embodiments, the solution comprises a luminogenic substrate.

[0390] After generating the solution or liquid formulation, the method includes applying the solution to the surface of a substrate or matrix. In some embodiments, the substrate or matrix is W-903 paper, FTA paper, FTA Elute paper, FTA DMPK paper, Ahlstrom A-226 paper, M-TFN paper, FTA paper, FP705 paper, Bode DNA collection paper, nitrocellulose paper, nylon paper, cellulose paper, Dacron paper, cotton paper, and polyester papers, or combinations thereof. In other embodiments, the substrate or matrix is a mesh comprising plastic, nylon, metal, or combinations thereof.

[0391] Embodiments of the method also include drying the substrate or matrix after the solution has been applied to the substrate or matrix. In some embodiments, drying the substrate or matrix containing the solution comprises drying the substrate or matrix at a temperature from

about 30°C to 65°C, from about 30°C to 60°C, from about 30°C to 55°C, from about 30°C to 50°C, from about 30°C to 45°C, or from about 30°C to 40°C. In some embodiments, the matrix or substrate is dried from about 15 mins to 8 hours, from about 30 mins to 7 hours, from about 45 mins to 6 hours, from about 1 hour to 5 hours, from about 2 hours to 4 hours, from about 30 mins to 2 hours, or from about 30 mins to 1 hour. In some embodiments, drying the substrate containing the solution comprises lyophilizing and/or freezing the substrate.

[0392] In some embodiments, the method includes drying the at least one target analyte binding agent and/or the complementary peptide or polypeptide component of the bioluminescent complex onto a first substrate, and drying the luminogenic substrate onto a second substrate. In some embodiments, the at least one target analyte binding agent and/or the complementary peptide or polypeptide component of the bioluminescent complex are dried onto a paper based substrate, and the luminogenic substrate is dried onto a mesh (see, e.g., FIGS. 42A-42E).

[0393] In accordance with these embodiments, the substrate or matrix can be used in a bioluminescent assay to detect a target analyte. For example, a bioluminescent signal can be generated upon exposure of the substrate or matrix containing the solution to the target analyte. In some embodiments, the bioluminescent signal is proportional to the concentration of the target analyte. In some embodiments, the at least one target analyte binding agent and/or the complementary peptide or polypeptide component of the bioluminescent complex exhibit(s) enhanced stability when dried on the substrate, as described further herein.

d. Solution Phase Components

[0394] In some embodiments, the present disclosure provides methods of manufacturing a solution phase detection platform (as described herein) that includes one or more detection regions and control regions (e.g., wells of a 96-well microtiter plate). For example, as shown in FIG. 33, embodiments of solution phase platforms of the present disclosure can include one or more components of the bioluminescent complexes described herein in a tablet or lyophilized cake that can be reconstituted in a solution (e.g., buffered solution) to facilitate analyte detection. In some embodiments, the tablet or lyocake can include all the reagents necessary to carry out a reaction to detect an analyte and are included as part of a solution phase detection platform (e.g., present in one or more wells of a 96-well microtiter plate). Such lyocakes or tablets are compatible with many different assay formats, including but not limited to, cuvettes, wells of

microtiter plates (e.g., 96-well microtiter plate), test tubes, large volume bottles, SNAP assays, and the like.

[0395] In some embodiments, one or more components of the bioluminescent complexes described herein can be added to a detection region and/or may already be present within a detection region, in the presence or absence of a sample. The detection reagents can then be reconstituted (e.g., rehydrated) as part of carrying out the detection of an analyte in the sample. In some embodiments, the detection reagent comprises a target analyte binding element and bioluminescent polypeptide or component of a bioluminescent complex, such as a bioluminescent polypeptide of SEQ ID NO: 5 (NanoLuc and variants thereof), a non-luminescent (NL) polypeptide of SEQ ID NO: 9 (LgBiT), an NL peptide of SEQ ID NO: 10 (SmBiT), an NL peptide of SEQ ID NO: 11 (HiBiT), an NL polypeptide of SEQ ID NO: 12 (LgTrip-3546), an NL peptide of SEQ ID NO: 13 (SmTrip), an NL peptide of SEQ ID NO: 14 (β9/β10 dipeptide), or variants thereof.

[0396] The solution phase detection platform can also include a luminogenic substrate, such as coelenterazine, coelenterazine-h, coelenterazine-h-h, furimazine, JRW-0238, JRW-1404, JRW-1482, JRW-1667, JRW-1743, JRW-1744, other coelenterazine analogs or derivatives, a pro-substrate, or other substrates (e.g., coelenterazine analog or derivative) described herein. In some embodiments, the luminogenic substrate is part of a composition comprising the luminogenic substrate and a polymer selected from pullulan, trehalose, maltose, cellulose, dextran, polystyrene, poly(meth)acrylate, and a combination of any thereof. In some embodiments, the luminogenic substrate is applied as part of a composition or solution, such as a protein buffer, detection reagent, or with the sample. In some embodiments, the luminogenic substrate is applied as a separate reagent as part of an assay method or system, and in other embodiments, it is part of a lyocake or tablet that includes one or more detection reagents.

6. Target Analytes

[0397] Embodiments of the present disclosure find use in the detection/quantification of target analytes and include target analyte binding agents capable of binding to or interacting with a target analyte via a target analyte binding element. In some embodiments, target analyte binding agents include target analyte binding elements capable of binding a group or class of analytes (e.g., protein L binding generally to antibodies), such binding elements may be referred to herein as “non-specific” or the like; in other embodiments, target analyte binding agents include target

analyte binding elements capable of binding a specific analyte (e.g., an antigen binding a monoclonal antibody), such binding elements may be referred to herein as "target specific" or the like.

[0398] In some embodiments, target analyte binding agents and corresponding target analyte binding elements are generated to detect one or more analytes associated with a disease state or environmental condition. Target analyte binding elements can be independently selected from the group consisting of an antibody (e.g., polyclonal, monoclonal, and/or recombinant), antibody fragment (e.g., Fab, Fab', $F(ab')_2$, Fv, scFv, Fd, variable light chain, variable heavy chain, diabodies, scFv, etc.), protein A, an Ig binding domain of protein A, protein G, an Ig binding domain of protein G, protein A/G, an Ig binding domain of protein A/G, protein L, a Ig binding domain of protein L, protein M, an Ig binding domain of protein M, an oligonucleotide probe, a peptide nucleic acid, a DARPin, an aptamer, an affimer, a purified protein (e.g., either the analyte itself or a protein that binds to the analyte), and analyte binding domain(s) of proteins.

[0399] In some embodiments, target analyte binding elements comprise an antigen or epitope recognized by an antibody (the target analyte) such as an antibody generated by a subject in response to an immunogenic reaction to a pathogen, which can indicate that the subject is infected with the pathogen. In some embodiments, the target analyte is an antibody against Zika virus, Dengue virus, West Nile virus, Yellow Fever virus, and/or Chikungunya virus, and the target analyte binding element is an immunogenic epitope specifically recognized by the antibody. In some embodiments, the target analyte is an antibody against Hep A, B, C, D or E. In some embodiments, the target analyte is an antibody against Mumps, measles, Rubella, RSV, EBV, Herpes, Influenza, Varicella-Zoster, prenatal Zika, or parainfluenza type 1, 2, or 3. In some embodiments, the target analyte is an antibody against Arbovirus, HIV, prenatal Hepatitis, CMV, Hantavirus, polio virus, of parvovirus. In some embodiments, the target analyte is an antibody against Tick borne disease (e.g., Lyme disease). In some embodiments, the target analyte is an antibody against Bordetella pertussis, pneumococcus, chlamydia, streptococcus, M. pneumoniea, S. pneumonie, shigella producing bacteria, E.coli, Enterobacter, syphilis, gonorrhea. In some embodiments, the target analyte is an autoantibody against ANA, Cardiolipin, celiac disease, insulin, GAD65, IA-2, Reticulin, Thyroglobulin, RNP, cytoplasmic neutrophil, thyrptropin receptor, thyroperoxidase, platelet antibody, PLAR2, myocardial, GBM, tissue transglutaminase, or thyroid stimulating. In some embodiments, the target analyte is a toxin or an antibody against

a toxin (e.g., diptheria, tetanus). In some embodiments, the target analyte is from a parasite or an antibody against a parasite (e.g., trichinella, trichinosis, trypanosoma cruzi, Toxoplasma gondii). In some embodiments, the target analyte is a therapeutic biologic or an antibody against the therapeutic biologic (Vedolizumab, Adalimumab, infliximab, certilizumab, entanercept, Opdivo, Keytruda, ipilimumab, Ustekinumab, secukinumab, guselkumab, Tocilizumab, rituximab, panitumumab, trastuzumab, cetuximab, ofatumumab, eptratuzumab, abatacept, tofacitinib).

[0400] Other target analytes include known biomarkers associated with a pathogenic organism, such as a virus, bacterium, protozoa, prion, fungus, parasitic nematode, or other microorganism. Disease biomarkers can include markers of the pathogenic organism itself and/or markers of a subject's reaction to an infection by the pathogenic organism. Diseases that can be detected using the assays and methods of the present disclosure include any of the following: Acinetobacter infections (Acinetobacter baumannii), Actinomycosis (Actinomyces sraelii, Actinomyces gerencseriae and Propionibacterium propionicus), African sleeping sickness or African trypanosomiasis (Trypanosoma brucei), AIDS (HIV), Amebiasis (Entamoeba histolytica), Anaplasmosis (Anaplasma species), Angiostrongyliasis (Angiostrongylus), Anisakiasis (Anisakis), Anthrax (Bacillus anthracis), Arcanobacterium haemolyticum infection (Arcanobacterium haemolyticum), Argentine Teagan fever (Junin virus), Ascariasis (Ascaris lumbricoides), Aspergillosis (Aspergillus species), Astrovirus infection (Astroviridae family), Babesiosis (Babesia species), Bacillus cereus infection (Bacillus cereus), Bacterial pneumonia (multiple bacteria), Bacteroides infection (Bacteroides species), Balantidiasis (Balantidium coli), Bartonellosis (Bartonella), Baylisascaris infection (Baylisascaris species), BK virus infection (BK virus), Black Piedra (Piedraia hortae), Blastocystosis (Blastocystis species), Blastomycosis (Blastomyces dermatitidis), Bolivian hemorrhagic fever (Machupo virus), Brazilian hemorrhagic fever (Sabiá virus), Brucellosis (Brucella species), Bubonic plague (Yersinia Pestis), Burkholderia infection (usually Burkholderia cepacia and other Burkholderia species), Buruli ulcer (Mycobacterium ulcerans), Calicivirus infection (Caliciviridae family), Campylobacteriosis (Campylobacter species), Candidiasis (usually Candida albicans and other Candida species), Carrion's disease (Bartonella bacilliformis), Cat-scratch disease (Bartonella henselae), Cellulitis (usually Group A Streptococcus and Staphylococcus), Chagas Disease (Trypanosoma cruzi), Chancroid (Haemophilus ducreyi), Chickenpox (Varicella zoster virus or VZV), Chikungunya (Alphavirus), Chlamydia (Chlamydia trachomatis), Cholera (Vibrio cholerae),

Chromoblastomycosis (usually Fonsecaea pedrosoi), Chytridiomycosis (Batrachochytrium dendrabatidis), Clonorchiasis (Clonorchis sinensis), Clostridium difficile colitis (Clostridium difficile), Coccidioidomycosis (Coccidioides immitis and Coccidioides posadasii), Colorado tick fever (Colorado tick fever virus or CTFV), Common cold (usually rhinoviruses and coronaviruses), Creutzfeldt–Jakob disease (PRNP), Crimean-Congo hemorrhagic fever (Crimean-Congo hemorrhagic fever virus), Cryptococcosis (Cryptococcus neoformans), Cryptosporidiosis (Cryptosporidium species), Cutaneous larva migrans (usually Ancylostoma braziliense; multiple other parasites), Cyclosporiasis (Cyclospora cayetanensis), Cysticercosis (Taenia solium), Cytomegalovirus infection (Cytomegalovirus), Dengue fever (Dengue viruses: DEN-1, DEN-2, DEN-3 and DEN-4), Desmodesmus infection (Green algae Desmodesmus armatus), Dientamoebiasis (Dientamoeba fragilis), Diphtheria (Corynebacterium diphtheriae), Diphyllobothriasis (Diphyllobothrium), Dracunculiasis (Dracunculus medinensis), Ebola hemorrhagic fever (Ebolavirus or EBOV), Echinococcosis (Echinococcus species), Ehrlichiosis (Ehrlichia species), Enterobiasis (Enterobius vermicularis), Enterococcus infection (Enterococcus species), Enterovirus infection (Enterovirus species), Epidemic typhus (Rickettsia prowazekii), Erythema infectiosum (Parvovirus B19), Exanthem subitum (Human herpesvirus 6 or HHV-6; Human herpesvirus 7 or HHV-7), Fasciolasis (Fasciola hepatica and Fasciola gigantica), Fasciolopsiasis (Fasciolopsis buski), Fatal familial insomnia (PRNP), Filariasis (Filarioidea superfamily), Fusobacterium infection (Fusobacterium species), Gas gangrene (usually Clostridium perfringens; other Clostridium species), Geotrichosis (Geotrichum candidum), Gerstmann-Sträussler-Scheinker syndrome (PRNP), Giardiasis (Giardia lamblia), Glanders (Burkholderia mallei), Gnathostomiasis (Gnathostoma spinigerum and Gnathostoma hispidum), Gonorrhea (Neisseria gonorrhoeae), Granuloma inguinale (Klebsiella granulomatis), Group A streptococcal infection (Streptococcus pyogenes), Group B streptococcal infection (Streptococcus agalactiae), Haemophilus influenzae infection (Haemophilus influenzae), Hand, foot and mouth disease (Enteroviruses, mainly Coxsackie A virus and Enterovirus 71 or EV71), Hantavirus Pulmonary Syndrome (Sin Nombre virus), Heartland virus disease (Heartland virus), Helicobacter pylori infection (Helicobacter pylori), Hemolytic-uremic syndrome (Escherichia coli O157:H7, O111 and O104:H4), Hemorrhagic fever with renal syndrome (Bunyaviridae family), Hepatitis A (Hepatitis A virus), Hepatitis B (Hepatitis B virus), Hepatitis C (Hepatitis C virus), Hepatitis D (Hepatitis D Virus), Hepatitis E (Hepatitis E virus), Herpes simplex (Herpes

simplex virus 1 and 2 (HSV-1 and HSV-2)), Histoplasmosis (Histoplasma capsulatum), Hookworm infection (Ancylostoma duodenale and Necator americanus), Human bocavirus infection (Human bocavirus or HBoV), Human ewingii ehrlichiosis (Ehrlichia ewingii), Human granulocytic anaplasmosis (Anaplasma phagocytophilum), Human metapneumovirus infection (Human metapneumovirus or hMPV), Human monocytic ehrlichiosis (Ehrlichia chaffeensis), Human papillomavirus (HPV) infection (Human papillomavirus or HPV), Human parainfluenza virus infection (Human parainfluenza viruses or HPIV), Hymenolepiasis (Hymenolepis nana and Hymenolepis diminuta), Epstein–Barr virus infectious mononucleosis (Epstein–Barr virus or EBV), Influenza (Orthomyxoviridae family), Isosporiasis (Isospora belli), Kingella kingae infection (Kingella kingae), Kuru (PRNP), Lassa fever (Lassa virus), Legionellosis (Legionella pneumophila), Legionellosis (Legionella pneumophila), Leishmaniasis (Leishmania species), Leprosy (Mycobacterium leprae and Mycobacterium lepromatosis), Leptospirosis (Leptospira species), Listeriosis (Listeria monocytogenes), Lyme disease (Borrelia burgdorferi, Borrelia garinii, and Borrelia afzelii), Lymphatic filariasis (Wuchereria bancrofti and Brugia malayi), Lymphocytic choriomeningitis (Lymphocytic choriomeningitis virus or LCMV), Malaria (Plasmodium species), Marburg hemorrhagic fever (Marburg virus), Measles (Measles virus), Middle East respiratory syndrome (Middle East respiratory syndrome coronavirus), Melioidosis (Burkholderia pseudomallei), Meningococcal disease (Neisseria meningitidis), Metagonimiasis (usually Metagonimus yokagawai), Microsporidiosis (Microsporidia phylum), Molluscum contagiosum (Molluscum contagiosum virus or MCV), Monkeypox (Monkeypox virus), Mumps (Mumps virus), Murine typhus (Rickettsia typhi), Mycoplasma pneumonia (Mycoplasma pneumoniae), Mycetoma (numerous species of bacteria (Actinomycetoma) and fungi (Eumycetoma)), Myiasis (parasitic dipterous fly larvae), Neonatal conjunctivitis (most commonly Chlamydia trachomatis and Neisseria gonorrhoeae), Norovirus (Norovirus), Nocardiosis (usually Nocardia asteroides and other Nocardia species), Onchocerciasis (Onchocerca volvulus), Opisthorchiasis (Opisthorchis viverrini and Opisthorchis felineus), Paracoccidioidomycosis (Paracoccidioides brasiliensis), Paragonimiasis (usually Paragonimus westermani and other Paragonimus species), Pasteurellosis (Pasteurella species), Pediculosis capitis (Pediculus humanus capitis), Pediculosis corporis (Pediculus humanus corporis), Pediculosis pubis (Phthirus pubis), Pertussis (Bordetella pertussis), Plague (Yersinia pestis), Pneumococcal infection (Streptococcus pneumoniae), Pneumocystis pneumonia (Pneumocystis

jirovecii), Pneumonia (multiple causes), Poliomyelitis (Poliovirus), Prevotella infection (Prevotella species), Primary amoebic meningoencephalitis (usually Naegleria fowleri), Progressive multifocal leukoencephalopathy (JC virus), Psittacosis (Chlamydophila psittaci), Q fever (Coxiella burnetiid), Rabies (Rabies virus), Relapsing fever (Borrelia hermsii, Borrelia recurrentis, and other Borrelia species), Respiratory syncytial virus infection (Respiratory syncytial virus (RSV)), Rhinosporidiosis (Rhinosporidium seeberi), Rhinovirus infection (Rhinovirus), Rickettsial infection (Rickettsia species), Rickettsialpox (Rickettsia akari), Rift Valley fever (Rift Valley fever virus), Rocky Mountain spotted fever (Rickettsia rickettsia), Rotavirus infection (Rotavirus), Rubella (Rubella virus), Salmonellosis (Salmonella species), Severe Acute Respiratory Syndrome (SARS coronavirus), Scabies (Sarcoptes scabiei), Scarlet fever (Group A Streptococcus species), Schistosomiasis (Schistosoma species), Sepsis (multiple causes), Shigellosis (Shigella species), Shingles (Varicella zoster virus or VZV), Smallpox (Variola major or Variola minor), Sporotrichosis (Sporothrix schenckii), Staphylococcal food poisoning (Staphylococcus species), Staphylococcal infection (Staphylococcus species), Strongyloidiasis (Strongyloides stercoralis), Subacute sclerosing panencephalitis (Measles virus), Syphilis (Treponema pallidum), Taeniasis (Taenia species), Tetanus (Clostridium tetani), Tinea barbae (usually Trichophyton species), Tinea capitis (usually Trichophyton tonsurans), Tinea corporis (usually Trichophyton species), Tinea cruris (usually Epidermophyton floccosum, Trichophyton rubrum, and Trichophyton mentagrophytes), Tinea manum (Trichophyton rubrum), Tinea nigra (usually Hortaea werneckii), Tinea pedis (usually Trichophyton species), Tinea unguium (usually Trichophyton species), Tinea versicolor (Malassezia species), Toxocariasis (Toxocara canis or Toxocara cati), Toxocariasis (Toxocara canis or Toxocara cati), Toxoplasmosis (Toxoplasma gondii), Trachoma (Chlamydia trachomatis), Trichinosis (Trichinella spiralis), Trichomoniasis (Trichomonas vaginalis), Trichuriasis (Trichuris trichiura), Tuberculosis (usually Mycobacterium tuberculosis), Tularemia (Francisella tularensis), Typhoid fever (Salmonella enterica subsp. enterica, serovar typhi), Typhus fever (Rickettsia), Ureaplasma urealyticum infection (Ureaplasma urealyticum), Valley fever (Coccidioides immitis or Coccidioides posadasii), Venezuelan equine encephalitis (Venezuelan equine encephalitis virus), Venezuelan hemorrhagic fever (Guanarito virus), Vibrio vulnificus infection (Vibrio vulnificus), Vibrio parahaemolyticus enteritis (Vibrio parahaemolyticus), Viral pneumonia (multiple viruses), West Nile Fever (West Nile virus), White piedra (Trichosporon beigelii), Yersinia

pseudotuberculosis infection (Yersinia pseudotuberculosis), Yersiniosis (Yersinia enterocolitica), Yellow fever (Yellow fever virus), Zygomycosis (Mucorales order (Mucormycosis) and Entomophthorales order (Entomophthoramycosis)), and Zika fever (Zika virus).

7. Methods of Detecting, Quantifying, and Diagnosing

[0401] Embodiments of the present disclosure include methods of detecting and/or quantifying a target analyte in a sample with an assay platform (e.g., solid phase detection platform or lateral flow assay) that uses bioluminescent polypeptides or bioluminescent complexes (and components thereof; e.g., non-luminescent peptide or polypeptides) for target analyte detection. Embodiments also include methods of diagnosing a disease state or evaluating an environmental condition based on detecting and/or quantifying a target analyte in a sample.

[0402] In some embodiments, a method of detecting an analyte in a sample includes using a lateral flow assay system or a solid phase detection platform as described herein. In accordance with these embodiments, the method includes applying a sample to a sample pad; facilitating flow of the sample from the sample pad to a conjugate pad, and then from the conjugate pad to a detection region and a control region on an analytical membrane. The method can include a first target analyte binding agent, a second target analyte binding agent, and a target analyte that form an analyte detection complex in the at least one detection region when the target analyte is detected in the sample. In some embodiments, methods comprise one or more steps of: sample addition, reagent (e.g., detection reagent) addition, washing, waiting, etc.

[0403] In some embodiments, the sample is a biological sample from a subject, such as blood, serum, plasma, urine, stool, cerebral spinal fluid, interstitial fluid, and saliva. In other embodiments, the sample is a sample from a natural or industrial environment, such as a water sample, a soil sample, a plant sample, a food sample, a beverage sample, an oil, and an industrial fluid sample. The method includes detecting the target analyte in the sample by detecting a bioluminescent signal generated from the analyte detection complex. In some embodiments, the target analyte in the sample is quantified based on the bioluminescent signal generated from the analyte detection complex. In some embodiments, the method includes diagnosing a subject from which the sample was obtained as having or not having a disease based on the detection of the analyte.

8. Competition

[0404] Some embodiments herein utilize competition between a labeled analyte and a target analyte in a sample to detect/quantify the target analyte in a sample. Exemplary embodiments comprise the use of (i) an analyte (e.g., identical or similar to the target analyte) labeled with detectable element described herein (e.g., NanoLuc®-based technology (e.g., NanoLuc, NanoBiT, NanoTrip, NanoBRET, or components (e.g., peptides, polypeptides, etc.) of variants thereof)), and (ii) a binding moiety for the target analyte (e.g., fused or linked to a second detectable element described herein (e.g., NanoLuc®-based technology (e.g., NanoLuc, NanoBiT, NanoTrip, NanoBRET, or components (e.g., peptides, polypeptides, etc.) of variants thereof)). In the absence of the target analyte from a sample, the detectable elements produce a detectable signal (e.g., via complementation between the detectable elements, via BRET, etc.) is produced by the system. When the system is exposed to a sample (e.g., biological sample, environmental sample, etc.), the bioluminescent signal is reduced if the target analyte is present in the sample (the labeled analyte will be competed out of the complex).

[0405] Various embodiments herein utilize such competition immunoassays for small molecule detection. In some embodiments, the target small molecule is a toxin (e.g., mycotoxin, etc.), metabolite (e.g., amino acid, glucose molecule, fatty acid, nucleotide, cholesterol, steroid, etc.), vitamin (e.g., vitamin A, vitamin B1, vitamin B2, Vitamin B3, vitamin B5, vitamin B7, vitamin B9, vitamin B12, vitamin C, vitamin D, vitamin E, vitamin H or vitamin K, etc.), coenzyme or cofactor (e.g., coenzyme A, coenzyme B, coenzyme M, coenzyme Q, cytidine triphosphate, acetyl coenzyme A, reduced nicotinamide adenine dinucleodtide (NADH), nicotinamide adenine (NAD+), nucleotide adenosine monophosphoate, nucleotide adenosine triphosphate, glutathione, heme, lipoamide, molybdopterin, 3'-phosphoadenosine-5'-phsphosulfate, pyrroloquinoline quinone, tetrahydrobiopterin, etc.), biomarker or antigen (e.g., erythropoietin (EPO), ferritin, folic acid, hemoglobin, alkaline phosphatase, transferrin, apolipoprotein E, CK, CKMB, parathyroid hormone, insulin, cholesteryl ester transfer protein (CETP), cytokines, cytochrome c, apolipoprotein AI, apolipoprotein AII, apolipoprotein BI, apolipoprotein B-100, apolipoprotein B48, apolipoprotein CII, apolipoprotein CIII, apolipoprotein E, triglycerides, HD cholesterol, LDL cholesterol, lecithin cholesterol acyltransferase, paraxonase, alanine aminotransferase (ALT), asparate transferase (AST), CEA, HER-2, bladder tumor antigen, thyroglobulin, alpha-fetoprotein, PSA, CA 125, CA 19.9, CA

15.3, leptin, prolactin, osteoponitin, CD 98, fascin, troponin I, CD20, HER2, CD33, EGFR, VEGFA, etc.), drug (cannabinoid (e.g., tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN), etc.), opioid (e.g., heroin, opium, fentanyl, etc.), stimulant (e.g., cocaine, amphetamine, methamphetamine, etc.), club drug (e.g., MDMA, flunitrazepam, gama-hydroxybutyrate, etc.), dissociative drug (e.g., ketamine, phencyclidine, salvia, dextromethorphan, etc.), hallucinogens (e.g., LSD, mescaline, psilocybin, etc.), etc.), explosive (e.g., 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), pentaerythritol tetranitrate (PETN), etc.), toxic chemical (e.g., tabun (GA), sarin (GB), soman (GD), cyclosarin (GF), 2-(dimethylamino)ethyl N, N-dimethylphosphoramidofluroidate (GV), VE, VG, VM, VP, VR, VS, or VX nerve agent), etc.

[0406] In some embodiments, small molecule detection immunoassays, such as the one exemplified in Example 5 and the like, are performed in the solid phase, lateral flow, and other assays and devices described herein.

9. Examples

[0407] It will be readily apparent to those skilled in the art that other suitable modifications and adaptations of the methods of the present disclosure described herein are readily applicable and appreciable and may be made using suitable equivalents without departing from the scope of the present disclosure or the aspects and embodiments disclosed herein. Having now described the present disclosure in detail, the same will be more clearly understood by reference to the following examples, which are merely intended only to illustrate some aspects and embodiments of the disclosure and should not be viewed as limiting to the scope of the disclosure. The disclosures of all journal references, U.S. patents, and publications referred to herein are hereby incorporated by reference in their entireties.

[0408] The present disclosure has multiple aspects, illustrated by the following non-limiting examples.

Example 1

Solid Phase Materials

[0409] As shows in FIG. 3, components of the bioluminescent complexes of the present disclosure produce detectable bioluminescence after being applied to a solid support substrate (e.g., membrane). Antibodies labeled with NanoLuc® components (e.g., target analyte binding

agents) were applied to a membrane that was either blocked (Buffer 1; upper two membranes on left and right panels) or unblocked (Buffer 2; lower two membranes on left and right panels) and then dried at room temperature with nitrogen or at 37°C without nitrogen. Using an Imagequant LAS4000 imaging platform (1 second exposure), detectable bioluminescence was produced under these conditions. These results demonstrate that components of the bioluminescent complexes of the present disclosure can be successfully used in solid phase and lateral flow assay platforms, which may involve drying reagents and application to solid phase materials, and exposure to various temperatures and processing conditions.

[0410] As shown in FIG. 4, components of the bioluminescent complexes produce detectable bioluminescence after being applied to membrane and paper-based solid support matrices. Compositions that included buffer, substrate (e.g., furimazine), and two complementary components of a bioluminescent complex (e.g., HiBiT and LgBiT) were applied to a nitrocellulose membrane (left three panels), or filter paper (Whatman 541 shown in the middle three panels; Whatman 903 shown in right three panels). These components were then dried, shipped at 4°C and then tested 24 hours later using an LAS4000 imaging platform (30 second and 5 min exposures). Detectable bioluminescence was produced under these conditions, with filter paper matrices allowing for brighter bioluminescent signal than nitrocellulose membranes. Matrices made with glass and synthetic fibers (e.g., Ahlstrom grade 8950) also yielded detectable bioluminescent signal (data not shown) demonstrating that components of the bioluminescent complexes of the present disclosure can be successfully used with various matrix materials.

Example 2

Detecting Target Analytes with Bioluminescent Complexes

[0411] As shown in FIG. 5, components of the bioluminescent complexes (e.g., non-luminescent peptides and polypeptides) of the present disclosure can be used as target analyte binding agents for target analyte recognition. For example, as shown in FIG. 5 (left panel), polyclonal goat anti-mouse IgG3 antibodies (e.g., target analyte binding elements) were conjugated to components of the bioluminescent complexes (e.g., LgBiT and SmBiT). In the presence of the target analyte (e.g., mouse IgG3), a bioluminescent complex was formed, and a bioluminescent signal was produced from the complementary interaction of the components of the bioluminescent complex (FIG. 5, right panel) with increased signal being produced as the

concentration of the target analyte increased. These results demonstrate the feasibility of detecting target analytes using the components of the bioluminescent complexes of the present disclosure.

[0412] As shown in FIG. 6, embodiments of the present disclosure include a solid phase assay platform using components of the bioluminescent complexes as target analyte binding agents for target analyte recognition. Four test spots were prepared on Whatman 903 filter paper as shown, and target analyte was added thereafter (FIG. 6, top panel). In one embodiment, 20 ng of goat-anti-mouse-conjugated to a component of the bioluminescent complex (e.g., SmBiT), and 20 ng of goat-anti-mouse-conjugated to a complementary component of the bioluminescent complex (e.g., LgBiT) were each prepared in 5 µl of protein buffer (20mM Na_3PO_4; 5% w/v BSA; 0.25% v/v Tween 20; 10% w/v sucrose) and dried for 1 hour at 37°C onto the paper in the locations indicated. Additionally, 5 µl of a 5 mM solution of furimazine in ethanol was applied to the spots as indicated under high vacuum for 15 mins (FIG. 6, top panel). The prepared spots were then stored for one week at 4°C. As demonstrated, in the presence of the target analyte (e.g., mouse IgG3; spot #2), a bioluminescent complex was formed, and a bioluminescent signal was produced from the complementary interaction of the components of the bioluminescent complex (FIG. 6, bottom panel). Although background bioluminescent signal was produced with no target analyte present (spot #4), the signal produced in the presence of the target analyte and the luminogenic substrate (e.g., furimazine) is substantially increased as compared to the signal produced with the luminogenic substrate alone (compare spots #2 and #4).

[0413] Additional tests of substrate and protein stability were performed and are depicted in FIGS. 7A-7E. These tests were performed as described above with the additional step of adding a fully functional bioluminescent complex (e.g., NanoLuc) after the addition of the target analyte to test luminogenic substrate stability. As demonstrated in FIGS. 7A-7C, components of the bioluminescent complex lose activity when stored at higher temperatures (e.g., 37°C) for two weeks. The loss of bioluminescent signal does not appear to be due to instability or breakdown of the luminogenic substrate, as the addition of a fully functional bioluminescent complex (e.g., NanoLuc) still produced a signal (FIG. 7D). Additionally, to test whether breakdown of one or more components of the bioluminescent complex was responsible for the reduced bioluminescent signal, a non-antibody conjugated component (e.g., HiBiT) was added that was not subject to storage conditions. As demonstrated in FIG. 7E, addition of the non-antibody

conjugated component led to the production of a bioluminescent signal at 4°C but not 37°C, thus indicating that the degradation of the complementary component of the bioluminescent complex (e.g., LgBiT) was likely leading to the loss of signal.

[0414] Additional tests of storage conditions were performed and are depicted in FIGS. 8A-8B. These tests were performed as described above, except that the test spots were stored for a total of 3 months. As shown in FIG. 8A, detectable bioluminescent signal was produced in the presence of the target analyte at both 4°C and 25°C even after 3 months of storage, albeit with somewhat reduced activity. The addition of a fully functional bioluminescent complex (e.g., NanoLuc) produced a signal (FIG. 8B), but the signal appeared to be dependent upon the use of protein buffer (compare spots #1 and #2) suggesting that the luminogenic substrate is stabilized by the protein buffer.

Example 3

Detecting Target Analytes in Complex Sampling Environments

[0415] FIGS. 9A-9C include representative images from a solid phase assay platform (e.g., spot test) testing whether bioluminescent complex formation and analyte detection could occur in complex sampling environments. As shown in FIG. 9A, a luminogenic substrate and two complementary components of a bioluminescent complex (HiBiT and LgBiT) were applied to Whatman 903 filter paper, with each component also having a target analyte-binding element (polyclonal anti-mouse IgM), as described above, and stored at 4°C for 6 weeks. An EDTA-collected whole blood sample (FIG. 9B) and a 100% serum sample (FIG. 9C) were each spiked with 10 pg mouse IgG3 (target analyte) and applied to the spots indicated in FIG. 9A. Corresponding control samples were not spiked with mouse IgG3. These results demonstrate the feasibility of detecting target analytes in complex sampling environments using the components of the bioluminescent complexes of the present disclosure.

Example 4

Qualitative and Quantitative Assessment

[0416] FIGS. 10A-10B include representative results of a solid phase assay demonstrating that bioluminescent signal can be both quantitatively (FIG. 10A) and qualitatively (FIG. 10B) assessed. As shown in FIG. 10A, 10 μM of luminogenic substrate (e.g., furimazine) was applied to filter paper and placed in a microtiter plate. PBS assay buffer containing NanoLuc® enzyme

was then added, and bioluminescent signal was quantitatively (FIG. 10A, right panel) and qualitatively assessed (FIG. 10B). Additionally, bioluminescent signal was effectively assessed using a luminometer (FIG. 10B, left panel) as well as a smart phone (FIG. 10B, right panel).

[0417] These results demonstrate that the assays and methods of the present disclosure can include comparing levels of bioluminescence corresponding to target analyte detection with various control samples to facilitate rapid quantitative and qualitative assessment. For example, assay formats can include a plurality of control samples with varying concentrations of target analyte that can act as standards against which test samples can be assessed.

[0418] In accordance with these methods, a bioluminescent signal can be assessed both quantitatively and qualitatively using a high affinity dipeptide capable of forming a bioluminescent complex with LgBiT or LgTrip. The results shown in FIGS. 11A-11B include representative graphs (RLUs in FIG. 11A; S/B in FIG. 11B) demonstrating the ability of a high affinity dipeptide, pep263, to form bioluminescent complexes with LgBiT and LgTrip. The high affinity dipeptide pep263 comprises the β9 and β10 stands of the NanoTrip complex. (See, e.g., U.S. Pat. App. 16/439,565 (PCT/US2019/036844), and U.S. Prov. Appln. Serial No. 62/941,255, both of which are herein incorporated by reference in their entirety.)

[0419] Additionally, FIG. 12 shows representative results of a solid phase assay demonstrating qualitative assessment of bioluminescence from paper punches placed into a standard microtiter plate using a standard camera from an iPhone or from an imager (e.g., LAS4000). This spot test assay assessed the functional stability of different LgBiT components dried onto Whatman 903 paper. Whatman 903 protein saver spot cards (1/8" punches) were used along with the following protein buffer: 20 mM Na_3PO_4, 5% w/v BSA, 0.25% v/v tween20, 10% w/v sucrose. A 1000x NanoLuc® stock solution was diluted 1:1000 in protein buffer. About 5 µL of this reaction solution was applied to Spot 1. For HT-LgBiT complexes, about 5 µL of 106.8 nM protein per spot was used. About 20 µM stock protein was diluted 1:100 in protein buffer. About 534 µL stock was diluted in 466 µL in protein buffer. About 5 µL of this conjugation solution was added to Spot 2. For LgTrip (2098) complexes, about 5 µL of 106.8 nM protein per spot was used. About 9.6 µM protein stock was obtained by diluting about 11.6 µL of stock in 988 µL of protein buffer to make 1 mL of 106.8 nM solution. About 5 µL of this conjugation solution was added to Spot 3. For LgTrip (3546) complexes, about 5 µL of 106.8 nM protein per spot. About 94 µM protein stock was obtained by diluting about 1.13 µL of

LgTrip stock into 998.87 µL protein buffer. About 5 µL of this conjugation solution was added to Spot 4. After all the protein was added, the samples were dried at 30°C for 1 hour at 4°C, 25°C, and 37°C.

[0420] Methods for assessing RLU activity for these experiments included imaging at day 6 for all at 25°C and 37°C (following the 4°C time frame of 1 or 2 days); day 8 at 4°C for LgTrip 3546; and day 9 for NanoLuc, LgBiT, and LgTrip 2098. Furimazine was tested at 50µM and about 1.2µM dipeptide was used for NanoBiT and NanoTrip experiments. All spots were placed into a plate with substrate reagents, images were captured with an iPhone and with an LAS4000 imaging system, then inserted into the plate reader. NanoGlo Live Cell Substrate cat #N205B (lot 189096) was used, along with assay buffer 1x PBS, pH 7.0).

[0421] FIGS. 13A-13B show quantitative analysis of the same solid phase assay depicted in FIG. 12, but luminescence was detected using a luminometer on day 3 at 25°C (RLUs in FIG. 13A; S/B in FIG. 13B). These quantitative data support the qualitative data from FIG. 12. Materials and methods used for FIG. 12 are the same used for FIGS. 13A-13B (e.g., add 1µM dipeptide + 50µM live cell substrate in PBS, pH 7.0 and read on a luminometer). In some cases, the elevated background of LgBiT can decrease the S/B ratio.

[0422] FIGS. 14A-14C show a quantitative time course of the same solid phase assay as depicted in FIGS. 12-13 demonstrating stability of all the proteins in the experimental conditions at all temps tested over the time frame. B_{max} RLU values at 50µM furimazine over time (0 to 60 days) are shown for 4°C (FIG. 14A), 25°C (FIG. 14B), and 37°C (FIG. 14C). These quantitative data are consistent with FIGS. 12 and 13, demonstrating stability in all the complexes tested and at all temps tested over the time frame. Materials and methods used for FIG. 12 are the same used for FIGS. 14A-14C.

Example 5

Buffer Compositions

[0423] Experiments were also conducted to test short-term, or accelerated, stability of the complexes in different buffer compositions from 0 to 90 minutes. Methods included using about a 1.068 nM concentration of each protein absorbed and dried on Whatman 903 paper spots (1/8"). Protein samples were prepared and dried on paper spots with either protein buffer or PBS buffer (see each figure for specific buffer composition used). Stock concentrations included

NanoLuc at 1000x (0.4 mg/mL), LgBiT-1672-11s-His at 20 μM, and LgTrip (3546) at 94 μM. Protein buffer was comprised of 20 mM Na_3PO_4, 5% w/v BSA, 0.25% v/v tween20, 10% w/v sucrose. Luminescence activity was tested using the dipeptide added with furimazine in 100μl assay buffer PBS, pH 7.0 (final [dipeptide] = 1nM; final [furimazine] = 50μM). Samples were read at time point 0 (fresh out of 4°C), then placed into 60°C and 25°C for continued testing. A 1000x stock solution of NanoLuc was diluted 1:1000 in protein buffer (1 mL), or 10 μL of stock was diluted into 990 μL of protein buffer for a 1.068 nM stock (see each figure for specific buffer composition used). About 5 μL of each concentration was added to a paper spot for testing. For each protein tested (LgBiT and LgTrip), appropriate dilutions were made in each buffer to ensure that about 5 μL of 1.068 nM protein was used per spot. After all protein was added, the samples were dried at 35°C for 1 hour, and 40 spots per condition and temperature were prepared.

[0424] FIGS. 15A-15D show representative results collected on day 0 of an accelerated stability study performed under two buffer conditions at 25°C and 60°C (FIGS.15A and 15C use protein buffer, whereas FIGS. 15B and 15D use PBS). These data demonstrate that the complexes tested did not tolerate PBS as the buffer condition for input into the Whatman 903 paper, as compared to the protein buffer. Buffer conditions appear to affect stability even at early time points. In some cases, LgTrip 3546 exhibited better activity, suggesting somewhat better chemical stability than NanoLuc and LgBiT under these conditions.

[0425] FIGS. 16A-16B show results for the accelerated stability study depicted in FIG. 15, but over a 0 to 50-day time course. FIG. 16A includes results of samples tested in protein buffer at 25°C, and FIG. 16B includes results of samples tested in protein buffer at 60°C. The same materials and methods were used as in FIG. 15. These results demonstrate that the complexes remain stable under these conditions (at 25°C and 60°C) up until at least 50 days.

[0426] FIG. 17 shows a comparison of the impact of buffer conditions on luminescence from NanoLuc dried onto a nitrocellulose membrane to assess NanoLuc® stability in the context of a lateral flow assay. Four different conditions were tested: Condition 1: Mouse-anti Hum + IgG–Nluc in PBS, pH 7.4; Condition 2: IgG—Nluc in PBS, pH 7.4; Condition 3: Mouse-anti Hum + IgG–Nluc in loading buffer (20 mM Na_3PO_4, 5% w/v BSA, 0.25% v/v tween20, 10% w/v sucrose); Condition 4: IgG—Nluc in loading buffer (20 mM Na_3PO_4, 5% w/v BSA, 0.25% v/v

tween20, 10% w/v sucrose). Each condition was applied to the membranes and either dried at RT or at 37°C.

[0427] For these experiments, the following solutions were prepared: (1) 5µl mouse/antihuman into 995µl Addition buffer (0.1 M PBS, pH 7.4); (2) 5µl anti-mouse-NanoLuc in 995µl Addition buffer (0.1 M PBS, pH 7.4); (3) 5µl mouse/antihuman in protein buffer (20mM Na_3PO_4, 5% w/v BSA, 0.25% v/v tween20, 10% w/v sucrose); and (4) 5µl anti-mouse-NanoLuc in 995µl protein buffer (20mM Na_3PO_4, 5% w/v BSA, 0.25% v/vtween20, 10% w/v sucrose).). About 0.5ml of solution (1) was loaded into an airbrush and applied to the left side of a nitrocellulose strip (Strip 1 and 2). The strips were allowed to dry either at RT or at 37°C for 1 hour. About 0.5ml of solution (2) for was applied to the entire surface of strip 1 and strip 2 and allowed to dry at RT or at 37°C; forming condition 1 and 2, respectively. About 0.5ml of solution (3) was loaded into an airbrush and applied to the left side of a nitrocellulose strip (Strip 3 and 4). The strip was allowed to dry either at RT or at 37°C for 1 hour. About 0.5ml of solution (4) for was applied to the entire surface of strip 3 and strip 4 and allow to dry at RT or 37°C; forming condition 3 and 4, respectively. For imaging, a 1x solution of substrate was prepared (4mls PBS + 1ml Nano-Glo LCS Dilution Buffer + 50ul Nano-Glo Live Cell Substrate) and overlaid on each strip with 1ml of substrate solution; imaging began immediately thereafter.

[0428] These data demonstrate that buffer formulations are important for activity in lateral flow membranes. In conditions 1 – 4, where protein was just applied to the membrane in PBS, very little to no light was observed when the membranes were exposed to freshly prepared Nano-Glo Live Cell substrate. In contrast, protein that was prepared with a loading buffer that contained additional components such as Na_3PO_4, BSA, Tween 20, and sucrose showed considerable light output. This suggests that the particular loading buffer used to add the protein to the surface of the membrane is important for stability and function (FIG. 17).

Example 6

Lateral Flow Assay Components

[0429] Experiments were conducted to test different membrane blocking agents and assay running buffers to facilitate proper movement of proteins and targets during a lateral flow assay. Four strips were used, and the design of each (with or without sucrose and blocking agent) is shown in the schematic below the far left image of FIG. 18. Briefly, strip 1 included a blocked

membrane with sucrose pre-treatment on a conjugation pad; strip 2 included a blocked membrane with no sucrose pre-treatment on a conjugation pad; strip 3 included an unblocked membrane with sucrose pre-treatment on a conjugation pad; and strip 4 included an unblocked membrane with no sucrose pre-treatment on a conjugation pad.

[0430] The blocking buffer was comprised of 1% w/v polyvinyl alcohol in 20mM tris, pH 7.4. Conjugation pre-treatment included 30% sucrose w/v in DI water. The conjugation pad was Ahlstrom grade 8950 (chopped glass with binder, 50 g/m^2), and the membrane was nitrocellulose. For blocking, the membrane was soaked in blocking buffer for 30min at RT, and subsequently remove from buffer, washed with DI water, and dried for 30min at 35°C. For secondary pre-treatment, sucrose solution was applied to the membrane pad near where conjugation reagent (substrate) will be applied. The membrane was dried for 1hr at 35°C. To prepare the proteins, about 5µL anti-mouse-NanoLuc was added to 995µl protein buffer. About 1ml of protein solution was placed into an airbrush and a light coating was applied to the conjugation pad. This was allowed to dry for 1hr at 35°C. Strips were then assembled on backing card. Additionally, for FIGS. 18-20, the following buffers compositions were tested: Buffer 1 was comprised of 20X SSC, 1% BSA, pH 7.0 + 10µM LCS (FIG. 18). Buffer 2 was comprised of 0.01 M PBS, 1% BSA, pH 7.0 + 10µM PCS (FIG. 19). And Buffer 3 was comprised of 5x LCS dilution buffer + 5x LCS – diluted to 1X in PBS (FIG. 20).

[0431] FIG. 18 shows the effects of membrane blocking and sucrose pre-treatment on lateral flow assays performed in a running buffer of 20X SSC, 1% BSA, pH 7.0 + 10µM LCS. FIG. 19 shows the effects of membrane blocking and sucrose pre-treatment on lateral flow assays performed in a running buffer of 0.01 M PBS, 1% BSA, pH 7.0 + 10µM Permeable Cell Substrate (PCS). FIG. 20 shows the effects of membrane blocking and sucrose pre-treatment on lateral flow assays performed in a running buffer of 5x LCS dilution buffer + 5x LCS – diluted to 1X in PBS. These data demonstrate that membrane treatment and protein buffers do affect assay fluid flow within the conjugation pad and across the lateral flow membrane.

[0432] Experiments were also conducted to assess different membranes and membrane properties within the context of a lateral flow assay such as the effects of membrane properties on absorption and capillary action. FIG. 21 shows the effects of membrane properties on bioluminescent reagent absorption and capillary action in a lateral flow assay. Membranes containing different pore sizes were tested for flow efficiency. Each membrane was unblocked

and contain a 30% w/v sucrose pretreatment on approximately the bottom 1/3 of the strip. Other materials included a Conjugation pad (Ahlstrom grade 8950, chopped glass with binder, 50 g/m^2); a Sample Pad (Cellulose glass fiber CFSP203000 (Millipore)); and an Absorption pad (Cotton linters, grade 238 (Ahlstrom)). The following membrane conditions were tested:

1. nitrocellulose FF170HP (Ahlstrom)
2. nitrocellulose Hi-Flow Plus HFC18002 (Millipore) – 180 sec/4cm
3. nitrocellulose Hi-Flow Plus HFC13502 (Millipore) – 135 sec/4cm
4. nitrocellulose Hi-Flow Plus HFC09002 (Millipore) – 90 sec/4cm
5. nitrocellulose Hi-Flow Plus HFC12002 (Millipore) – 120 sec/4cm
6. nitrocellulose Hi-Flow Plus HFC07502 (Millipore) – 75 sec/4cm
7. nitrocellulose FF170HP (Ahlstrom) - NEGATIVE CONTROL.

[0433] Running buffer was comprised of 5x LCS dilution buffer + 5x LCS –diluted to 1X in PBS. Membranes were pre-treated by applying 30% sucrose solution to the membrane, covering ~1.5 cm of the bottom of the strip, the allowed to dry at 35°C for 1 hour. Proteins were prepared by adding about 5 µL anti-mouse-NanoLuc in 995 µL protein buffer. About 1 mL of protein solution was added to an airbrush, which was used to lightly coat conjugation pad. This was allowed to dry at 35°C for 1 hour. The negative control for these experiments contained protein buffer without protein, which was applied with an airbrush in the same manner as the test conditions. Strips were assembled on backing card. The conjugation pad, sample pad, and wicking pad were cut to be 2 cm x 1 cm. The sample pad and conjugation pad were overlapped by ~1.8 cm. The total dimensions of the strip were about 6 cm x 1 cm.

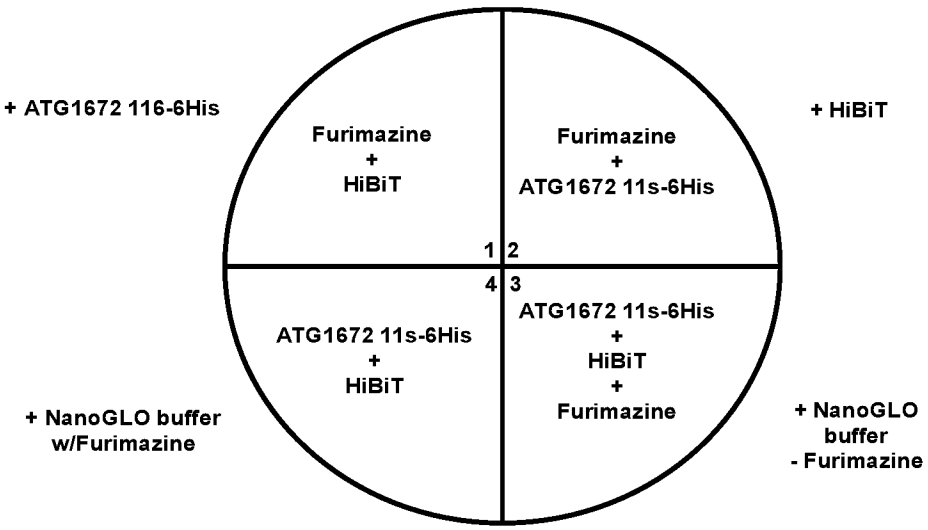
[0434] An imaging program was created to capture 5 sec exposure images every 30 seconds for a total of about 10 minutes. Imaging was repeated if it appeared that there was still NanoLuc flowing across the membrane. Images were stacked into movies using ImageJ, and the final images included in FIG. 21 are the accumulative signal of all images taken over time.

[0435] These results suggest that strips 4 and 6 (boxed in FIG. 21) had the most complete NanoLuc traveling out of conjugation pad and into sample reservoir, based on the conditions used in these experiments.

Example 7

Bioluminescent Complex Formation

[0436] Experiments were conducted to evaluate bioluminescent complex formation in the presence of various reagents on membrane and filter paper. Experiments were designed and conducted according to the schematic below, which shows the four different conditions tested.



[0437] For these experiments, 2.5 µL of HaloTag-HiBiT was added to 498 µL protein buffer. About 5 µL of this solution was spotted on both the membrane and filter paper in quadrants 1, 3, and 4 (see above schematic) and allowed to dry at 37°C for 1 hour. About 2.5 µL of ATG-1672-11S-6His was diluted in 498 µL of protein buffer, and about 5 µL was spotted directly onto nitrocellulose membrane and filter paper in quadrants 2, 3, and 4 (see above schematic). Membranes were allowed to dry at RT for 1 hour. Furimazine was prepared as a 5 mM stock solution in EtOH. About 5 µL of this solution was spotted onto both the membrane, and the filter paper in quadrants 1, 2, and 3 and immediately placed under high vacuum for 15 minutes. About 2.5 µL of stock protein (20 µM) was diluted in 498 µL of NanoGLO buffer, which does not contain substrate. About 5 µL was added to the quadrant indicated above and subsequently read in a luminometer.

[0438] FIGS. 22A-22B show bioluminescent signal from NanoBiT/HiBiT complementation on nitrocellulose (left) and Whatman grade 541 (right) papers (FIG. 22A), and a compilation image from a corresponding movie taken across total exposure time (movies can be made available upon request). Images were captured at increasing exposure times starting with 1 sec

and ending with 10 min exposure (1s, 3s, 10s, 30s, 1m, 2, 3, 4, 5, 10m) for a total time (26 min) after the addition of the reagents indicated 26.

[0439] These results suggest that filter paper may provide an increased signal as compared to the membrane. Also, the conditions present in quadrant 4 did not produce detectable luminescence, which could indicate that complex formation was impeded by one or more of the other reagents present.

[0440] Experiments were conducted to assess the effects of increased substrate concentration on complex formation. FIG. 23 shows bioluminescent signal from NanoBiT/HiBiT complementation on Whatman grade 903 paper, with a spike of additional substrate and liquid at 20 minutes. FIG. 23 is a representative compilation image from a corresponding movie taken across total exposure time (movies can be made available upon request). About 2.5µl of purified LgBiT or HiBiT was diluted in 498µl 1X LCS Buffer and added directly to the filter paper (consistent with the conditions in quadrant 1) in a 10µL volume (2:1 LgBiT to HiBiT ratio). The original substrate was NanoBRET NanoGlo (5 µl was added at 5mM), and the additional submerging substrate was NanoBRET NanoGlo (5mM stock), diluted 1:5 in 1X NanoGlo buffer, which was diluted to 1X in PBS. About 500µl was added to cover the filter paper. Images were captured at repeating 30 sec exposures during the entire time duration.

[0441] Spiking in additional substrate (furimazine) in an excess of liquid volume showed that signal returns, suggesting that as components start to move within the additional fluid, more complexes may be forming due to their increased mobility. This experiment also indicates that the enzyme retains activity with substrate concentration being the limiting factor that can be remedied by the addition of excess substrate.

[0442] FIG. 24 shows bioluminescent signal from NanoBiT/HiBiT complementation on Whatman 903 paper, instead of Whatman 541 paper, with the experimental conditions consistent with those in the above schematic diagram (quadrants 1-4 in FIG. 22). Buffer was added to rehydrate the membrane near the end of the experiment. FIG. 24 is a representative compilation image from a corresponding movie taken across total exposure time (movies can be made available upon request). The conditions in quadrant 2 appear to provide the strongest luminescent signal.

Example 8

Spot Tests with LgTrip and Substrate

[0443] Experiments were conducted to assess the feasibility of an "all-in-one" spot by first testing paper matrix containing LgTrip 3546 and furimazine to which an analyte-of-interest can be added (e.g., dipeptide). FIGS. 25A-25C show bioluminescent signal resulting from reconstitution with dipeptide of LgTrip 3546 and substrate in Whatman 903 paper, in the presence (FIG. 25B) and absence (FIG. 25A) of BSA, along with a serial dilution of the dipeptide with BSA (FIG. 25C). Two sets of spots were made, each spot being comprised of the following components: 1) 5 mM ATT, 5 mM ascorbate, 5 μM LgTrip 3546, and 1 mM furimazine; 2) 5% BSA, 5 mM ATT, 5 mM ascorbate, 5 μM LgTrip 3546, and 1 mM furimazine.

[0444] To prepare the spots, a vial containing 200 μL of 5 μM LgTrip 3546, 5 mM ATT, and 5 mM ascorbic acid was prepared. About 5 μL of this solution was added to each spot, and the spots were then allowed to dry at 35°C for 1 hour. After drying, 1 mM stock of furimazine in ethanol was prepared. About 5 μL of this solution was added to each spot and allowed to dry at 35°C for an additional 30 minutes. For luminescent measurements, at the time of testing, 1.2 mM dipeptide stock in water was serial diluted down to $1e^{-10}$ M in PBS, pH 7.0. About 100 μL of each dipeptide stock was added to a 96-well plate containing a spot and kinetic measurements were started immediately.

[0445] These data demonstrate that a stable, concentration dependent response was observed with the addition of the dipeptide (FIG. 25). This experiment highlights that a paper-format containing LgTrip 3546 and substrate can be made and then reconstituted in buffered aqueous media containing a potential analyte of interest (e.g., dipeptide). Different materials were then tested with substrate and LgTrip 3546 input. Either fresh dipeptide was added at 1 nM to test NanoTrip and substrate activity, or fresh Nluc was added to isolate the substrate. FIG. 27 shows bioluminescent signal in three different solid phase materials (Whatman 903, Ahlstrom 237, and Ahlstrom 6613H) resulting from reconstitution with dipeptide of LgTrip 3546 and substrate, or NanoLuc added to dried LgTrip 3546 and substrate. Alhstrom 6613H seems to be detrimental to signal output over time as it appears that the luminescent signal is decreased in both conditions. Overall, the stability of the assay components can be affected by the composition of the solid matrix materials in which they are imbedded.

[0446] FIG. 28 shows bioluminescent signal from Whatman 903 paper that contains both LgTrip 3546 as well as substrate and stored under ambient conditions for over 25 days. Spots were exposed to 1 nM dipeptide in PBS at the time of testing. Overall, this experiment shows that there is no significant loss of signal from the materials after extended storage times under ambient temperature.

Example 9

Lyophilized Cake Containing LgTrip and Substrate

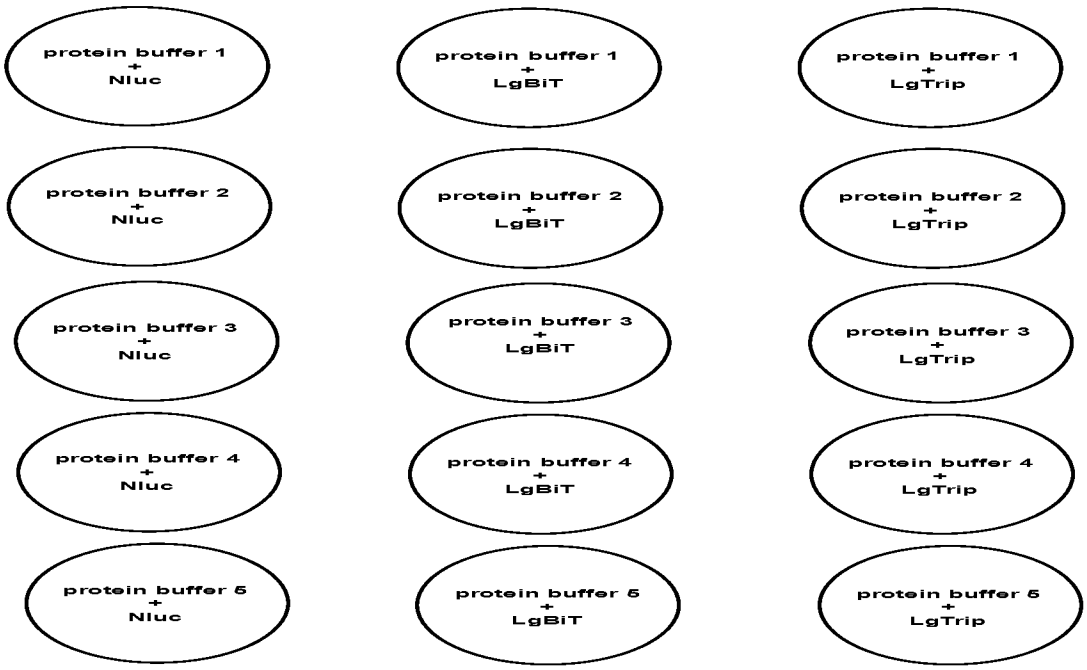
[0447] FIGS. 26A-26B show bioluminescent signal resulting from reconstitution with dipeptide of LgTrip 3546 and substrate from a lyocake (FIG. 26A) along with the summary data of the titration of the dipeptide (FIG. 26B). To prepare the lyocakes, 5% w/v pullulan was added to water containing 26.3 mM ATT and 11.3 mM ascorbic acid (solution 1). Solution 1 was then aliquoted out into 35 µL volumes in snap-cap vials. About 10 µL of 95 µM LgTrip 3546 protein was then added to each vial and pipetted to mix (solution 2). A 10 mM stock solution of furimazine in ethanol was prepared, and 5 µL of this solution was added to each vial and mixed (solution 3). Vials containing solution 3 were placed on dry ice to freeze for 1 hour, and then lyophilized overnight. For luminescent measurements, at the time of testing, 1.2 mM dipeptide stock added to water was serial diluted down to $1e^{-10}$ M in PBS, pH 7.0. About 100 µL of each dipeptide stock was added to a lyophilized vial containing LgTrip 3546 and substrate, pipetted briefly to mix, and then placed into a 96-well plate, and kinetic measurements were started immediately.

[0448] These data demonstrate that a stable, concentration dependent bioluminescent response was observed with the addition of the dipeptide (FIG. 26). This experiment highlights that a solid format lyophilized cake or tablet containing LgTrip 3546 and substrate can be made and then reconstituted in aqueous media containing a potential analyte of interest (e.g., dipeptide).

Example 10

Protein Buffer Formulations

[0449] For FIGS. 29-33, experiments were conducted to test the compatibility of protein components with different protein buffer formulations, according to the experimental design shown in the schematic diagram below.



[0450] For these experiments, Whatman 903 protein saver spot cards were used with the following protein buffer formulations:

Protein buffer 1: 20 mM Na_3PO_4, 5% w/v BSA, 0.25% v/v tween20, 10% w/v sucrose

Protein buffer 2: 20 mM Na_3PO_4, 0.25% v/v tween20, 10% w/v sucrose

Protein buffer 3: 20 mM Na_3PO_4, 5% w/v BSA, 0.25% v/v tween20

Protein buffer 4: 20 mM Na_3PO_4, 5% w/v BSA, 0.25% v/v tween20, 2.5% pullulan

Protein buffer 5: 20 mM Na_3PO_4, 0.25% v/v tween20, 2.5% pullulan.

[0451] For NanoLuc, a 1000x stock solution was diluted 1:1000 in protein buffer (1 mL). For a 1.068 nM stock solution, 3µl was diluted into 297 µl of protein buffer. About 5 µL of each concentration was spotted on the filter paper. For LgBiT-1672-11s-His, 5 µL of 1.068 nM protein per spot was used. About 10µL was diluted in 990 µL protein buffer for a $2e^{-7}$ M stock. About 100 µL of a 100 nM protein solution was then prepared, and about 10 µL stock was diluted into 990 µL protein buffer for 1 nM stock. About 5 µL of each concentration was spotted onto filter paper. For LgTrip 3546, about 5 µL of 1.068 nM protein was used per spot. About 1.1 µL of LgBiT-1672 stock was diluted into 998.94 µL protein buffer. About 3 µL stock was diluted into 297 µL protein buffer. About 5µL of each concentration was spotted onto filter paper. After all protein was added, the samples were dried at 30°C for about 1 hour. About 40 spots were made for each condition (see above schematic diagram). Spots were tested on day 0

for a baseline and then placed at 60°C and tested 6 days later. RLU activity was tested by addition of 1nM of high affinity dipeptide + 50μM live cell substrate in PBS, pH 7.0.

[0452] FIGS. 29A-29C show bioluminescent signal, measured by RLUs, in the various protein buffer formulations described above for NanoLuc (FIG. 29A), LgBiT-1672 (FIG. 29B), and LgTrip 3546 (FIG. 29C), and FIGS. 30A-30C show bioluminescent signal, measured by B_{max}, in various protein buffer formulations for NanoLuc (FIG. 30A), LgBiT-1672 (FIG. 30B), and LgTrip 3546 (FIG. 30C). Together, these data suggest that BSA is an important component in the protein buffer formulations tested, with NanoLuc and LgTrip 3546 exhibiting the largest decreases in RLU (buffers 2 and 5).

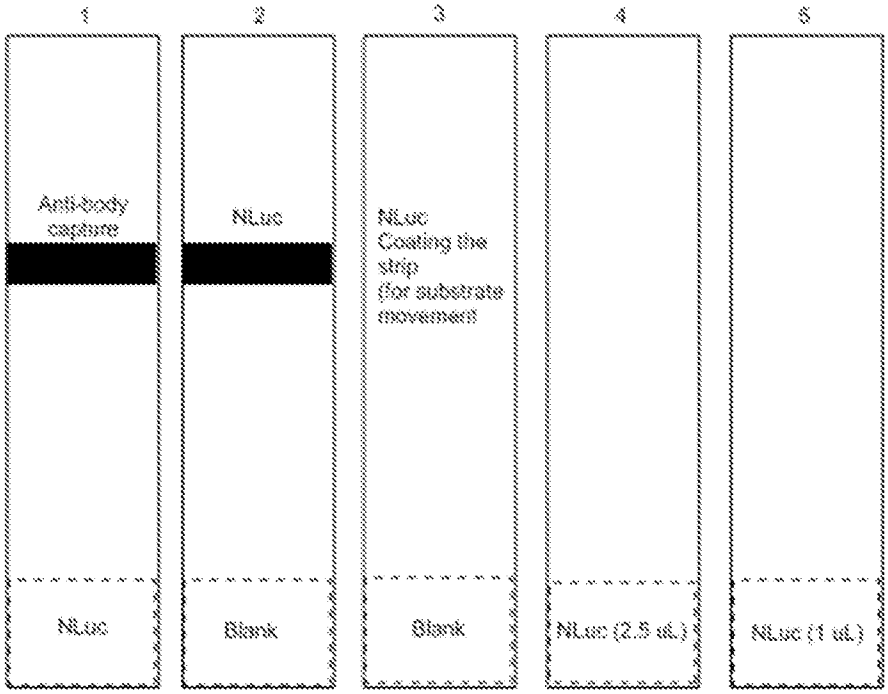
[0453] Experiments were also conducted to assess luminescent background levels in the various protein buffer compositions described above. FIGS. 31A-31B show bioluminescent background levels in various protein buffer compositions for LgBiT-1672 (FIG. 31A) and LgTrip 3546 (FIG. 31B). These data suggest that BSA or pullulan are important components of the protein buffer formulations for LgBiT-1672 for minimizing background luminescence, but there appears to be little to no effect on LgTrip 3546 background levels under these conditions.

[0454] In FIGS. 32A-32F, the kinetics of the above conditions were assessed after addition of dipeptide and substrate in PBS. More specifically, FIGS. 32A-32F show bioluminescent signal (RLUs in FIGS. 32A-32C; B_{max} in FIGS. 32D-32F) in various protein buffer formulations for NanoLuc® (FIGS. 32A and 32D), LgBiT-1672 (FIGS. 32B and 32E), and LgTrip 3546 (FIGS. 32C and 32F), after 6 days at 60°C. These data indicate that proteins are stable and maintain activity after 6 days at 60°C under these conditions, and suggest that BSA is an important component for all proteins buffer formulations. Additionally, FIG. 33 includes representative embodiments of all-in-one lyophilized cakes ("lyocakes") or tablets containing all the necessary reagents to perform an analyte detection test supporting several types of assay formats, including but not limited to, cuvettes, test tubes, large volumes in bottles, snap test type assays, and the like.

Example 11

Lateral Flow Assays

[0455] For FIGS. 34 and 35, lateral flow assays were performed using the information obtained in the above experiments, and according to the experimental design shown in the schematic diagram below.



[0456] The materials used for these experiments included a Conjugation pad (Ahlstrom grade 8950, chopped glass with binder, 50 g/m^2), a Sample Pad (Cellose glass fiber CFSP203000 (Millipore)), an Absorption pad (Cotton linters, grade 238 (Ahlstrom)), a Membrane (nitrocellulose Hi-Flow Plus HFC07502 (Millipore), #6 from strip-test 2), and Running buffer (5x LCS dilution buffer + 5x LCS diluted to 1X in PBS). Membranes were prepared by applying 30% sucrose solution to the membrane covering about 1.5 cm of the bottom of the strip. The membrane was allowed to dry at 35°C for 1 hour. Strips were initially cut to be 4.5 cm x 1 cm.

[0457] Protein preparations were carried out according to the conditions below:

Condition 1: 5 µL mouse anti-NanoLuc antibody diluted in 995 µL protein buffer, applied evenly across the conjugation pad with an air brush, and dried in oven at 37°C. Dilute 2.5 µL mouse antibody in 0.5 mL of protein buffer and applied directly to membrane.

Condition 2: Dilute 2.5 µL of NanoLuc in 0.5 mL of protein buffer and applied directly to membrane. Allowed to dry at 37°C for 1 hour.

Condition 3: Treat entire membrane directly with 5 µL of NanoLuc diluted to 1 mL in protein buffer. Applied evenly with airbrush. Allowed to dry at 37°C for 1 hour.

Condition 4: 2.5 µL mouse anti-NanoLuc antibody in 997 µL protein buffer. Applied evenly across conjugation pad with airbrush. Allowed to dry at 37°C for 1 hour.

Condition 5: 1 µL mouse anti-NanoLuc antibody in 999 µL protein buffer. Applied evenly across conjugation pad with airbrush. Allowed to dry at 37°C for 1 hour.

[0458] Strips were assembled on backing card with conjugation pad, sample pad, and wicking pad cut to 1 cm x 1 cm. Once strips were assembled, they were cut in half lengthwise to a final dimension of 4.5 cm x 0.5 cm. For imaging analysis, about 250µl 1X LCS buffer + LCS was diluted in PBS. Images were captured at 5 sec exposures with 5 sec wait time in between images; representative images are compilation images from corresponding movies taken across total exposure time (movies can be made available upon request). Total read time was 2:40 minutes.

[0459] FIG. 34 shows bioluminescent signal from substrate movement across a lateral flow strip from a compilation image corresponding to a movie taken across total exposure time. Substrate was added to the sample window of the lateral flow assay cassette and real time imaging shows substrate movement across the strip, and NanoLuc® activity can be seen throughout the test window (strip #3 in schematic above). By 70 seconds, the substrate flowed across the entire sample window.

[0460] FIG. 35 shows bioluminescent signal from NanoLuc® movement across a lateral flow strip from a compilation image corresponding to a movie taken across total exposure time (strip #s 4 and 5 in the schematic above). Under these conditions, strip #5 appeared to outperform strip #4 with, as demonstrated by the NanoLuc® flowing out of the conjugation pad and into the liquid flow across the membrane to the strip containing the mouse anti-NanoLuc antibody.

Example 12

Fumonisin Detection

[0461] Experiments were conducted during development of embodiments herein to demonstrate the use of NanoLuc®-based technologies in a competition-type immunoassay for the detection of a fumonisin B1, an exemplary small molecule toxin. Such assays can be

performed in the devices and systems described herein, and with other small molecule targets and target analytes.

[0462] In an exemplary assay, tracers were generated by tethering fumonisin B1 to a NLpeptide tag (e.g., a peptide tag comprising SEQ ID NO: 10) via a biotin/streptavidin linkage, via a HaloTag linkage, or directly (FIG. 36). In some embodiments, the tracers can be combined with an anti-fumonisin B1 antibody linked to a polypeptide complement of the NLpeptide tag (e.g., a complement comprising SEQ ID NO: 9). A bioluminescent complex can form between the peptide tag and the polypeptide component upon binding of the antibody to the fumonisin B1. Exposure to varying concentrations of unlabeled Fumonisin B1 disrupts the bioluminescent complex and results in decreased luminescence, and the ability to detect/quantify the amount of fumonisin B1 in a sample (FIG. 37).

Example 13

Lyophilized Cake Containing LgBiT and Substrate

[0463] FIGS. 38A-38B show bioluminescent signal resulting from reconstitution with dipeptide of LgBiT and substrate from a lyocake (FIG. 38A) along with a titration of the dipeptide (FIG. 38B). To prepare a lyocake with LgBiT: 5% w/v pullulan in water containing 5 mM ATT and 5 mM ascorbic acid was prepared (solution 1). Solution 1 was then aliquoted out into 45 µl volumes in snap-cap vials. About 5 µl of 20 µM LgBiT protein was then added to each vial and pipetted to mix (solution 2). A 10 mM stock solution of furimazine in ethanol was prepared, and 5 µl of this solution was added to each vial and mixed (solution 3). Vials containing solution 3 were placed on dry ice to freeze for 1 hour, and then lyophilized overnight.

[0464] For luminescent measurements, at time of testing, 1.2 mM dipeptide stock in water was serial diluted down to $1e^{-10}$ M in PBS, pH 7.0. 100 µl of each dipeptide stock was added to a lyophilized vial containing LgBiT and substrate, pipetted briefly to mix, and then placed into a 96-well plate and kinetic measurements were started immediately.

[0465] These data demonstrate that a stable, concentration dependent bioluminescent response was observed with the addition of the dipeptide. This experiment highlights that a solid format containing LgBiT and substrate can be made and then reconstituted in aqueous media containing a potential analyte of interest (e.g., dipeptide).

Example 14

Substrate and LgTrip 3546 or LgBiT Lyophilization

[0466] FIG. 39 shows bioluminescent signal resulting from reconstitution with dipeptide of LgBiT, or LgTrip 3546, and substrate from a lyocake prepared directly into a standard 96-well tissue culture treated plate (Costar 3917). To prepare a lyocake in plates: 2.5% w/v pullulan in water containing 5 mM ATT and 5 mM ascorbic acid was prepared (solution 1, pH 6.5). Solution 1 was then aliquoted out into 45 µl volumes into each well of the plate. 2.6 µl of 95 µM LgTrip 3546 protein was then added to each vial and pipetted to mix forming condition 1 (LgTrip 3546 alone). Additionally, 5 µl of 20 µM LgBiT protein was added to each vial and pipetted to mix, forming condition 2 (LgBiT alone). 5 µl of ethanol was then add to each well of condition 1 and 2 as a vehicle control.

[0467] Conditions 3 (LgTrip 3546/substrate) and 4 (LgBiT/substrate) were prepared as described above: 2.5% w/v pullulan in water containing 5 mM ATT and 5 mM ascorbic acid was prepared (solution 1, pH 6.5). Solution 1 was then aliquoted out into 45 µl volumes into each well of the plate. About 2.6 µl of 95 µM LgTrip 3546 protein or 5 µl of 20 µM LgBiT protein was added to each vial and pipetted to mix. Approximately 5 µl of 10 mM furimazine in ethanol was then added to each well forming condition 3 and 4 respectively. The plate was then placed in a cooler with dry ice to freeze for 1 hour, followed by lyophilization overnight.

[0468] For luminescent measurements, at time of testing, 1.2 mM dipeptide stock in water was serial diluted down to $1e^{-9}$ M in PBS, pH 7.0 (FIG. 39). Fresh NanoGlo® substrate was then added to this stock for a final concentration of 10 µM substrate. 100 µl of this solution was added to wells that contained condition 1 (LgTrip 3546) and 2 (LgBiT). Conditions 3 (LgTrip 3546/substrate) and 4 (LgBiT/substrate) only received 100 µl of $1e^{-9}$ M dipeptide in PBS. After testing, the plates were wrapped in tin foil and left on the bench at ambient temperature.

[0469] This data demonstrates that a lyocake containing either LgBiT or LgTrip 3546 and substrate can be prepared directly within a 96-well plate and reconstituted in the presence of an analyte of interest (dipeptide) leading to stable and robust signal.

Example 15

Paper based all-in-one analyte detection systems

[0470] Experiments were conducted to test the efficacy of paper-based detection platforms containing NanoBiT (FIGS. 40A-40B) and NanoTrip (FIG. 41A) complementation systems. Paper spots were created from punching 1/8" diameter circles from Whatman903 spot paper. The spots were treated with 5 µl of a master mix solution containing: 5% w/v BSA, 5 mM ATT, 5 mM ascorbate, 40 nM LgBiT-protein G fusion, and 20 nM SmBiT-TNFα in water, pH 6.5. The spots were allowed to dry at 35C for 1 hour. A 200 µM solution of furimazine in ethanol was prepared, and 5 µl of this solution was added to each spot. The spots were allowed to dry for an additional 30-60 minutes at 35°C. At the time of testing, spots were plated into individual wells of a 96-well NBS plate (Costar 3917), and reconstituted with Opti-MEM assay buffer that contained either 0 nM (blank), 1 nM, or 100 nM Remicade.

[0471] FIGS. 40A-40B include assay results using NanoBiT components. In the condition where the spots were exposed to assay buffer containing 1 nM Remicade, there was an increase in overall light output compared to the blank condition/control, which contained no Remicade. An increase in signal is observed as the concentration of Remicade was increased to 100 nM. As shown in FIG. 40B, Remicade was prepared in opti-MEM assay buffer at 100nM, 10nM, 1nM, and 0.1 nM concentrations. At time of testing, 100 µl of each solution containing Remicade was added to a well of a 96-well plate containing a spot, and RLU output was measured.

[0472] Similar experiments were performed, as shown in FIG. 41A using NanoTrip components. Spots were created from punching 1/8" diameter circles from Whatman903 spot paper. Each the spot was treated with 5 µl of a master mix solution containing: 5% w/v BSA, 5 mM ATT, 5 mM ascorbate, 20 µM LgTrip 3546, 100 nM TNFα-15gs-VSHiBiT, SmTrip9 Pep521-15gs-protein G in water, pH 6.5. The spots were allowed to dry at 35°C for 1 hour. A 200 µM solution of furimazine in ethanol was prepared and 5 µl of this solution was added to each spot. The spots were allowed to dry for an additional 30 minutes at 35°C. At the time of testing, spots were plated into individual wells of a 96-well NBSplate (Costar 3917), and reconstituted with opti-MEM assay buffer that contained either 0 nM (blank), 1 nM, or 100 nM Remicade. The results are shown in FIG. 41A.

[0473] These experiments show that it is possible to build and all-in-one, paper-based bioluminescent assay platforms for the detection of an analyte-of-interest using both NanoBiT

and NanoTrip complementation systems. In addition, these experiments demonstrate that it is possible to quantify the amount of analyte present in the sample matrix based on a change in overall light output. Increasing the concentration of the analyte-of-interest (i.e. Remicade) led to a proportional increase in the bioluminescent signal (the bioluminescent signal generated from the analyte detection complex is proportional to the concentration of the analyte).

Example 16

Lyocake based all-in-one analyte detection systems

[0474] Experiments were also conducted to test the efficacy of lyocake-based detection platforms containing NanoBiT (FIG. 40C) and NanoTrip (FIGS. 41B-41C) complementation systems.

[0475] As shown in FIG. 40C, stability conditions were tested when drying down the components of the bioluminescent complexes. About 45 µl of a master mix solution was added to 1.5 mL, plastic snap-cap vials. The master mix included: 5% w/v pullulan, 5 mM ATT, 5 mM ascorbate, 40 nM LgBiT-protein G fusion, and 20 nM SmBiT-TNFα, at pH 6.5. About 5-10 µl of the substrate furimazine in ethanol was added to each vial, mixed, and placed in dry ice for about 1 hour. The frozen samples were then lyophilized overnight to form a lyocake. At the time of testing, solutions of 100 nM and 10 nM Remicade were prepared in Opti-MEM assay buffer. About 100 µl of these solutions were added to the vials containing the NanoBiT Cake, pipetted to mix, and then transferred to a Costar 3600 96-well plate. A blank control was prepared that lacked the analyte Remicade. The results in FIG. 40C demonstrate a proportional increase in signal as the analyte concentration increased, even when all the components of the bioluminescent complex, including the substrate, are frozen and stored in the form of a lyocake, and subsequently exposed to the analyte-of-interest.

[0476] In FIGS. 41B-41C, stability conditions were tested when drying down the components of the bioluminescent complexes. About 45 µl of a master mix solution was added to 1.5 mL, plastic snap-cap vials. The master mix included: 5% w/v pullulan, 5 mM ATT, 5 mM ascorbate, 9 µM LgTrip 3546, 225 nM SmTrip9-Protein G, and 45 nM SmBiT-TNFα, at pH 6.5. About 5-10 µl of the substrate furimazine in ethanol was added to each vial, mixed, and placed in dry ice for about 1 hour. The frozen samples were then lyophilized overnight to form a lyocake. At the time of testing, solutions of 100 nM, 10 nM and 1 nM Remicade were prepared in Opti-MEM

assay buffer. About 100 µl of these solutions were added to the vials containing the NanoTrip Cake, pipetted to mix, and then transferred to a Costar 3600 96-well plate. A blank control was prepared that lacked the analyte Remicade. The results in FIG. 41B-41C demonstrate a proportional increase in signal as the analyte concentration increased, even when all the components of the bioluminescent complex, including the substrate, are frozen and stored in the form of a lyocake, and subsequently exposed to the analyte-of-interest.

[0477] In the condition where the spots were exposed to assay buffer containing 1 nM Remicade, there was an increase in overall light output compared to the blank condition, which contained no Remicade. An increase in signal was observed as the concentration of Remicade increased to 100 nM. These experiments show that it is possible to build and all-in-one lyocake-based, bioluminescent-based assay platforms for the detection of an analyte-of-interest using both NanoBiT and NanoTrip complementation systems. In addition, these experiments demonstrate that it is possible to quantify the amount of analyte present in the sample matrix based on a change in overall light output. Increasing the concentration of the analyte-of-interest (i.e. Remicade) led to a proportional increase in the bioluminescent signal (the bioluminescent signal generated from the analyte detection complex is proportional to the concentration of the analyte).

Example 17

Mesh-based systems to separate substrate from bioluminescent complexes for analyte detection

[0478] Experiments were conducted to investigate the conditions required to generate a bioluminescent signal when peptide and polypeptide components of the bioluminescent complexes provided herein were produced in a format that does not include the substrate. For example, in one embodiment, an amount of a solution (e.g., containing an analyte-of-interest) is added to a mesh or matrix that has the luminogenic substrate adhered ("caked") to it. Addition of the solution acts to reconstitute the substrate on the mesh, and this solution subsequently interacts with the surface of paper containing the dried down peptides and polypeptides of the bioluminescent complexes of the present disclosure, thus generating a bioluminescent signal (FIG. 42A). The mesh format does not hinder the ability to detect the bioluminescent signal; any bioluminescence detected comes from the surface of the paper, and not from any solution phase that is formed during the experiment.

[0479] As shown in FIG. 42A, bioluminescence is detectable using this format. Whatman 903 paper spots were made to have about 0.25 inch diameters, similar to the nylon mesh. The master mix, which was used to generate the paper spots containing the bioluminescent peptide/polypeptide components, included: 5% w/v BSA, 5 mM ATT, 5 mM ascorbate, 10 μM NanoLuc, at pH 6.5. About 10-20 μl of the master mix was added to the spots and then dried at about 35°C for about 1 hour. To generate the mesh containing the substrate, a solution of about 0.75% pullulan in water was prepared. About 450 μl of this solution was added to a plastic snap-cap vial. About 50 μl of 10 mM furimazine in EtOH was added to the vial and pipetted to mix. About 25 μl of this solution was added to the top of the mesh-spots. The mesh spots were then frozen on dry-ice, and lyophilized overnight. At time of testing, the mesh containing the lyocake substrate was placed on top of the spots containing the NanoLuc® protein. The complete system was then added to the well of a 96-well costar 3600 plate. About 10 μl of PBS was then added to the top of the mesh to reconstitute the material and the plate was read for RLU light output.

[0480] Experiments were also conducted using LgTrip 3546 bioluminescent components with the mesh-based format. The master mix, which was used to generate the paper spots containing the bioluminescent peptide/polypeptide components, included: 5% w/v BSA, 5 mM ATT, 5 mM ascorbate, 100 nM LgTrip 3546, at pH 6.5. About 10-20 μl of the master mix was added to the spots and then dried at about 35°C for about 1 hour. To generate the mesh containing the substrate, a solution of about 0.75% pullulan in water was prepared. About 450 μl of this solution was added to a plastic snap-cap vial. About 50 μl of 10 mM furimazine in EtOH was added to the vial and pipetted to mix. About 25 μl of this solution was added to the top of the mesh-spots. The mesh spots were then frozen on dry-ice, and lyophilized overnight. At the time of testing, dipeptide ranging from 100 nM to 0.1 nM was prepared in PBS. The spots were placed in wells, and the screen containing the substrate was placed on the surface of the spots. About 10 μl of the solutions containing each concentration of peptide was added to the surface of the screen and RLU's were recorded (FIGS. 42B-42C). The blank control did not contain any dipeptide.

[0481] Experiments were also conducted using LgTrip 3546 bioluminescent components with the mesh-based format and by forming a pullulan film. The master mix, which was used to generate the paper spots containing the bioluminescent peptide/polypeptide components, included: 5% w/v BSA, 5 mM ATT, 5 mM ascorbate, 100 nM LgTrip 3546, at pH 6.5. About

10-20 µl of the master mix was added to the spots and then dried at about 35°C for about 1 hour. To generate the mesh containing the substrate, a solution of about 2.0% pullulan in water was prepared. About 450 µl of this solution was added to a plastic snap-cap vial. About 50 µl of 10 mM furimazine in EtOH was added to the vial and pipetted to mix. About 25 µl of this solution was added to the top of the mesh-spots. The spots were then allowed to dry under ambient conditions, in the dark, overnight. This method resulted in the formation of a pullulan film that filled the holes of the mesh. At the time of testing, dipeptide ranging from 100 nM to 0.1 nM was prepared in PBS. The spots were placed in wells, and the screen containing the substrate was placed on the surface of the spots. About 10 µl of the solutions containing each concentration of peptide was added to the surface of the screen and RLU's were recorded (FIGS. 42D-42E). The blank control did not contain any dipeptide.

[0482] These experiments show that it is feasible to detect bioluminescent signal in a mesh-based format in which the peptide/polypeptide components are separate from the substrate. In addition, in the context of this format, these experiments demonstrate that increasing the concentration of the analyte-of-interest (i.e. dipeptide) leads to a proportional increase in the bioluminescent signal (the bioluminescent signal generated from the analyte detection complex is proportional to the concentration of the analyte).

Example 18

Testing different formulated, lyophilized substrates for cake appearance, reconstituted kinetic activity performance, and accelerated thermal stability

[0483] To evaluate the potential application of lyophilization for preservation of the furimazine substrate, formulations containing furimazine were prepared. The 20X stock formulations were as follows:

[0484] Condition 1: 100 µM furimazine in ethanol, 5 mM azothiothymine, 5 mM ascorbic acid, 2.5% pullulan w/v, ddH20 (Millipore);

[0485] Condition 3: 100 µM furimazine in ethanol, 5 mM azothiothymine, 5 mM ascorbic acid, 2.5% pullulan w/v, 20 mM HEPES buffer (pH 8.0), 90 mM glycine, 20 mM histidine, 25 mg/ml sucrose, 0.01% polysorbate 80;

[0486] Condition 5: 40 µM furimazine in 85% ethanol + 15% glycol, 200 mM MES buffer (pH 6.0), 200 mM hydroxyproyl beta cyclodextrin (m.w. 1396 Da), 600 mM sodium ascorbate, 2.5% pullulan w/v; and

[0487] Condition 7: 20 μM furimazine in ethanol, 200 mM MES buffer (pH 6.0), 200 mM hydroxyproyl beta cyclodextrin (m.w. 1396 Da), 600 mM sodium ascorbate, 2.5% pullulan w/v.

[0488] One mL aliquots of 20X stock solution was dispensed into 10 mL amber glass vials, and a runner stopper was partially inserted into the vial. Vials were loaded into a lyophilizer (Virtis Genesis 12EL lyophilizer) with shelves pre-chilled to 4.7°C. Product then underwent a freezing step with a shelf temperature of -50°C for 2 hr after which time the condenser step started. During the run, the condenser temperature ran between -5°C and -87°C. A vacuum pull down ran next at the pressure set-points of 75 and 200 mTorr. Sublimation lasted ~7.5 hr, and desorption lasted ~16.1 hr. At the end of the lyophilization process, the vials were back-filled with nitrogen and sealed with fully inserted stoppers at ~600 Torr of pressure.

[0489] Vials were stored at 25°C or 60°C and tested at various timepoints post-lyophilization. For activity-based assays, furimazine cakes were reconstituted with 10 mL of PBS containing 0.01% BSA. The vials were shaken manually and allowed to equilibrate at room temperature for 5 minutes. Fifty μl of the reconstituted substrate was added to 50 μl of 1 ng/mL purified NANOLUC enzyme (Promega) that was reconstituted in the same BSA buffer (final [NanoLuc] = 0.5 ng/ml). The controls used were the NANOGLO Live Cell Substrate (Promega Cat. N205) or NANOGLO substrate (Promega Cat. N113) according to manufacturer's protocol, but were diluted into PBS containing 0.01% BSA instead of the dilution buffer provided in the kit (Promega). Assays were performed in solid, white, nonbinding surface (NBS) plates (Costar) and analyzed on a GLOMAX Discover Multimode Microplate Reader (Promega) collecting total luminescence using kinetic or endpoint reads, depending on the experiment. For analysis of absolute [furimazine], reconstituted samples were analyzed on HPLC for absorbance spectra at wavelength 245 nm and the absolute amount remaining from day 0 was plotted.

[0490] The appearance of the lyophilized cakes resulting from these formulations are displayed in FIG. 43, which shows that all 4 conditions tested produced an intact cake, although conditions 5 and 7 did display some cracking. A pH indicator that was supplied for these vials indicated that the resulting cakes had pH values of about 2-3 for Condition 1, pH values of about 7.5 for Condition 3, and pH values of about 6 for Conditions 6 and 7. Signal kinetics of the reconstituted furimazine, when tested with purified NanoLuc, compared to that of furimazine in standard organic storage buffer (N113 and N205) and maintained at -20°C, indicated there was

no observable loss in performance due to the formulated buffer and lyophilization process itself, with an improved half-life for conditions 5 and 7 (FIG. 44).

[0491] Accelerated thermal stability studies indicated no loss of activity for 3 months for the formulated and lyophilized furimazine for Condition 1, which in stark contrast to the furimazine stored in organic solvent, which lost all activity in about 10 days when stored at this elevated temperature (FIG. 45). HPLC analysis for the absolute [furimazine] remaining after storage at 25°C and 60°C supported the activity findings with the formulated and lyophilized substrate containing significantly higher purity of furimazine relative to furimazine in the standard organic storage buffer (FIGS. 46A and 46B). To determine the liquid stability of the formulated, lyophilized furimazine, vials were reconstituted with water and allowed to remain in solution for 12 days prior to analysis by HPLC for total remaining furimazine as compared to day 0. Liquid stability of conditions 5 and 7 were found to be superior (FIG. 47).

Example 19

Development of a solution-based, homogeneous human Interleukin-6 tripartite immunoassay using HaloTag-peptide fusions to chemically conjugate monoclonal antibody pairs

[0492] The basic principle of the homogeneous NanoLuc tripartite (NanoTrip) immunoassay is depicted in FIG. 48. First, a pair of antibodies that target non-overlapping epitopes on IL-6 are chemically conjugated to SmTrip9 (SEQ ID NO: 13) or HiBiT (SEQ ID NO: 11) using the HaloTag® technology. When the labeled antibodies bind an IL-6 analyte, the complementary subunits are brought into proximity thereby reconstituting a bright luciferase in the presence of the LgTrip 3546 protein (SEQ ID NO: 12) and furimazine substrate. This assay is quantitative because the amount of luminescence generated by a standard plate-reading luminometer is directly proportional to the amount of target analyte present.

[0493] Genetic fusions containing the SmTrip9 variants (SmTrip9 Pep521; SEQ ID NO: 16) or SmTrip10 variants (SmTrip10 Pep289 or VSHiBiT; SEQ ID NO: 17 separated by either a 2X or 3X Gly-Ser-Ser-Gly linker to the amino terminus of HaloTag was achieved using the pFN29A HIS_6HaloTag T7 Flexi Vector (Promega). Glycerol stocks of *E. coli* expressing HisTag-HaloTag fusion protein was used to inoculate 50mL starter cultures, which were grown overnight at 37°C in LB media containing 25 ug/ml kanamycin. Starter cultures were diluted 1:100 into 500 mL fresh LB media containing 25 ug/mL kanamycin, 0.12% glucose, and 0.2% rhamnose. Cultures

were grown for 22-24 h at 25°C. Cells were pelleted by centrifugation (10,000 rpm) for 30 min at 4°C and re-suspended in 50 mL PBS. 1 mL protease inhibitor cocktail (Promega), 0.5 mL RQ1 DNase (Promega), and 0.5 mL of 10 mg/mL lysozyme (Sigma) were added, and the cell suspension was incubated on ice with mild agitation for 1 h. Cells were lysed by sonication at 15% power at 5 s intervals for 1.5 min (3 min total) and subsequently centrifuged at 10,000 rpm for 30 min at 4°C. Supernatant was collected, and protein purified using HisTag columns (GE) following manufacturer's recommended protocol. Protein was eluted using 500 mM imidazole, dialyzed in PBS, characterized using SDS-PAGE gel and was > 95% pure. Proteins were stored in 50% glycerol at -20°C.

[0494] To chemically conjugate the antibodies to the HaloTag-peptide fusion proteins, antibodies were buffered exchanged 2x into 10mM sodium bicarbonate buffer (pH 8.5) using Zeba spin desalting columns (ThermoFisher). Antibodies were then primed with 200µM amine-reactive HaloTag Succinimidyl Ester (04) Ligand (Promega) for 2 hr shaking at 1000 rpm at 22°C. Unreacted ligand was removed with two passes through Zeba spin columns in PBS buffer. Then, antibodies were covalently labeled with 30µM of the HaloTag fusion protein overnight at 4°C while shaking. Excess unreacted HaloTag fusion protein was removed using HaloLink Resin (Promega). Non-denaturing SDS-PAGE gel was used to characterize the conjugated antibodies. Mouse anti-human IL-6 monoclonal antibodies used in the human IL-6 immunoassay were clone 5IL6 (Thermo cat# M620) and clone 505E 9A12 A3 (Thermo cat# AHC0662). SDS-PAGE gels were performed on the labeled antibodies and it was determined that each antibody was labeled with a variable number of peptide-HaloTag fusion proteins, with the primary species containing 3-5 peptide labels (FIG. 49).

[0495] Binding kinetic studies were performed to establish maximum light output and signal duration of the fully complemented system as show in FIG. 50. The signal kinetics were compared between conditions: (1) peptide labeled antibodies and LgTrip 3546 (SEQ ID NO: 12) were pre-equilibrated with rhIL-6 for 90 minutes with addition of furimazine at time 0, (2) peptide labeled antibodies are pre-equilibrated with rhIL-6 for 90 minutes with addition of LgTrip 3546 and furimzine at time 0, and (3) all assay reagents are added to rhIL-6 at time 0. Condition 2 tracks the binding kinetics of LgTrip 3546 (SEQ ID NO: 12) to the peptide labeled antibodies:rhIL-6 complex. Condition 3 tracks the binding kinetics of the antibodies to the analyte and the LgTrip 3546 to the peptides. FIG. 50A displays the raw RLUs and FIG. 50B

displays the fold response as calculated by taking the RLU value generated in the presence of 5 ng/ml rhIL-6 divided by the background signal generated in the absence of rhIL-6. The assay buffer used was 0.01% BSA in PBS, pH 7.0, and assay reagent concentrations were 7 ng/ml for each peptide labeled antibody, 1 μM LgTrip 3546 (SEQ ID NO: 12) protein, and furimazine. FIG. 51 displays the dose response curve for the solution-based homogenous IL-6 immunoassay performed in a standard assay buffer consisting of 0.01% BSA in PBS, pH 7.0. This assay was shown to be extremely sensitive with a limit of detection (LOD) of 2.1 pg/ml, which resulted in a broad dynamic range of over 3-4 orders of magnitude, and maintained low variability (CVs <10%) throughout the linear range. For these experiments, 7 ng/ml of each peptide labeled antibody and 1 μM LgTrip 3546 (SEQ ID NO: 12) protein were incubated in the presence of rhIL-6 for 90 minutes. Furimazine was added, and luminescence signal analyzed.

Example 20

Lyophilized, single-reagent tripartite immunoassays in vials

[0496] To evaluate the potential application of lyophilization for preservation of the entire IL-6 tripartite immunoassay in a single vial, formulations containing peptide labeled antibodies (SmTrip9 Pep521 (SEQ ID NO: 16) and SmTrip10 Pep289 (SEQ ID NO: 17)), LgTrip 3546 (SEQ ID NO: 12), and furimazine were prepared. The 20X stock formulations are as follows:

[0497] Formulation A: 20 mM HEPES buffer (pH 8.0), 90 mM glycine, 20 mM histidine, 25 mg/ml sucrose, 0.01% polysorbate 80, 0.6 ug/ml clone 5IL6 antibody labeled with HaloTag-SmTrip9 Pep521 (SEQ ID NO: 16), 1.2 ug/ml 505E A12 A3 antibody labeled with HaloTag-SmTrip10 Pep289 (SEQ ID NO: 17), and 20 μM LgTrip 3546 (SEQ ID NO: 17).

[0498] Formulation B: 20 mM HEPES buffer (pH 8.0), 90 mM glycine, 20 mM histidine, 25 mg/ml sucrose, 0.01% polysorbate 80, 0.6 ug/ml clone 5IL6 antibody labeled with HaloTag-SmTrip9 Pep521 (SEQ ID NO: 16), 1.2 ug/ml 505E A12 A3 antibody labeled with HaloTag-SmTrip10 Pep289 (SEQ ID NO: 17), 20 μM LgTrip 3546 (SEQ ID NO: 12), and 100μM furimazine in ethanol.

[0499] Formulation C: 5 mM azothiothymine, 5 mM ascorbic acid, 2.5% pullulan w/v, 20 mM HEPES buffer (pH 8.0), 90 mM glycine, 20 mM histidine, 25 mg/ml sucrose, 0.01% polysorbate 80, 0.6 ug/ml clone 5IL6 antibody labeled with HaloTag-SmTrip9 Pep521 (SEQ ID

NO: 16) 1.2 ug/ml 505E A12 A3 antibody labeled with HaloTag-SmTrip10 Pep289 (SEQ ID NO: 17), 20 μM LgTrip 3546 (SEQ ID NO: 12), and 100μM furimazine in ethanol.

[0500] One mL aliquots of 20X stock solution was dispensed into 10 mL amber glass vials, and a runner stopper was partially inserted into the vial. Vials were loaded into the lyophilizer (Virtis Genesis 12EL lyophilizer) with shelves pre-chilled to 4.7°C. Product then underwent a freezing step with a shelf temperature of -50°C for 2 hr after which time the condenser step started. During the run, the condenser temperature ran between -5°C and -87°C. A vacuum pulled down ran next at the pressure set-points of 75 and 200 mTorr. Sublimation lasted ~7.5 hr, and desorption lasted ~16.1 hr. At the end of the lyophilization process, the vials were back-filled with nitrogen and sealed with fully inserted stoppers at ~600 Torr of pressure.

[0501] FIG. 52A displays the resulting lyophilized product for single-reagent, IL-6 NanoTrip (tripartite NanoLuc) immunoassays using formulations A and B..

[0502] Vials were stored at 25°C and tested at various timepoints post-lyophilization. For activity-based assays, single-reagent cakes were reconstituted with 10 mL of PBS containing 0.01% BSA. The vials were shaken manually and allowed to equilibrate at room temperature for 5 minutes. 50 μl of the reconstituted substrate was added to 50 μl of recombinant human IL-6 (source) reconstituted in the same BSA buffer. Formulation A requires the addition of furimazine, in which NANOGLO Live Cell Substrate (Promega N205) was used. Assays were performed in solid, white, nonbinding surface (NBS) plates (Costar) and analyzed on a GLOMAX Discover Multimode Microplate Reader (Promega) collecting total luminescence using kinetic or endpoint reads, depending on the experiment. FIG. 52B displays the signal/background assay performance of formulation A over a two-week time course at ambient temps showing that this formulation is shelf-stable and displays an excellent dose response curve over the time tested. However, when furimazine is added (i.e. Formulation B), reduced shelf-stability is observed (FIG. 52C).

[0503] FIG. 53A displays the resulting lyophilized product for a single-reagent, IL-6 NanoTrip (tripartite NanoLuc) immunoassay using formulation C. This formula results in a very desirable cake that is intact and mobile from the glass sides without any fragmenting. FIG. 53B displays the signal/background assay performance of formulation C over a 3 month time course of storage at ambient temperatures showing that this formulation is shelf-stable and displays an excellent dose response curve that is unchanged over the time tested. FIG. 54 shows the kinetic

profile of an IL-6 dose response of lyophilized formulation C post reconstitution in PBS containing 0.01% BSA.

[0504] To determine the lyophilized assay compatibility with complex human matrices, lyophilized cakes produced with formulation C were reconstituted in PBS (pH 7.0) containing 0.01% BSA. 50 µl was added to wells of 96-well microtiter plates containing 50 ul of rhIL-6 in 20% normal pooled human serum, citrate collected plasma, or urine. In all experiments, plates were incubated at room temperature for 90 minutes. Final concentration of the assay reagents in all experiments were 60 ng/ml SmTrip10-labeled antibody, 30 ng/ml SmTrip9-labeled antibody, 1 µM LgTrip 3546, and 5 µM furimazine. Luminescence was analyzed. FIG. 55 displays the signal/background results from these experiments indicating complex sample matrix compatibility with the single-reagent IL-6 NanoTrip immunoassay produced with formulation C.

Example 21

Lyophilized, single-reagent tripartite immunoassays in pre-filled, 96-well microtiter plates

[0505] To evaluate the potential application of lyophilization for preservation of the entire IL-6 NanoTrip (tripartite NanoLuc) immunoassay directly into a 96-well microtiter plates, formulations containing 5 mM azothiothymine, 5 mM ascorbic acid, 2.5% pullulan w/v, 20 mM HEPES buffer (pH 8.0), 90 mM glycine, 20 mM histidine, 25 mg/ml sucrose, 0.01% polysorbate 80, 0.12 ug/ml clone 5IL6 antibody labeled with HaloTag-SmTrip9 Pep521 (SEQ ID NO: 16), 0.24 ug/ml 505E A12 A3 antibody labeled with HaloTag-SmTrip10 Pep289 (SEQ ID NO: 17), 4 µM LgTrip 3546 (SEQ ID NO: 12), and 100µM furimazine in ethanol (same as formulation C in the previous example, but with a 4x reagent addition instead of a 20x stock reagent as used in the vials) were used.

[0506] Approximately 25 µl aliquots of 4X stock solution was dispensed into 96-well microtiter plates. Two types of plates were used: non-binding surface (Costar 3600) and non-treated surface (Costar 3912). Plates were loaded into the lyophilizer (Virtis Genesis 12EL lyophilizer) with shelves pre-chilled to 4.7°C. Product then underwent a freezing step with a shelf temperature of -50°C for 2 hr after when time the condenser step started. During the run, the condenser temperature ran between -5°C and -87°C. A vacuum pull down ran next at the pressure set-points of 75 and 200 mTorr. Sublimation lasted ~7.5 hr, and desorption lasted ~ 16.1

hr. At the end of the lyophilization process, the plates were back-filled with nitrogen and sealed with adhesive plate cover.

[0507] FIG. 56A depicts one of the plates with the lyophilized material in the bottom of the wells. The lyophilized cakes stayed in an intact cake, but were mobile when using the nonbinding surface plates. The lyophilized material stayed "stuck" on the bottom of the wells in the non-treated plates. FIG. 56B shows the resulting bioluminescence when 1X rhIL-6 was added directly to the wells and analyzed for luminescence using a GLOMAX luminometer. The resulting dose response curve showed excellent reconstitution and performance in both plates.

Example 22

Testing the effects of individual excipients in formulations using the solution-based, homogeneous IL-6 tripartite immunoassay

[0508] To determine the effects of assay performance of individual excipients used in the lyophilized formulations for the single-reagent NanoTrip (tripartite NanoLuc) immunoassays, the IL-6 model system in the solution-based assay was used with the effects of various excipients analyzed. FIG. 57A displays the assay background signals for the solution-based homogenous IL-6 immunoassay performed in a standard assay buffer consisting of 0.01% BSA in PBS, pH 7.0, and with the addition of various individual excipients as indicated on the X-axis. FIG. 57B displays the IL-6 dose response curve when the assay was performed in different buffers consisting of formulation C from Example 20 and modified versions of formulation C. For these experiments, 30 ng/ml 5IL6 antibody labeled with HaloTag-SmTrip9 Pep521 (SEQ ID NO: 16), 60 ng/ml 505E A12 A3 antibody labeled with HaloTag-SmTrip10 Pep289 (SEQ ID NO: 17), and 1 μM LgTrip 3546 (SEQ ID NO: 12) were incubated in the presence of rhIL-6 for 90 minutes. Furimazine (Promega Live Cell Substrate N205) was added according to manufacturer's instruction, but using the formulation indicated as buffer. Luminescent signal was analyzed using a GLOMAX luminometer. These experiments demonstrated that iterative experimentation is required to determine appropriate buffer components for NanoTrip immunoassays.

Example 23

Creating a solution-based and lyophilized, single-reagent tripartite immunoassays in vials for the target analyte human cardiac troponin I

[0509] The basic principle of the homogeneous NanoTrip (NanoLuc tripartite) cardiac troponin I immunoassay is depicted in FIG. 58. First, a pair of antibodies that target non-overlapping epitopes on human cardiac troponin I were chemically conjugated to SmTrip9 (or variants thereof) or HiBiT (or variants thereof) using the HaloTag® technology. When the labeled antibodies bind a cardiac troponin I analyte, the complementary subunits are brought into proximity thereby reconstituting a bright luciferase in the presence of the LgTrip 3546 protein and furimazine substrate. This assay is quantitative because the amount of luminescence generated by a standard plate-reading luminometer is directly proportional to the amount of target analyte present.

[0510] Genetic fusions containing SmTrip9 Pep521 (SEQ ID NO: 16) or SmTrip10 Pep289 (SEQ ID NO: 17) separated by either a 2X or 3X Gly-Ser-Ser-Gly linker to the amino terminus of HaloTag was achieved using the pFN29A HIS_6HaloTag T7 Flexi Vector (Promega). Glycerol stocks of *E. coli* expressing HisTag-HaloTag fusion protein were used to inoculate 50mL starter cultures, which were grown overnight at 37°C in LB media containing 25 ug/ml kanamycin. Starter cultures were diluted 1:100 into 500 mL fresh LB media, containing 25 ug/mL kanamycin, 0.12% glucose, and 0.2% rhamnose. Cultures were grown for 22-24 h at 25°C. Cells were pelleted by centrifugation (10,000 rpm) for 30 min at 4°C and re-suspended in 50 mL PBS. 1 mL protease inhibitor cocktail (Promega), 0.5 mL RQ1 DNase (Promega), and 0.5 mL of 10 mg/mL lysozyme (Sigma) were added, and the cell suspension was incubated on ice with mild agitation for 1 h. Cells were lysed by sonication at 15% power at 5 s intervals for 1.5 min (3 min total) and subsequently centrifuged at 10,000 rpm for 30 min at 4°C. Supernatant was collected, and protein purified using HisTag columns (GE) following the manufacturer's recommended protocol. Protein was eluted using 500 mM imidazole, dialyzed in PBS, characterized using SDS-PAGE gel and was > 95% pure. Proteins were stored in 50% glycerol at -20°C.

[0511] To chemically conjugate the antibodies to the HaloTag-peptide fusion proteins, antibodies were buffered exchanged 2x into 10mM sodium bicarbonate buffer (pH 8.5) using Zeba spin desalting columns (ThermoFisher). Antibodies were then primed with 200μM amine reactive HaloTag Succinimidyl Ester (04) Ligand (Promega) for 2 hr shaking at 1000 rpm at

22°C. Unreacted ligand was removed with two passes through Zeba spin columns in PBS buffer. Then, antibodies were covalently labeled with 30μM of the HaloTag fusion protein overnight at 4°C while shaking. Excess unreacted HaloTag fusion protein was removed using HaloLink Resin (Promega). Non-denaturing SDS-PAGE gel was used to characterize the conjugated antibodies. Anti-human cardiac troponin I monoclonal antibodies used in the human cardiac troponin I immunoassay were recombinant rabbit clone 1H11L19 (Invitrogen) and monoclonal mouse antibody clone 16A11 (Invitrogen).

[0512] FIG. 59A (raw RLUs) and 59B (signal/background) display the dose response curve for the solution-based homogenous cardiac troponin I immunoassay performed in a standard assay buffer consisting of 0.01% BSA in PBS, pH 7.0. Purified recombinant human cardiac troponin I (Fitzgerald) was used to generate the dose response curve. For these experiments, 2 ng/ml of clone 1H11L19 labeled with HaloTag-24gly/ser-SmTrip9 Pep521 (SEQ ID NO: 16), 40 ng/ml of clone 16A11 labeled with HaloTag-8gly/ser-SmallTrip10 Pep289 (SEQ ID NO: 17), and 1 μM LgTrip 3546 (SEQ ID NO: 12) protein were incubated in the presence of recombinant human cardiac troponin I for 90 minutes. Furimazine (Promega Live Cell Substrate N205) was added according to the manufacturer's instructions, but using 0.01% BSA in PBS as the buffer. Luminescent signal was analyzed on a GLOMAX luminometer.

[0513] To evaluate the potential application of lyophilization for preservation of the entire cardiac troponin I tripartite immunoassay in a single vial, formulations containing the peptide labeled antibodies (SmTrip9 Pep521 (SEQ ID NO: 16) and SmTrip10 Pep289 (SEQ ID NO: 17)), LgTrip 3546 (SEQ ID NO: 12), and furimazine were prepared. The 20X stock formulations are as follows:

[0514] Approximately, 5 mM azothiothymine, 5 mM ascorbic acid, 2.5% pullulan w/v, 20 mM HEPES buffer (pH 8.0), 90 mM glycine, 20 mM histidine, 25 mg/ml sucrose, 0.01% polysorbate 80, 0.08 ug/ml clone 1H11L19 antibody labeled with HaloTag-SmTrip9 Pep521 (SEQ ID NO: 16), 1.6 ug/ml of clone 16A11 antibody labeled with HaloTag-SmTrip10 Pep289 (SEQ ID NO: 17), 20 μM LgTrip 3546 (SEQ ID NO: 12), and 200μM furimazine (Promega NANOGLO substrate N113).

[0515] One mL aliquots of 20X stock solution were dispensed into 10 mL amber glass vials, and a runner stopper was partially inserted into the vial. Vials were loaded into the lyophilizer (Virtis Genesis 12EL lyophilizer) with shelves pre-chilled to 4.7°C. Product then underwent a

freezing step with a shelf temperature of -50°C for 2 hr after which time the condenser step started. During the run, the condenser temperature ran between -5°C and -87°C. A vacuum pull down ran next at the pressure set-points of 75 and 200 mTorr. Sublimation lasted ~7.5 hr, and desorption lasted ~16.1 hr. At the end of the lyophilization process, the vials were back-filled with nitrogen and sealed with fully inserted stoppers at ~600 Torr of pressure.

[0516] For activity-based assays, single-reagent cakes were reconstituted with 10 mL of PBS containing 0.01% BSA. The vials were shaken manually and allowed to equilibrate at room temperature for 5 minutes. 50 µl of the reconstituted single-reagent cardiac troponin I NanoTrip (tripartite NanoLuc) immunoassay was added to 50 µl of recombinant human cardiac troponin I (Fitzgerald) that was reconstituted in the same BSA buffer or with 20% human serum diluted in General Serum Diluent (Immunochemistry Technologies). Assays were performed in solid, white, nonbinding surface (NBS) plates (Costar) and analyzed on a GLOMAX Discover Multimode Microplate Reader (Promega) collecting total luminescence using an endpoint read. FIG. 60 shows the cardiac troponin I dose response curve of the resulting bioluminescence upon reconstitution of the single-reagent troponin NanoTrip immunoassay with the sample in 0.01% BSA in PBS buffer or in the presence of the complex matrix sample of human serum diluted in General Serum Diluent. Troponin was effectively detected even in the presence of serum using this immunoassay.

Example 24

Investigating and mitigating the effects of complex sample matrices on tripartite immunoassay performance

[0517] A solution-based, homogeneous IL-6 NanoTrip (tripartite NanoLuc) immunoassay was tested to determine if the assay was compatible with human sample types commonly analyzed for clinical biomarkers, and factors in the samples that might affect the performance of the assay and possible solutions to mitigate these effects were investigated. This is critical because sample matrix interference effects in immunoassays, defined as the effect of a substance present in the sample that alters the correct value of the result, are a common phenomenon especially in homogenous formats due to the removal of the wash steps.

[0518] Reagents used for the following experiments were the HaloTag-peptide labeled antibodies described in Example 19. 30 ng/ml clone 5IL6 antibody labeled with HaloTag-SmTrip9 Pep521 (SEQ ID NO: 16), 60 ng/ml 505E A12 A3 antibody labeled with HaloTag-

SmTrip10 Pep289 (SEQ ID NO: 17), 1 μM LgTrip 3546 (SEQ ID NO: 12), and NANOGLO Live Cell Substrate (Promega N205) or NANOGLO substrate (Promega N113), which were used according to the manufacturer's instructions, but were diluted in the given buffer for that experiment. Assays were performed +/- 50 ng/ml recombinant human IL-6 (R&D Systems) with assay backgrounds, and Bmax analyzed. Assays were allowed to incubate on the bench for 90 minutes prior to addition of substrate. Assays were performed in solid, white, nonbinding surface (NBS) plates (Costar) and analyzed on a GLOMAX Discover Multimode Microplate Reader (Promega) collecting total luminescence using an endpoint read.

[0519] FIG.61 shows the solution-based, homogeneous IL-6 NanoTrip (tripartite NanoLuc) assay background in the presence of increasing normal, pooled human serum when the assay was performed in (A) 0.01% BSA in PBS (pH 7.0) assay buffer or (B) in General Serum Diluent (Immunochemistry Technologies) and using NANOGLO Live Cell Substrate (Promega N205). General Serum Diluent mitigated non-specific IgG effects and had a positive effect by decreasing the assay background. FIG. 62 shows the bioluminescent response when in the presence of 50 ng/ml rhIL-6 and increasing human serum when the assay was performed in (A) 0.01% BSA in PBS (pH 7.0) assay buffer or (B) General Serum Diluent and using NANOGLO Live Cell Substrate (Promega N205). General Serum Diluent displayed a slightly lower Bmax overall, but less of a loss in signal with increasing human serum. FIG. 63A-D shows the fold response of results when the rhIL-6 screening assays were performed with 0.01% BSA in PBS (pH 7.0) or General Serum Diluent and using NANOGLO Live Cell Substrate (Promega N205) or NANOGLO substrate (Promega N113) and testing in increasing amounts of normal, pooled human serum or plasma. Overall, using General Serum Diluent paired with the NANOGLO Live Cell Substrate (Promega N205) provided the best assay results in these complex sample matrices.

[0520] Next, the effects of endogenous IgG in human serum samples had on assay performance was determined. Using the solution-based, homogeneous IL-6 NanoTrip assay +/- 50 ng/ml rhIL-6 in General Serum Diluent, the bioluminescent response when running the assay in normal, pooled human serum or in serum that had been depleted of endogenous IgG was analyzed. FIG. 64 shows the fold response of this experiment, which indicates that endogenous IgG is one of the components in serum that negatively effects the performance of the immunoassay.

[0521] Next, the effects of blood biochemistry on the solution-based, homogenous IL-6 tripartite immunoassay was investigated using the VeriChem reference plus chemistry kit, which contains the following:

Analyte	**Units**	**Level A**	**Level B**	**Level C**	**Level D**	**Level E**
Glucose	mg/dL	5	40	75	110	145
Urea Nitrogen	mg/dL	1.0	7.5	14.0	20.5	27.0
Creatinine	mg/dL	0.04	1.24	2.44	3.64	4.84
Calcium	mg/dL	1.0	1.5	2.0	2.5	3.0
Phosphorus	mg/dL	0.2	0.7	1.2	1.7	2.2
Magnesium	mg/dL	0.16	0.46	0.76	1.06	1.36
Magnesium	mEq/L	0.132	0.38	0.63	0.87	1.12
Triglyceride	mg/dL	2	49	240	143	190

[0522] The IL-6 NanoTrip assay was run in the presence of Level A-E diluted in general serum diluent and using NANOGLO Live Cell Substrate (Promega N205) to determine the effects of increasing these blood chemistry components on assay performance. FIG. 65A shows the assay background in raw RLUs, FIG. 65B shows the Bmax signal when in the presence of 50 ng/ml rhIL-6, and FIG. 65C shows the signal over background results. The results indicate that increasing these chemistry components had an effect on increasing assay background as well as decreasing the Bmax impacting the overall signal to background of the assay performance.

[0523] To determine the effects of urine on the solution-based, homogeneous IL-6 NanoTrip immunoassay performance, a IL-6 screening assay in the presence of increasing normal, pooled human urine diluted in General Serum Diluent and NANOGLO substrate (Promega N113) or NANOGLO Live Cell Substrate (Promega N205) was performed. FIG. 66A shows the assay background in raw RLUs, FIG. 66B shows the Bmax signal when in the presence of 50 ng/ml rhIL-6, and FIG. 66C shows the signal over background results. The results indicate that the IL-6 NanoTrip immunoassay was compatible with human urine when using the General Serum Diluent paired with the NANOGLO Live Cell Substrate (Promega N205).

Example 25

Creating a stable, lyophilized substrate and LgTrip cake reagent in a single vial

[0524] To evaluate the potential application of lyophilization for preservation of furimazine, LgTrip and furimazine were paired with LgTrip 3546 used as a general detection reagent for tripartite applications and supplied in a single vial. Formulations containing furimazine, LgTrip 3546 (SEQ ID NO: 12), and furimazine with LgTrip 3546 were prepared. The 20X stock formulations are as follows:

[0525] Furimazine only formulation: 5 mM azothiothymine, 5 mM ascorbic acid, 2.75% pullulan w/v, 200 µM furimazine in ethanol, and ddH20 millipore

[0526] LgTrip 3546 only formulation: 5 mM azothiothymine, 5 mM ascorbic acid, 2.75% pullulan w/v, 20 µM LgTrip 3546 (SEQ ID NO: 12), and ddH_20 (Millipore)

[0527] Furimazine with LgTrip 3546formulation: 5 mM azothiothymine, 5 mM ascorbic acid, 2.75% pullulan w/v, 200 µM furimazine in ethanol, 20 µM LgTrip 3546 (SEQ ID NO: 12) and ddH_20 (Millipore).

[0528] One mL aliquots of 20X stock solution was dispensed into 10 mL amber glass vials, and a runner stopper was partially inserted into the vial. Vials were loaded into the lyophilizer (Virtis Genesis 12EL lyophilizer) with shelves pre-chilled to 4.7°C. Product then underwent a freezing step with a shelf temperature of -50°C for 2 hr after when time the condenser step started. During the run, the condenser temperature ran between -5°C and -87°C. A vacuum pull down ran next at the pressure set-points of 75 and 200 mTorr. Sublimation lasted ~7.5 hr and desorption lasted ~16.1 hr. At the end of the lyophilization process, the vials were back-filled with nitrogen and sealed with fully inserted stoppers at ~600 Torr of pressure.

[0529] Vials were stored at 25°C or 60°C and tested at various time points post-lyophilization. For activity-based assays, lyophilized cakes were reconstituted with 10 mL of PBS containing 0.01% BSA. The vials were shaken manually and allowed to equilibrate at room temperature for 5 minutes. 50 µl of the reconstituted substrate was added to 50 µl of purified NANOLUC enzyme (Promega) or dipeptide (SEQ ID NO: 14) that was reconstituted in the same BSA buffer. LgTrip 3546 only formulations required the addition of furimazine in which NANOGLO Live Cell Substrate (Promega N205) was used. Assays were performed in solid, white, nonbinding surface (NBS) plates (Costar) and analyzed on a GLOMAX Discover Multimode Microplate Reader (Promega) collecting total luminescence using an endpoint read. FIG. 67 displays the Bmax signal produced for (A) furimazine only formulation when in the presence of NanoLuc, (B) LgTrip 3546 only formulation when in the presence of the dipeptide, and (C) furimazine with LgTrip 3546 formulation when in the presence of dipeptide. All formulations displayed thermal stability at all temperatures tested for the 100 day duration of the storage conditions, as opposed to the N205 substrate which is predissolved in organic solvent.

Example 26

Creating a solution-based and lyophilized, single-reagent tripartite immunoassays in vials for the target analytes anti-TNFα biologics

[0530] The basic principle of the homogeneous anti-TNFα biologics NanoTrip (tripartite NanoLuc) immunoassay is depicted in FIG. 68. In this model, protein G-SmTrip9 (or variants thereof) fusion proteins and TNFα-HiBiT (or variants thereof) fusion proteins were used. Protein G will bind the Fc region of the anti-TNFα biologic antibody analyte, and the analyte itself will bind the TNFα thus bringing the complementary subunits into proximity, thereby reconstituting a bright luciferase in the presence of the LgTrip 3546 protein and furimazine substrate. This assay

is quantitative because the amount of luminescence generated by a standard plate-reading luminometer is directly proportional to the amount of target analyte present.

[0531] ***6xHis-TNFa-15GS-HiBiT (ATG-3998).*** Genetic fusions containing the SmTrip10 (SEQ ID NO: 15) separated by a 15GS linker (SSSGGGGSGGGSSGG) to the carboxyl-terminus of TNFα was achieved using the pF4Ag CMV Flexi Vector (Promega). Purified plasmid DNA of the TNFα-strand 10 fusion was transformed into Shuffle T7 *E. coli* K12 (New England Biolabs) and plated at a 1:100 dilution on an LB plate containing 100 µg/ml ampicillin and incubated overnight at 37°C. A colony from this plate was used to inoculate 50 mL starter cultures, which were grown overnight at 37°C in LB media containing 100 µg/ml ampicillin. Starter cultures were diluted 1:100 into 500 mL fresh LB media containing 100 µg/ml ampicillin and were incubated at 37°C until it reached an OD of 0.6, at which time a final concentration of 1 mM IPTG was added to the sample. After IPTG inoculation, cultures were grown overnight at 25°C. Cells were pelleted by centrifugation (10,000 rpm) for 30 min at 4°C and re-suspended in 50 mL TBS, 1 mL protease inhibitor cocktail (Promega), 0.5 mL RQ1 DNase (Promega), and 1 mL of 10 mg/mL lysozyme (Sigma), and the cell suspension was incubated on ice with mild agitation for 1 h. Cells were lysed by three freeze-thaw cycles from -80°C freezer to a 37°C water bath and subsequently centrifuged at 10,000 rpm for 30 min at 4°C. Supernatant was collected and protein was purified using Ni Sepharose 6 Fast Flow resin (GE), following manufacturer's recommended protocol. Protein was eluted using a step-wise imidazole elution starting at 100mM imidazole and reaching up to 500 mM imidazole, dialyzed in TBS, characterized using SDS-PAGE gel and was > 95% pure. Proteins were stored in 50% glycerol at -20°C.

[0532] ***SmTrip9(521)-15GS-PtnG-6xHis (ATG4002).*** Genetic fusions containing the SmTrip9 (SEQ ID NO: 13) separated by a linker (GSSGGGGSGGGGSSG) to the amino terminus of Protein G was achieved using the pF1A T7 Flexi Vector (Promega). Glycerol stocks of E. coli expressing SmTrip9(521)-PtnG fusion protein was used to inoculate 50mL starter cultures, which were grown overnight at 37°C in LB media containing 100 µg/ml ampicillin. Starter cultures were diluted 1:100 into 500 mL fresh LB media, containing 100 µg/mL ampicillin, 0.15% glucose, and 0.1% rhamnose. Cultures were grown for 16-24 h at 25°C. Cells were pelleted by centrifugation (10,000 rpm) for 30 min at 4°C and re-suspended in 50 mL TBS. 1 mL protease inhibitor cocktail (Promega), 0.5 mL RQ1 DNase (Promega), and 1 mL of 10 mg/mL lysozyme (Sigma) were added, and the cell suspension was incubated on ice with mild

agitation for 1 h. Cells were lysed by three freeze-thaw cycles from -80°C freezer to a 37°C water bath and subsequently centrifuged at 10,000 rpm for 30 min at 4°C. Supernatant was collected and protein purified using HisTag columns (GE), following manufacturer's recommended protocol. Protein was eluted using gradient elution with a 500 mM imidazole final concentration, dialyzed in TBS, characterized using SDS-PAGE gel and was > 95% pure. Proteins were stored in 50% glycerol at -20°C.

[0533] FIG. 69 displays the dose response curves for the solution-based homogenous anti-TNFα biologics immunoassay performed in a standard assay buffer consisting of 0.01% BSA in PBS, pH 7.0. For these experiments, 10 nM of protein G-15gly/ser-SmTrip9 Pep521 (SEQ ID NO: 16), 10 nM TNFα-15 gly/ser–SmTrip10 Pep289 (SEQ ID NO: 17), and 1 μM LgTrip 3546 (SEQ ID NO: 12) protein were incubated in the presence of (A) Remicade, (B) Humira, and (C) Enbrel for 90 minutes. Furimazine (NANOGLO Live Cell Substrate; Promega N205) was added, and total luminescence signal was analyzed using a GLOMAX Discover.

[0534] To evaluate the potential application of lyophilization for preservation of the entire anti-TNFαTNFα biologics, NanoTrip and NanoBiT immunoassays in single vial formulations containing peptide-labeled fusion proteins and LgTrip 3546 (SEQ ID NO: 12; for NanoTrip assays) and furimazine were prepared. The 20X stock formulations are as follows:

[0535] NanoTrip anti-TNFα biologics immunoassay: 5 mM azothiothymine, 5 mM ascorbic acid, 2.75% w/v pullulan, ddH20 (Millipore), 200μM furimazine in ethanol, 20 μM LgTrip 3546 protein (SEQ ID NO:12), 200 nM protein G-SmTrip9 Pep521 (SEQ ID NO: 16) fusion protein, and 200 nM TNFα-SmTrip10 Pep289 (SEQ ID NO:17) fusion protein.

[0536] NanoBiT anti-TNFα biologics immunoassay: 5 mM azothiothymine, 5 mM ascorbic acid, 2.75% w/v pullulan, ddH20 (Millipore), 200 μM furimazine in ethanol, 200 nM protein G-SmBiT (SEQ ID NO:10) fusion protein, and 200 nM TNFα-LgBiT (SEQ ID NO: 12) fusion protein.

[0537] One mL aliquots of 20X stock solution was dispensed into 10 mL amber glass vials, and a runner stopper was partially inserted into the vial. Vials were loaded into the lyophilizer (Virtis Genesis 12EL lyophilizer) with shelves pre-chilled to 4.7°C. Product then underwent a freezing step with a shelf temperature of -50°C for 2 hr after which time the condenser step started. During the run, the condenser temperature ran between -5°C and -87°C. A vacuum pull down ran next at the pressure set-points of 75 and 200 mTorr. Sublimation lasted ~7.5 hr and

desorption lasted ~16.1 hr. At the end of the lyophilization process, the vials were back-filled with nitrogen and sealed with fully inserted stoppers at ~600 Torr of pressure.

[0538] For activity-based assays, single-reagent cakes were reconstituted with 10 mL of PBS containing 0.01% BSA. The vials were shaken manually and allowed to equilibrate at room temperature for 5 minutes. 50 µl of the reconstituted single-reagent anti-TNFα biologics NanoTrip and NanoBiT immunoassays were added to 50 µl of Remicade in a titration that was reconstituted in the same BSA buffer. Assays were performed in solid, white, nonbinding surface (NBS) plates (Costar) and analyzed on a GLOMAX Discover Multimode Microplate Reader (Promega) collecting total luminescence using a kinetic read. FIG. 70 shows the Remicade dose response curves of the resulting bioluminescence upon reconstitution of the single-reagent Remicade (A) NanoTrip immunoassay or (B) NanoBiT immunoassay.

[0539] Testing the thermal stability of these lyophilized, single-reagent anti-TNFα biologics NanoTrip and NanoBiT immunoassays when stored at ambient temperatures indicated that both assays, when reconstituted in 0.01% BSA in PBS (pH 7.0) in the presence or absence of 100 nM Remicade, displayed shelf stability and a significant increase in signal when the analyte Remicade is present. Results are shown in FIG. 71.

Example 27

Developing stable, lyophilized tripartite and NanoBiT immunoassay using a split-reagent approach

[0540] To evaluate the potential application of lyophilization for preservation of separate components of the anti-TNFα biologics, NanoTrip and NanoBiT immunoassays that are then combined in a single vial formulations containing the peptide labeled fusion proteins and LgTrip 3546 (SEQ ID NO: 12; for NanoTrip assays) and furimazine were prepared. The 20X stock formulations are as follows:

[0541] NanoBiT anti-TNFα biologics immunoassay:

[0542] Furimazine with LgBiT-TNFα: 5 mM azothiothymine, 5 mM ascorbic acid, 2.75% w/v pullulan, ddH20 (Millipore), 200 µMfurimazine in ethanol, and 200 nM TNFα-LgBiT (SEQ ID NO: 12) fusion protein.

[0543] NanoBiT protein G: 5 mM azothiothymine, 5 mM ascorbic acid, 2.75% w/v pullulan, ddH20 millipore, 200 nM protein G-SmBiT (SEQ ID NO: 10) fusion protein

[0544] NanoTrip anti-TNFα biologics immunoassay:

[0545] Furimazine with LgTrip 3546: 5 mM azothiothymine, 5 mM ascorbic acid, 2.75% w/v pullulan, ddH_20 (Millipore), 200 μMfurimazine in ethanol, 20 μM LgTrip 3546 protein (SEQ ID NO: 12),

[0546] Protein G with TNFα: 5 mM azothiothymine, 5 mM ascorbic acid, 2.75% w/v pullulan, ddH20 (Millipore), 200 nM protein G-SmTrip9 Pep521 (SEQ ID NO: 16) fusion protein, and 200 nM TNFα-SmTrip10 Pep289 (SEQ ID NO: 17) fusion protein.

[0547] Formulations were lyophilized as separate components then manually combined to create the complete immunoassay. Cakes were reconstituted with Opti-MEM (Gibco), and 50 ul added to 50 μl of Remicade in a dose titration. Assays were performed in solid, white, nonbinding surface (NBS) plates (Costar) and analyzed on a GLOMAX Discover Multimode Microplate Reader (Promega) collecting total luminescence using a kinetic read. FIG. 72 displays the process and assay results for the NanoBiT anti-TNFα biologics "split-cake" lyophilized immunoassay. FIG. 72A depicts the independent lyophilized products. FIG. 72B depicts the results after manually combining the two separate cakes into one microcentrifuge tube. FIG. 72C depicts the lyophilized products after reconstitution with Opti-MEM buffer. FIG. 72D displays the kinetic bioluminescence results when in the presence of increasing amounts of Remicade. FIG. 73 displays the kinetic bioluminescence results for the anti-TNFα biologics NanoTrip assay using a kinetic read for bioluminescence in the presence of Remicade after following the same process laid out in FIG. 72. The dual cake format also created a successful immunoassay for Remicade.

Example 28

Developing a cell-based, homogeneous tripartite assay for the quantitation of anti-EGFR biologics

[0548] A bulk transfection was performed on HEK293 cells by preparing a 10 μg/ml solution of DNA with a 1:10 dilution of IL6-VSHiBiT-15GS-EGFR (GSSGGGGSGGGGSS) (ATG-4288) and pGEM3Z carrier DNA (Promega). FuGENE HD was added to the DNA mixture to form a lipid:DNA complex. This complex was added to HEK293 cells with an adjusted cell density of $2x10^5$ cells/ml and incubated at 37°C and 5% CO_2 overnight.

[0549] Transfected HEK293 cells were added to 96-well NBS plates (a separate plate for each SmTrip-15GS-G being tested) at a final concentration of $2x10^5$ cells/well. A reagent mixture of LgTrip 3546 and SmTrip9-G was added to the cells at a final concentration of 1 μM LgTrip 3546

and 10nM SmTrip9-15GS-G. A 24-point panitumumab titration was added to each well with a final starting concentration of 100 nM and diluted 1:2 with a final ending concentration of 0 nM. All plates were covered and incubated for an hour at 37°C and 5% CO_2. NANOLUC Live Cell Substrate was added to all wells at a final concentration of 10 μM, and luminescence of each plate was subsequently read on a luminometer. The following SmTrip9-G constructs were tested: ATG4002 SmTrip9(521)-15GS-G (SEQ ID NO: 724); ATG4496 SmTrip9(743)-15GS-G (SEQ ID NO: (726); ATG4558 SmTrip9(759)-15GS-G (SEQ ID NO: 728); and ATG4551 SmTrip9(760)-15GS-G (SEQ ID NO: 730). Each configuration was successful in quantitatively detecting panitumumab.

Example 29

Testing various SmTrip9-protein G fusion proteins in solution-based, homogeneous anti-TNFα biologics tripartite immunoassays

[0550] FIG. 77 displays the dose response curves for the solution-based homogenous anti-TNFα biologics immunoassay using SmTrip9 variants SmTrip9 pep521 (SEQ ID NO: 16), SmTrip9 pep743 (SEQ ID NO: 21), SmTrip9 pep759 (SEQ ID NO: 22), or SmTrip 9 pep760 (SEQ ID NO: 23) in a standard assay buffer consisting of 0.01% BSA in PBS, pH 7.0. For these experiments, 10 nM of protein G-15gly/ser-SmTrip9 variant, 10 nM TNFα-15 gly/ser–SmTrip10 Pep289 (SEQ ID NO: 17), and 1 μM LgTrip 3546 (SEQ ID NO: 12) protein were incubated in the presence of Remicade for 90 minutes. Furimazine (NANOGLO Live Cell Substrate; Promega N205) was added, and total luminescence signal was analyzed using a GLOMAX Discover. All of the SmTrip9 variants were successful in the assay detecting Remicade, albeit with different levels of background and Bmax.

[0551] To evaluate the potential application of lyophilization for preservation of the entire anti-TNFα biologics, NanoTrip immunoassays in single vial formulations containing peptide-labeled fusion proteins and LgTrip 3546 (SEQ ID NO: 12) and furimazine were prepared. The 20X stock formulations are as follows:

[0552] NanoTrip anti-TNFα biologics immunoassay: 5 mM azothiothymine, 5 mM ascorbic acid, 2.75% w/v pullulan, ddH20 (Millipore), 200μM furimazine in ethanol, 20 μM LgTrip 3546 protein (SEQ ID NO:12), 200 nM protein G-SmTrip9 variant fusion protein, and 200 nM TNFα-SmTrip10 Pep289 (SEQ ID NO:17) fusion protein.

[0543] One mL aliquots of 20X stock solution was dispensed into 10 mL amber glass vials, and a runner stopper was partially inserted into the vial. Vials were loaded into the lyophilizer (Virtis Genesis 12EL lyophilizer) with shelves pre-chilled to 4.7°C. Product then underwent a freezing step with a shelf temperature of -50°C for 2 hr after which time the condenser step started. During the run, the condenser temperature ran between -5°C and -87°C. A vacuum pull down ran next at the pressure set-points of 75 and 200 mTorr. Sublimation lasted ~7.5 hr and desorption lasted ~16.1 hr. At the end of the lyophilization process, the vials were back-filled with nitrogen and sealed with fully inserted stoppers at ~600 Torr of pressure. FIG. 77B provides the dose response curve for Remicade using the lyophilized anti-TNFα biologics immunoassay.

Example 30

Direct-labeling of antibodies via reactive peptides for development of solution-based, homogenous IL-6 immunoassays

[0553] The basic principle of homogeneous NanoLuc tripartite immunoassays with directly-labeled antibodies is depicted in FIG. 78. First, a pair of antibodies that target non-overlapping epitopes on IL-6 are chemically conjugated to SmTrip9 or SmTrip10-based reactive peptides. When the labeled antibodies bind IL-6 analyte, the complementary subunits are brought into proximity, thereby reconstituting a bright luciferase that produces a bioluminescent signal in the presence of the LgTrip protein and furimazine substrate. The amount of luminescence generated by this assay configuration is directly proportional to the amount of target analyte.

[0554] SmTrip9 variants such as Pep693 (SEQ ID NO: 20), Pep895 (SEQ ID NO: 24), and Pep929 (SEQ ID NO: 25) or SmTrip10 variants such as Pep691 (SEQ ID NO: 18) and Pep692 (SEQ ID NO: 19) were individually dissolved in DMF to 5mM. Antibodies were buffered exchanged 2x into 10mM sodium bicarbonate buffer (pH 8.5) using Zeba spin desalting columns (ThermoFisher). Subsequently, these antibodies were combined with 20x molar excess of a reactive peptide for 1 hr at 4°C while shaking in order to covalently label the proteins. Unreacted label was removed with two passes through Zeba spin columns in PBS buffer. To create the reagents for the exemplary human IL-6 immunoassay, the mouse anti-human IL-6 monoclonal antibodies clone 5IL6 (Thermo cat# M620) and clone 505E 9A12 A3 (Thermo cat# AHC0662) were used. SmTrip9 reactive peptides were used to label antibody 5IL6 while SmTrip10 reactive peptides were used to label antibody 505E. The denaturing SDS-PAGE gel shown in FIG. 79

was used to characterize the conjugated antibodies. The gel revealed that the degree of antibody labeling was dependent on the peptide sequence and chemical structure of the label.

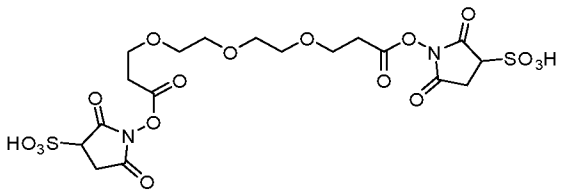
[0555] FIGS. 80-82 display raw RLU dose response curves for antibody conjugates in the presence of a rhIL-6 titration series. For these experiments, rhIL-6 and antibody conjugates were incubated for 90 minutes with 1 μM LgTrip 3546 (SEQ ID NO: 12) in PBS (pH 7.0) with 0.01% BSA. After addition of N205, luminescence signal was measured. Data in FIG. 80 were generated using 15 ng/ml of SmTrip9-labeled variant (HW-0984 or HW-1010) 5IL6 antibody and 60 ng/ml of SmTrip10-labeled variant (HW-0977) 505E antibody. Data in FIG. 81 were generated using 62.5 ng/ml of SmTrip9-labeled (HW-0984) 5IL6 antibody and 60 ng/ml of SmTrip10-labeled (HW-1053) 505E antibody. Data in FIG. 82 were generated using the following concentrations of antibody conjugates: 15 ng/ml HW-1043 (SEQ ID NO: 24) + 30 ng/ml HW-1053 (SEQ ID NO: 18), 15 ng/ml HW-1052 (SEQ ID NO: 25) + 15 ng/ml HW-1053, (SEQ ID NO: 18) 15 ng/ml HW-1055(SEQ ID NO: 25) + 15 ng/ml HW-1053 (SEQ ID NO: 18), 60 ng/ml HW-1042 (SEQ ID NO: 20) + 8 ng/ml HW-1053 (SEQ ID NO: 18), and 60 ng/ml HW-1050 (SEQ ID NO: 27) + 8 ng/ml HW-1053 (SEQ ID NO: 18). In this experiment, SmTrip9 variant labels HW-1050 (SEQ ID NO: 27) and HW-1043 (SEQ ID NO: 24) gave the best signal to background displaying close to 10^6 RLUs in the presence of high rhIL-6 concentrations and low light output in the absence of the analyte. In contrast, SmTrip9 variant labels HW-1055(SEQ ID NO: 25 (SulfoSE-PEG3)) and HW-1052 (SEQ ID NO: 25 (SulfoSE-PEG6)) had high signal even in the absence of rhIL-6 suggesting these labels spontaneously assemble into the reconstituted luciferase. FIG. 83 displays light output from titration of individual antibody conjugates in PBS (pH 7.0) with 0.01% BSA, 1 μM LgTrip 3546 (SEQ ID NO: 12), and N205. Most conjugates show RLUs equivalent to furimazine background (~100 RLU), and no increase in RLU with increasing concentration of labeled antibodies. Conjugates HW-0984 (SEQ ID NO: 20) and HW-1053 (SEQ ID NO: 19) were exceptions, generating increasing RLUs with concentration and reaching over 1,000 at concentrations above 100 ng/ml. In FIG. 84, two SmTrip9 conjugates with high S/B (labeled with HW-1050 (SEQ ID NO: 27) and HW-1043 (SEQ ID NO: 24)) were assayed under conditions described for FIG. 82, but with 1 μM LgTrip 5146 (SEQ ID NO: 451), producing results similar to LgTrip 3546 (SEQ ID NO: 12), demonstrating the feasibility of using different LgTrp variants to construct these assays.

[0556] Components for homogeneous tripartite NanoLuc immunoassays can also be constructed by direct-labeling antibodies with SmTrip9 or SmTrip10 variants that contain a fluorophore such as tetramethylrhodamine (TMR). This is depicted schematically in FIG. 85 including the expected BRET from the luciferase to the fluorophore labels. Kinetic reads for BRET with labels HW-0987 (SmTrip9 variants with TMR) and HW-0992 (SmTrip10 variants with TMR) in the IL-6 immunoassay are shown in FIG. 86. BRET was observed only in the presence of rhIL-6 analyte demonstrating the complementation and energy transfer are occurring when the analyte brings these components together.

Example 31

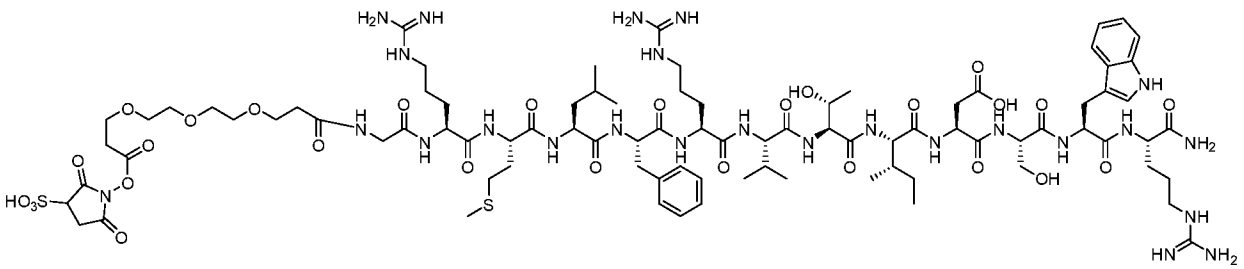
SulfoSE-PEG3-SmTrip9 Pep693 (HW-0984)

[0557] PEG3 bis Sulfo-SE



[0558] 3,3'-((oxybis(ethane-2,1-diyl))bis(oxy))dipropionic acid (55 mg, 0.22 mmol) was dissolved in anhydrous DMF, and then diisopropylethylamine (120 mg, 0.88 mmol) and HATU (176 mg, 0.45 mmol) added. The mixture was stirred for five minutes. Meanwhile, N-hydroxy-2,5-dioxopyrrolidine-3-sulfonic acid (90 mg, 0.46 mmol) was dissolved in 5 ml DMSO and then added to the previous solution dropwise. The mixture was stirred for another hour until LC-MS shows disappearance of acid. The solution was directly used in the next step. Calculated: m/z = 603.05 [M^-]; measured (ESI): m/z = 603.04 [M^-].

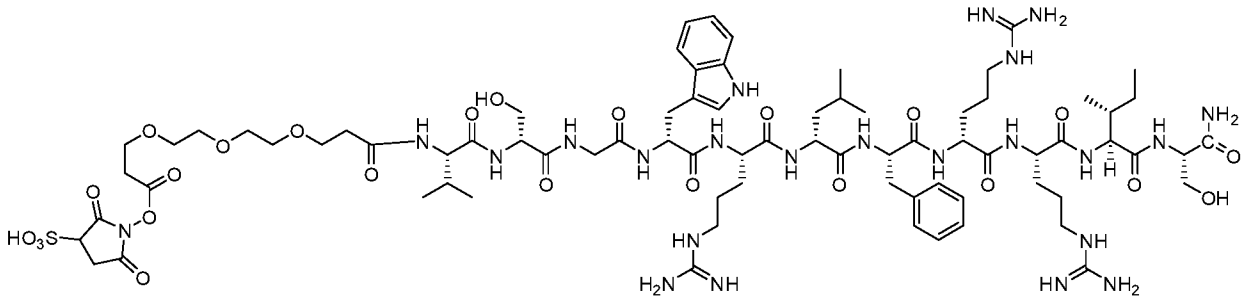
[0559] SulfoSE-PEG3-SmTrip9 Pep693 (HW-0984)



[0560] SmTrip9 Pep693 (GRMLFRVTINSWR, 27mg, 0.045mmol) was dissolved in DMF. The solution was then added to the previous PEG3 bis Sulfo-SE solution. The mixture was then stirred for another hour and directly purified by preparative HPLC. Calculated: m/z = 1022.98 $[M+2H]^{2+}$; measured (ESI): m/z = 1023.09 $[M+2H]^{2+}$.

Example 32

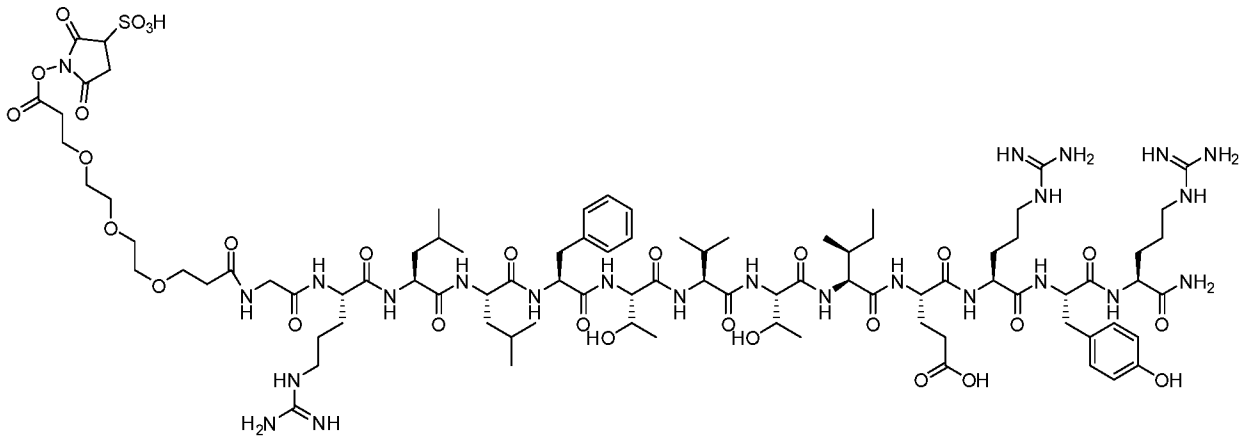
SulfoSE-PEG3-SmTrip10 Pep691 (HW-0977)



[0561] HW-0977 was synthesized by the same method as HW-0984. Calculated: m/z = 892.93 $[M+2H]^{2+}$; measured (ESI): m/z = 893.61 $[M+2H]^{2+}$.

Example 33

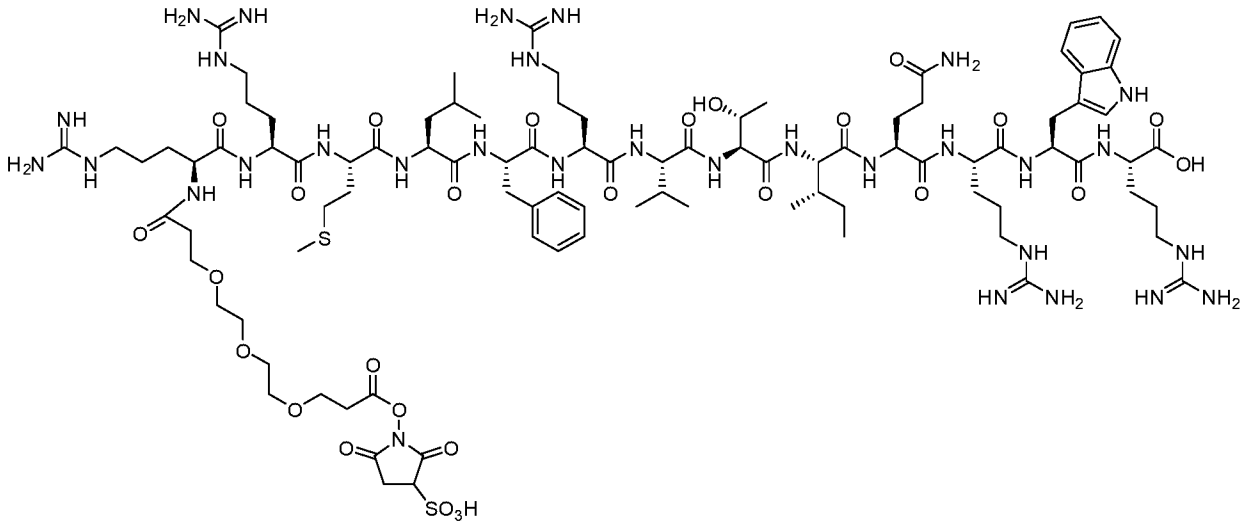
SulfoSE-PEG3-SmTrip9 Pep895 (HW-1010)



[0562] HW-1010 was synthesized by the same method as HW-0984. Calculated: m/z = 1016.51 $[M+2H]^{2+}$; measured (ESI): m/z = 1016.92 $[M+2H]^{2+}$.

Example 34

SulfoSE-PEG3-SmTrip9 Pep929 (HW-1055)

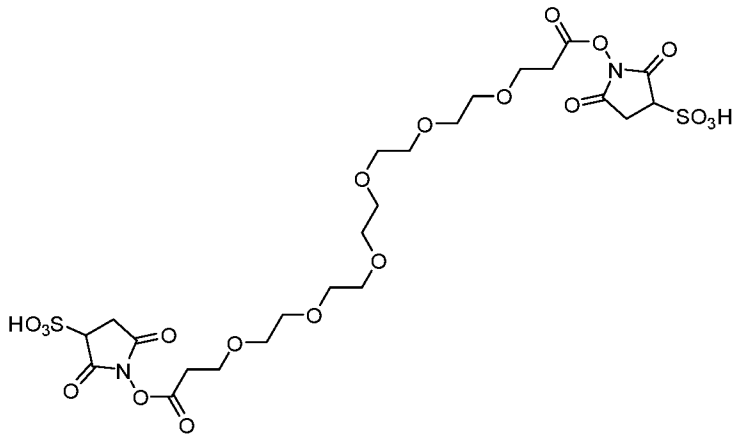


[0563] HW-1055 was synthesized by the same method as HW-0984. Calculated: m/z = 1114.06 $[M+2H]^{2+}$; measured (ESI): m/z = 1113.95 $[M+2H]^{2+}$.

Example 35

SulfoSE-PEG6-SmTrip9 Pep693 (HW-1042)

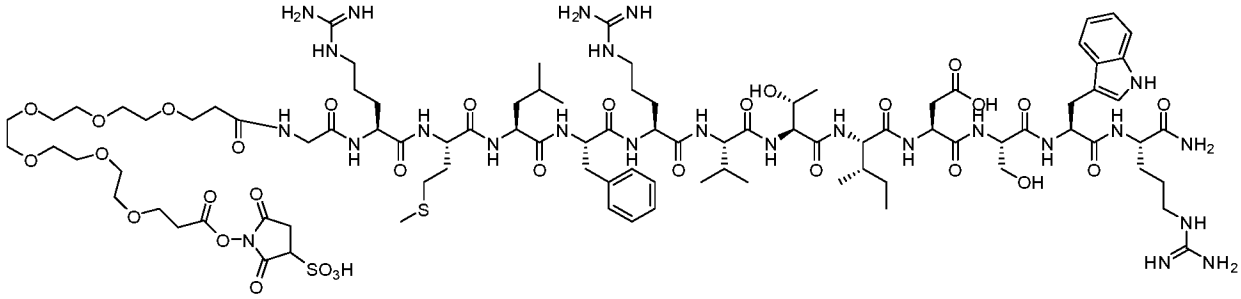
[0564] **PEG6 bis Sulfo-SE**



[0565] Bis PEG6-acid (39 mg, 0.10 mmol) was dissolved in anhydrous DMF and then diisopropylethylamine (53 mg, 0.4 mmol) and HATU (78 mg, 0.20 mmol) added. The mixture was stirred for five minutes. Meanwhile, N-hydroxy-2,5-dioxopyrrolidine-3-sulfonic acid (40 mg, 0.20 mmol) was dissolved in 5 ml DMSO and then added to the previous solution dropwise.

The mixture was stirred for another hour until LC-MS shows disappearance of acid. The solution was directly used in the next step. Calculated: m/z = 735.13 [M^-]; measured (ESI): m/z = 735.04 [M].

[0566] SulfoSE-PEG6-SmTrip9 Pep693 (HW-1042)

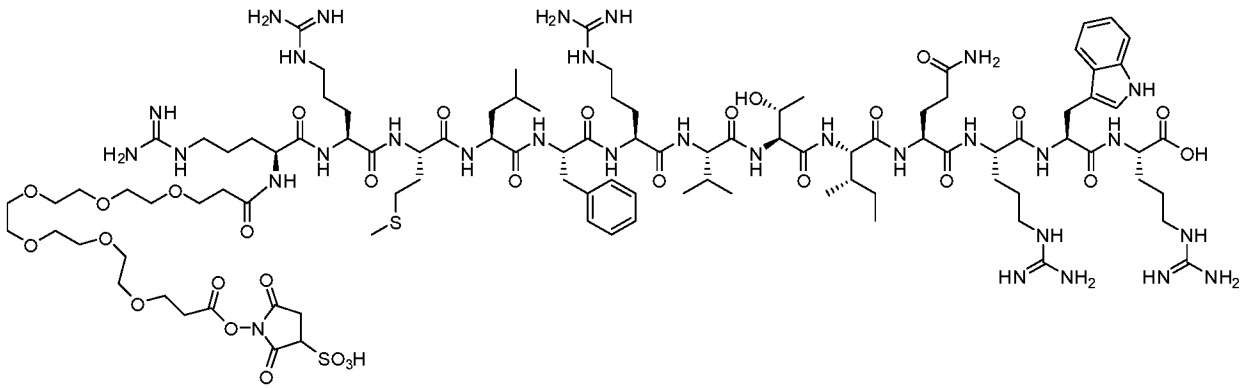


[0567]

[0568] SmTrip9 Pep693 (GRMLFRVTINSWR, 20mg, 0.013mmol) was dissolved in DMF. The solution was then added to the previous PEG6 bis Sulfo-SE solution. The mixture was then stirred for another hour and directly purified by preparative HPLC. Calculated: m/z = 1089.02 $[M+2H]^{2+}$; measured (ESI): m/z = 1088.94 $[M+2H]^{2+}$.

Example 36

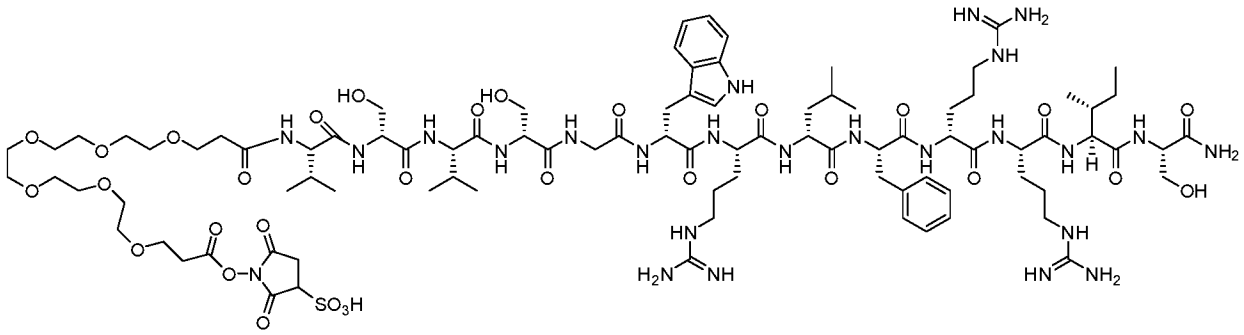
SulfoSE-PEG6-SmTrip9 Pep929 (HW-1052)



[0569] HW-1052 was synthesized by the same method as HW-1042. Calculated: m/z = 1180.10 $[M+2H]^{2+}$; measured (ESI): m/z = 1179.82 $[M+2H]^{2+}$.

Example 37

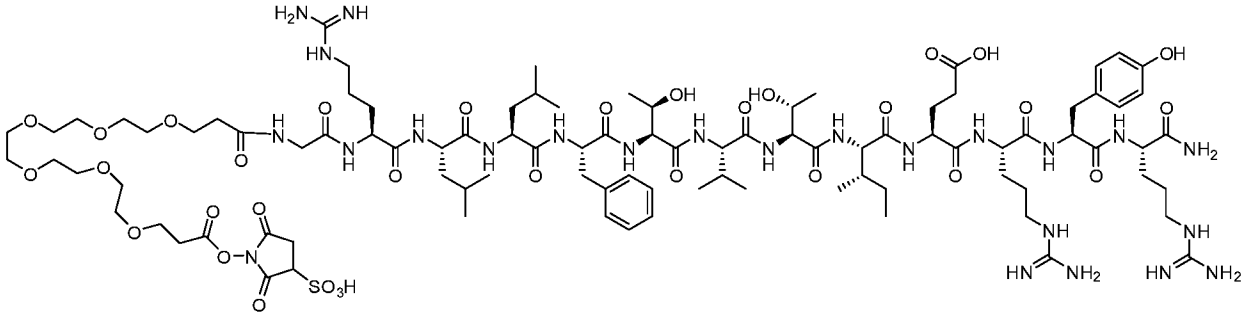
SulfoSE-PEG6-SmTrip10 Pep692 (HW-1053)



[0570] HW-1053 was synthesized by the same method as HW-1042. Calculated: m/z = 1052.03 $[M+2H]^{2+}$; measured (ESI): m/z = 1051.92 $[M+2H]^{2+}$.

Example 38

SulfoSE-PEG6-SmTrip9 Pep895 (HW-1043)

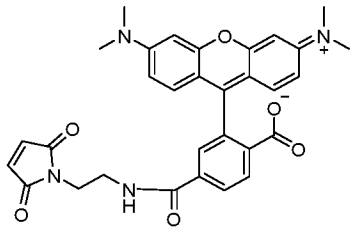


[0571] HW-1043 was synthesized by the same method as HW-1042. Calculated: m/z = 1082.55 $[M+2H]^{2+}$; measured (ESI): m/z = 1082.34 $[M+2H]^{2+}$.

Example 39

SulfoSE-PEG3-SmTrip9 Pep938-TAMRA (HW-0992)

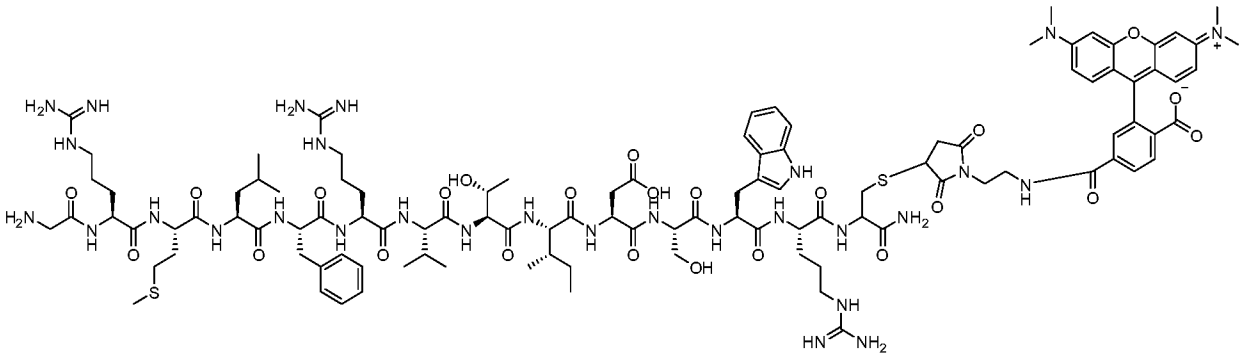
[0572] **TAMRA-Maleimide**



[0573] 5-TAMRA (50 mg, 0.116 mmol) was dissolved in DMF. Diisopropylethylamine (45 mg, 0.128 mmol) was added followed by TSTU (38 mg, 0.128 mmol). The mixture was stirred

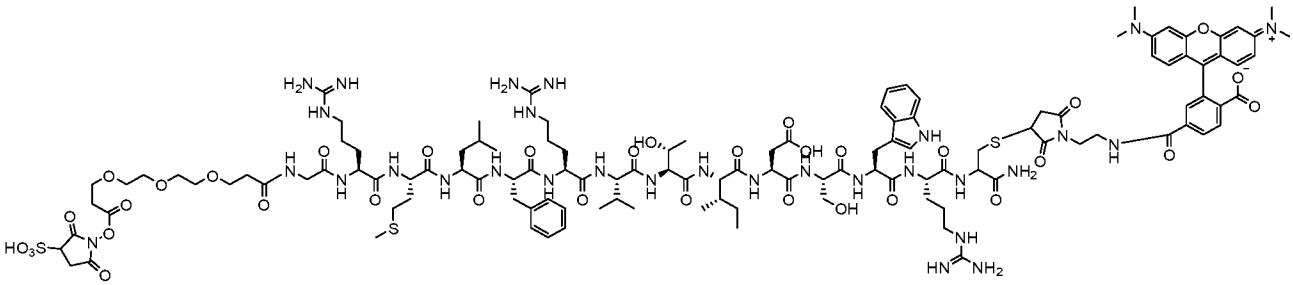
for 20 min, 1-(2-aminoethyl)-1H-pyrrole-2,5-dione (18 mg, 0.128 mmol) added, and the resulting reaction mixture was stirred for another hour and directly purified by preparative HPLC. Calculated: m/z = 553.20 $[M+H]^+$; measured (ESI): m/z = 553.40 $[M+H]^+$.

[0574] SmTrip9 Pep938-TAMRA



[0575] TAMRA-Maleimide (8 mg, 0.014 mmol) was dissolved in DMF. A solution of SmTrip9 (Pep938) (GRMLFRVTINSWRC, 25 mg, 0.014 mmol) in PBS buffer (pH 7.4, 200mM) was added. The reaction mixture was stirred for two hours and directly purified by preparative HPLC. Calculated: m/z = 1146.05 $[M+2H]^{2+}$; measured (ESI): m/z = 1146.33 $[M+2H]^{2+}$.

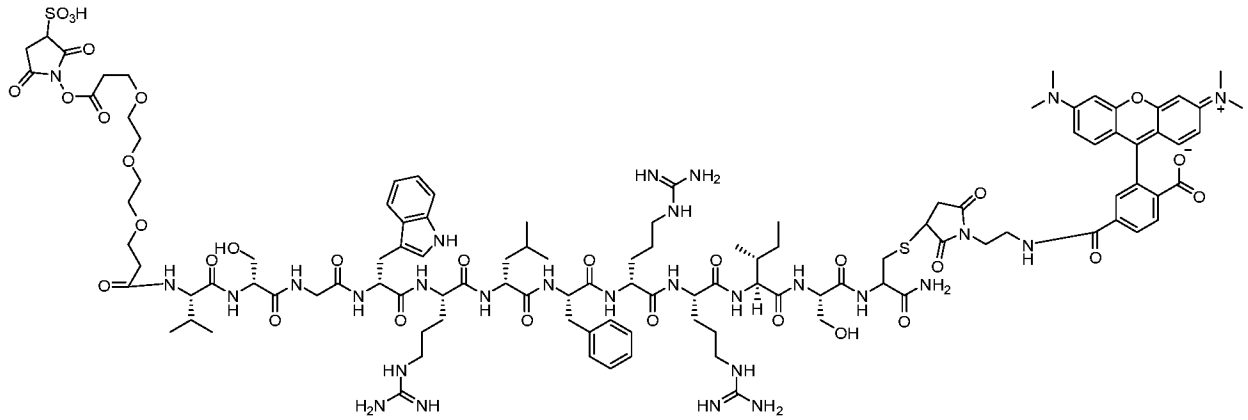
[0576] SulfoSE-PEG3-SmTrip9 Pep938-TAMRA (HW-0992)



[0577] SmTrip9 Pep938-TAMRA (8.5 mg, 0.0038 mmol) was dissolved in DMF. The solution was then added to PEG3 bis Sulfo-SE prepared as shown in synthesis of HW-0984. The reaction mixture was stirred for two hours and directly purified by preparative HPLC. Calculated: m/z = 901.05 $[M+3H]^{3+}$; measured (ESI): m/z = 901.20 $[M+3H]^{3+}$.

Example 40

SulfoSE-PEG3-Strnd 9 (Pep937)-TAMRA (HW-0987)

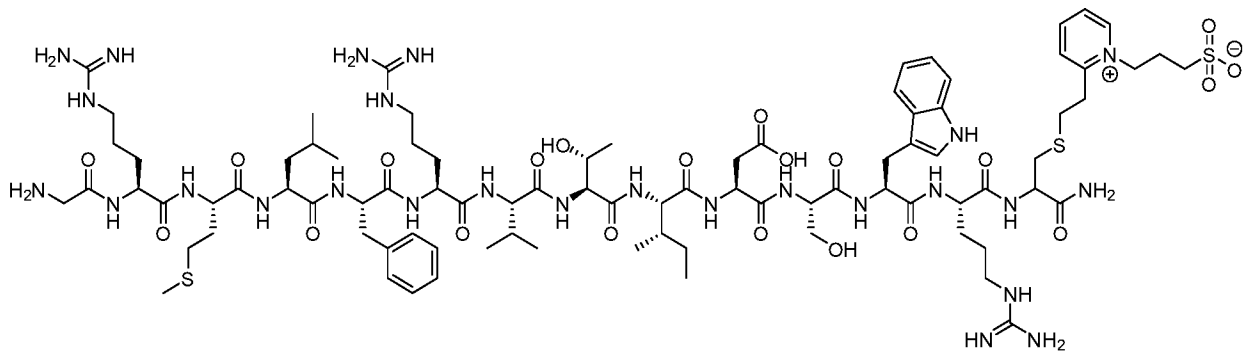


[0578] HW-0987 was synthesized by the same method as HW-0992. Calculated: m/z = 814.03 $[M+3H]^{3+}$; measured (ESI): m/z = 814.40 $[M+3H]^{3+}$.

Example 41

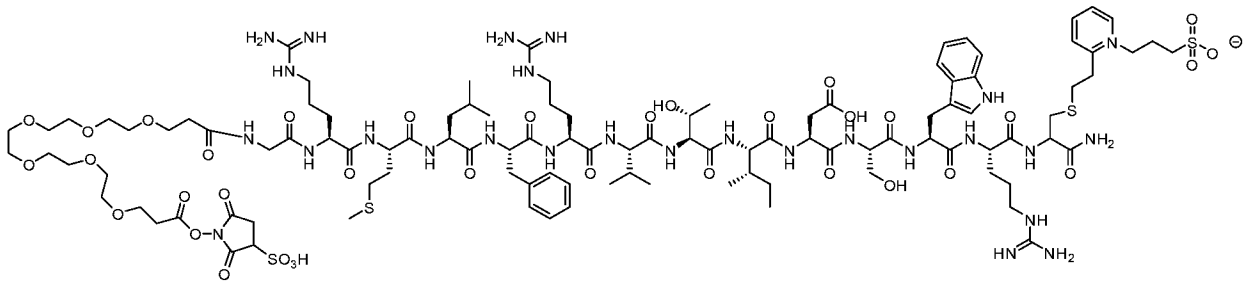
SulfoSE-PEG3- SmTrip9 Pep938-SA (HW-1050)

[0579] **SmTrip9 Pep938-SA**



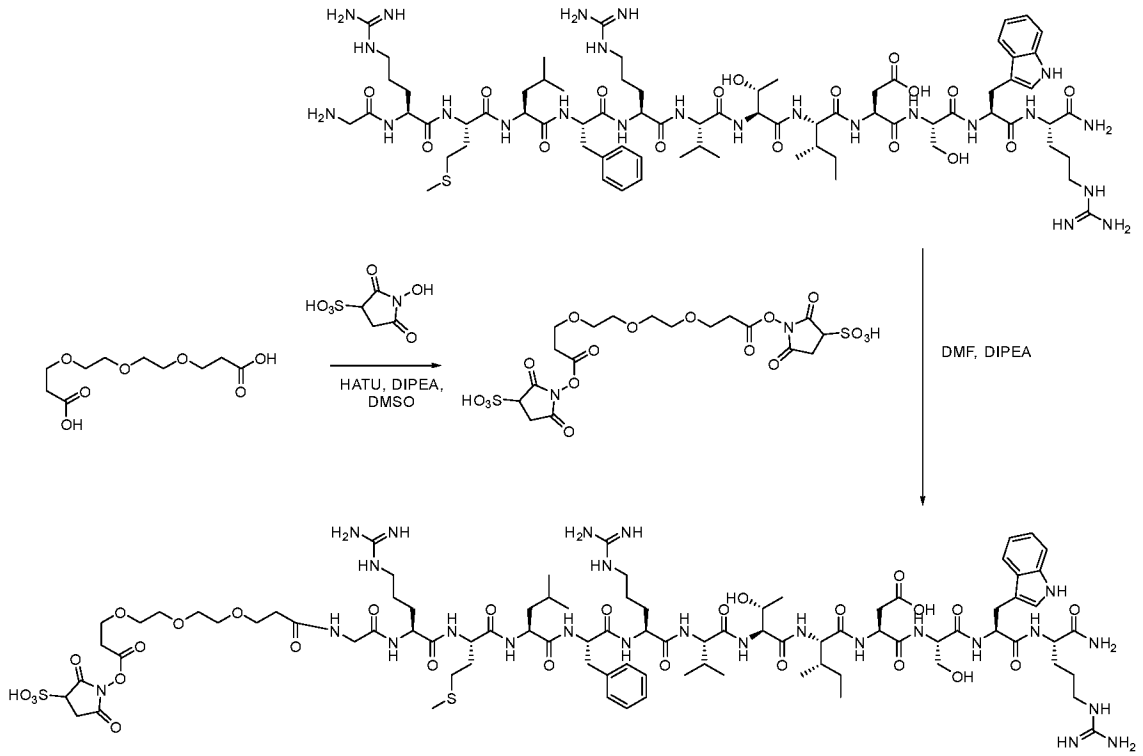
[0580] SmTrip9 Pep938 (GRMLFRVTINSWR, 26 mg, 0.015 mmol) was dissolved in DMSO. 1-(3-Sulfopropyl)-2-vinylpyridinium Hydroxide Inner Salt (3.40 mg 0.015 mmol) was dissolved in phosphate buffer (pH = 7.4, 100mM) and was added slowly to the peptide solution. The mixture was stirred for another three hours and directly purified by preparative HPLC. Calculated: m/z = 983.48 $[M+2H]^{2+}$; measured (ESI): m/z = 983.39 $[M+2H]^{2+}$.

[0581] **SulfoSE-PEG3- SmTrip9 Pep938-SA (HW-1050)**

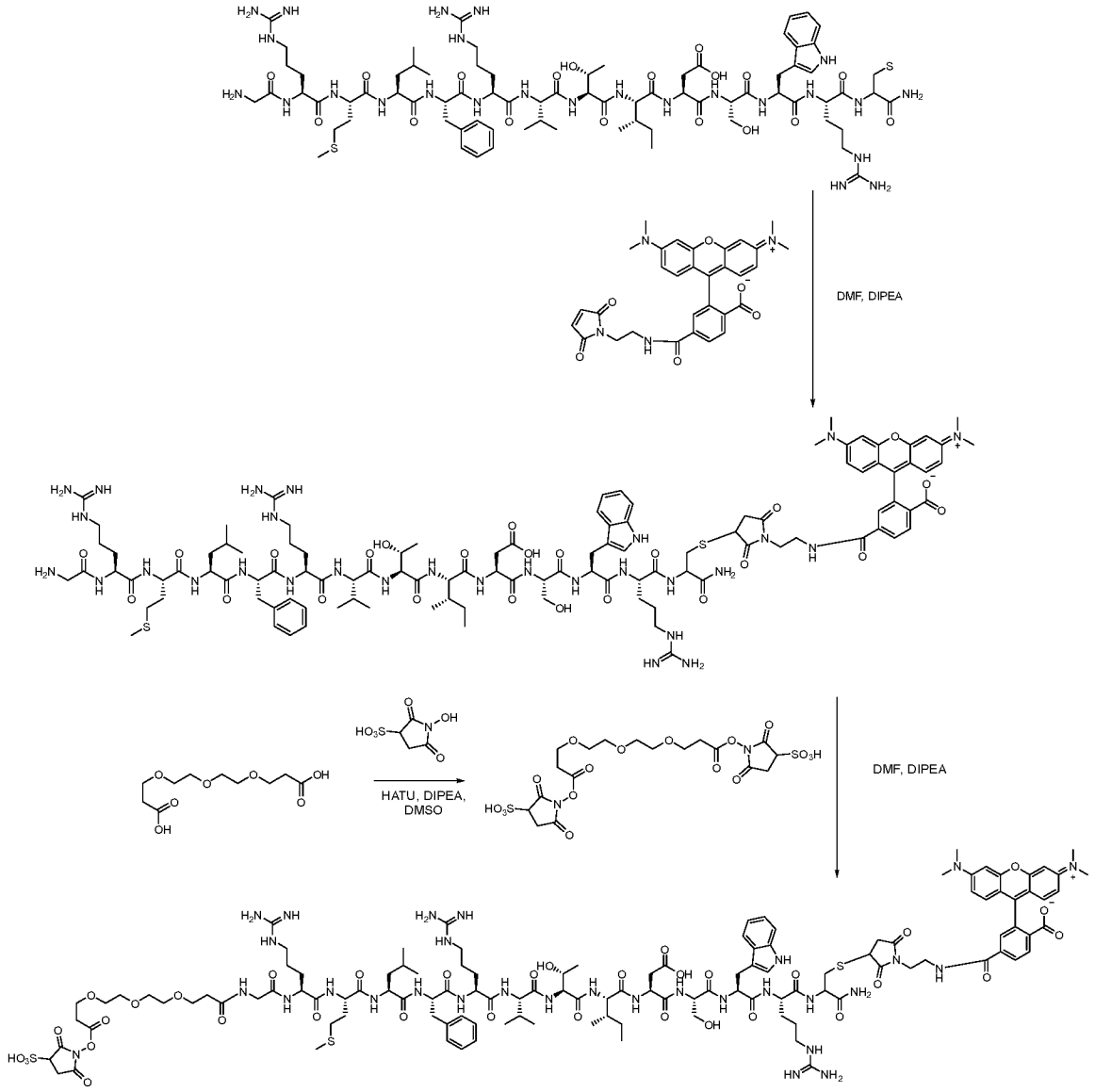


[0582] SmTrip9 Pep938-SA (10 mg, 0.005 mmol) was dissolved in DMF. The solution was then added to PEG6 bis Sulfo-SE prepared as shown in HW-0984. The reaction mixture was stirred for two hours and directly purified by preparative HPLC. Calculated: m/z = 1254.05 $[M+2H]^{2+}$; measured (ESI): m/z = 1253.98 $[M+2H]^{2+}$.

[0583] Shown below is a representative scheme for the synthesis of PEG-linked peptide SulfoSE.



[0584] Shown below is a representative scheme for the synthesis of PEG-linked peptide SulfoSE linked to a fluorophore.



Example 42

Investigating Luminescence in Complex Sample Matrices on Performance of Coelenterazine Derivatives JRW-1404 and JRW-1482

[0585] FIG. 87 displays the luminescence derived from coelenterazine derivative substrates JRW-1404 and JRW-1482 in complex sample matrices. 100% samples of plasma (12/28/18), urine (Innovative research 2/25/19), and Human-Sera (2/11/19) were diluted to 10%, 20%, 0%, and 80% in PBS. The sample with "0%" is PBS. In duplicate, 50 µl of each sample was combined with 50 µl NanoLuc diluted to 0.4ng/ml in PBS. Each substrate was diluted to 20 µM

PBS and then 100µl of each diluted substrate was added to the NanoLuc/sample mixtures. Luminescence was measured on a GloMax® Discover plate luminometer.

[0586] It is understood that the foregoing detailed description and accompanying examples are merely illustrative and are not to be taken as limitations upon the scope of the disclosure, which is defined solely by the appended claims and their equivalents.

[0587] Various changes and modifications to the disclosed embodiments will be apparent to those skilled in the art. Such changes and modifications, including without limitation those relating to the chemical structures, substituents, derivatives, intermediates, syntheses, compositions, formulations, or methods of use of the disclosure, may be made without departing from the spirit and scope thereof.

SEQUENCES

[0588] The following polypeptide sequences each comprise an N-terminal methionine residue or corresponding ATG codon; polypeptide sequences lacking the N-terminal methionine residue or corresponding ATG codon are also within the scope herein and are incorporated herein by reference.

[0589] The following peptide sequences each lack an N-terminal methionine residue; peptide sequences comprising an N-terminal methionine residue are also within the scope herein and are incorporated herein by reference.

[0590] **Table 2. Exemplary peptide, dipeptide, and polypeptide sequences.**

SEQ ID NO	Name	Sequence
1	WT OgLuc	MFTLADFVGDWQQTAGYNQDQVLEQGGLSSLFQALGVSVTPIQKV VLSGENGLKADIHVIIPYEGLSGFQMGLIEMIFKVVYPVDDHHFKIIL HYGTLVIDGVTPNMIDYFGRPYPGIAVFDGKQITVTGTLWNGNKIYD ERLINPDGSLLFRVTINGVTGWRLCENILA
28	WT OgLuc	atggtgtttaccttggcagatttcgttggagactggcaacagacagctggatacaaccaagatcaagtgttaga acaaggaggattgtctagtctgttccaagccctgggagtgtcagtcaccccaatccagaaagttgtgctgtctg gggagaatgggttaaaagctgatattcatgtcatcatcccttacgagggactcagtggttttcaaatgggtctga ttgaaatgatcttcaaagttgtttacccagtggatgatcatcatttcaagattattctccattatggtacactcgttatt gacggtgtgacaccaaacatgattgactactttggacgcccttaccctggaattgctgtgtttgacggcaagca gatcacagttactggaactctgtggaacggcaacaagatctatgatgagcgcctgatcaacccagatggttca ctcctcttccgcgttactatcaatggagtcaccggatggcgcctttgcgagaacattcttgcc

5	NanoLuc	MVFTLEDFVGDWRQTAGYNLDQVLEQGGVSSLFQNLGVSVTPIQRI VLSGENGLKIDIHVIIPYEGLSGDQMGQIEKIFKVVYPVDDHHFKVIL HYGTLVIDGVTPNMIDYFGRPYEGIAVFDGKKITVTGTLWNGNKIID ERLINPDGSLLFRVTINGVTGWRLCERILA
29	NanoLuc	atgaaacatcaccatcaccatcatgcgatcgccatggtcttcacactcgaagatttcgttggggactggcgac agacagccggctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgtttcagaatctcggggt gtccgtaactccgatccaaaggattgtcctgagcggtgaaaatgggctgaagatcgacatccatgtcatcatc ccgtatgaaggtctgagcggcgaccaaatgggccagatcgaaaaaatttttaaggtggtgtaccctgtggat gatcatcactttaaggtgatcctgcactatggcacactggtaatcgacggggttacgccgaacatgatcgacta tttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaa cggcaacaaaattatcgacgagcgcctgatcaaccccgacggctccctgctgttccgagtaaccatcaacgg agtgaccggctggcggctgtgcgaacgcattctggcggtt
2	WT OgLuc Lg	MFTLADFVGDWQQTAGYNQDQVLEQGGLSSLFQALGVSVTPIQKV VLSGENGLKADIHVIIPYEGLSGFQMGLIEMIFKVVYPVDDHHFKIIL HYGTLVIDGVTPNMIDYFGRPYPGIAVFDGKQITVTGTLWNGNKIYD ERLINPD
3	WT OgLuc β9	GSLLFRVTIN
4	WT OgLuc β10	GVTGWRLCENILA
6	WT NanoLuc Lg	MVFTLEDFVGDWRQTAGYNLDQVLEQGGVSSLFQNLGVSVTPIQRI VLSGENGLKIDIHVIIPYEGLSGDQMGQIEKIFKVVYPVDDHHFKVIL HYGTLVIDGVTPNMIDYFGRPYEGIAVFDGKKITVTGTLWNGNKIID ERLINPD
7	WT NanoLuc β9	GSLLFRVTINV
8	WT NanoLuc β10	GVTGWRLCERILA
9	LgBit	MVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRI VRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVIL PYGTLVIDGVTPNMLNYFGRPYEGIAVFDGKKITVTGTLWNGNKIID ERLITPDGSMLFRVTIN
30	LgBit	atggtcttcacactcgaagatttcgttggggactgggaacagacagccgcctacaacctggaccaagtccttg aacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccggag cggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggc ccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggca cactggtaatcgacggggttacgccgaacatgctgaactatttcggacggccgtatgaaggcatcgccgtgtt cgacggcaaaaagatcactgtaacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgatca cccccgacggctccatgctgttccgagtaaccatcaacagccatcatcaccatcaccac
10	SmBit	VTGYRLFEEIL
31	SmBit	gtgaccggctaccggctgttcgaggagattctg
11	HiBit	VSGWRLFKKIS
32	HiBit	gtgagcggctggcggctgttcaagaagattagc
33	LgTrip 2098	MVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRI VRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVIL PYGTLVIDGVTPNMLNYFGRPYEGIAVFDGKKITVTGTLWNGNKIID ERLITPD

34	LgTrip 2098	atggtcttcacactcgaagatttcgttggggactgggaacagacagccgcctacaacctggaccaagtccttg aacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccggag cggtgaaaatgCcctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggc ccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggca cactggtaatcgacggggttacgccgaacatgctgaactatttcggacggccgtatgaaggcatcgccgtgtt cgacggcaaaaagatcactgtaacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgatca cccccgac
35	LgTrip 3092 His	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPV DDHHFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITVTGT LWNGNKIIDERLITPD
36	LgTrip 3092 His	atgaaacatcaccatcaccatcatgtcttcacactcgaagatttcgttggggactgggaacagacagccgcct acaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactcc gatccaaaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggt ctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcacttta aggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacatgctgaactatttcggacggc cgtatgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaacggcaacaaa attatcgacgagcgcctgatcacccccgac
37	LgTrip 3092	MVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIMRI VRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVIL PYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITVTGTLWNGNKIID ERLITPD
38	LgTrip 3092	atggtcttcacactcgacgatttcgttggggactgggaacagacagccgcctacaacctggaccaagtccttg aacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatcatgaggattgtccggag cggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggc ccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggca cactggtaatcgacggggttacgccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgtgt tcgacggcaaaaagatcactgtaacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgatc acccccgac
13	SmTrip9	GSMLFRVTINS
39	SmTrip9	ggctccatgctgttccgagtaaccatcaacagc
15	SmTrip10	VSGWRLFKKIS
40	SmTrip10	gtgagcggctggcggctgttcaagaagattagc
41	5P-B9	MVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLFQNLAVSVTPIQRI VLSGENALKIDIHVIIPYEGLSADQMAQIEKIFKVVYPVDDHHFKVIL HYGTLVIDGVTPNMINYFGRPYEGIAVFDGKKITVTGTLWNGNKIID ERLITPD
42	5P-B9	atggtcttcacactcgaagatttcgttggggactgggaacagacagccgcctacaacctggaccaagtccttg aacagggaggtgtgtccagtttgtttcagaatctcgccgtgtccgtaactccgatccaaaggattgtcctgagc ggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcc cagatcgaaaaaatttttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgcactatggcaca ctggtaatcgacggggttacgccgaacatgatcaactatttcggacggccgtatgaaggcatcgccgtgttcg acggcaaaaagatcactgtaacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgatcacc cccgac
43	5P(147-157)	GSMLFRVTINV
44	5P(147-157)	ggctccatgctgttccgagtaaccatcaac

45	LgTrip 2098 His	MKHHHHHHVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIQRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVD DHHFKVILPYGTLVIDGVTPNMLNYFGRPYEGIAVFDGKKITVTGTL WNGNKIIDERLITPD
46	LgTrip 2098 His	atgaaacatcaccatcaccatcatgtcttcacactcgaagatttcgttggggactgggaacagacagccgcct acaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactcc gatccaaaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggt ctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcacttta aggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacatgctgaactatttcggacggc cgtatgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaacggcaacaaa attatcgacgagcgcctgatcacccccgac
14	SmTrip9/10 Dipeptide (pep263)	GSMLFRVTINSVSGWRLFKKIS
47	SmTrip9/10 Dipeptide (Pep263)	ggctccatgctgttccgagtaaccatcaacagcgtgagcggctggcggctgttcaagaagattagc
48	SmTrip9+ (pep286)	SSWKRGSMLFRVTINS
49	SmTrip9+ (pep286)	Agcagctggaagcgcggctccatgctgttccgagtaaccatcaacagc
50	LgTrip 3440	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPV DDHHFKVILPYGTLVIDGDTPNKLNYFGRPYDGIAVFDGKKITVTGT LWNGNKIIDERLITPD
51	LgTrip 3440	atgaaacatcaccatcaccatcatgtcttcacactcgacgatttcgttggggactgggaacagacagccgcct acaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactcc gatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggt ctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcacttta aggtgatcctgccctatggcacactggtaatcgacggggatacgccgaacaagctgaactatttcggacggc cgtatgatggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaacggcaacaaa attatcgacgagcgcctgatcacccccgac
52	LgTrip 3121	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPV DDHHFKVILPYGTLVIDGVTPSKLNYFGRPYEGIAVFDGKKITVTGTL WNGNKIIDERLITPD
53	LgTrip 3121	atgaaacatcaccatcaccatcatgtcttcacactcgacgatttcgttggggactgggaacagacagccgcct acaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactcc gatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggt ctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcacttta aggtgatcctgccctatggcacactggtaatcgacggggttacgccgagcaagctgaactatttcggacggc cgtatgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaacggcaacaaa attatcgacgagcgcctgatcacccccgac
54	LgTrip 3482	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPV DDHHFKVILPYGTLVIDGVTPNKLNYFGRPYEGFAVFDGKKITVTGT LWNGNKIIDERLITPD

55	LgTrip 3482	atgaaacatcaccatcaccatcatgtcttcacactcgacgatttcgttggggactgggaacagacagccgcct acaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactcc gatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggt ctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcacttta aggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggc cgtatgaaggcttcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaacggcaacaaa attatcgacgagcgcctgatcacccccgac
56	LgTrip 3497	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPV DDHHFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVCDGKKITVTGT LWNGNKIIDERLITPD
57	LgTrip 3497	atgaaacatcaccatcaccatcatgtcttcacactcgacgatttcgttggggactgggaacagacagccgcct acaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactcc gatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggt ctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcacttta aggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggc cgtatgaaggcatcgccgtgtgcgacggcaaaaagatcactgtaacagggaccctgtggaacggcaacaa aattatcgacgagcgcctgatcacccccgac
58	LgTrip 3125	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPV DDHHFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKISVTGT LWNGNKIIDERLITPD
59	LgTrip 3125	atgaaacatcaccatcaccatcatgtcttcacactcgacgatttcgttggggactgggaacagacagccgcct acaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactcc gatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggt ctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcacttta aggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcgggcggc cgtatgaaggcatcgccgtgttcgacggcaaaaagatctctgtaacagggaccctgtggaacggcaacaaa attatcgacgagcgcctgatcacccccgac
60	LgTrip 3118	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPV DDHHFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITATGT LWNGNKIIDERLITPD
61	LgTrip 3118	atgaaacatcaccatcaccatcatgtcttcacactcgacgatttcgttggggactgggaacagacagccgcct acaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactcc gatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggt ctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcacttta aggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggc cgtatgaaggcatcgccgtgttcgacggcaaaaagatcactgcaacagggaccctgtggaacggcaacaa aattatcgacgagcgcctgatcacccccgac
12	LgTrip 3546	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPV DDHHFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTL WNGNKIIDERLITPD

62	LgTrip 3546	atgaaacatcaccatcaccatcatgtcttcacactcgacgatttcgttggggactgggaacagacagccgcct acaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactcc gatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggt ctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcacttta aggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggc cgtatgaaggcatcgccgtgttcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaa aattatcgacgagcgcctgatcacccccgac
63	LgTrip 3546+G (ATG 3572)	MVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIMRI VRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVIL PYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDE RLITPDG
64	LgTrip 3546+G (ATG 3572)	atggtcttcacactcgacgatttcgttggggactgggaacagacagccgcctacaacctggaccaagtccttg aacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatcatgaggattgtccggag cggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggc ccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggca cactggtaatcgacggggttacgccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgtgt tcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgatc acccccgacggc
65	LgTrip 3546-D (ATG 3573)	MVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIMRI VRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVIL PYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDE RLITP
66	LgTrip 3546-D (ATG 3573)	atggtcttcacactcgacgatttcgttggggactgggaacagacagccgcctacaacctggaccaagtccttg aacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatcatgaggattgtccggag cggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggc ccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggca cactggtaatcgacggggttacgccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgtgt tcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgatc accccc
67	LgTrip 3546-PD (ATG 3574)	MVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIMRI VRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVIL PYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDE RLIT
68	LgTrip 3546-PD (ATG 3574)	atggtcttcacactcgacgatttcgttggggactgggaacagacagccgcctacaacctggaccaagtccttg aacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatcatgaggattgtccggag cggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggc ccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggca cactggtaatcgacggggttacgccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgtgt tcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgatc acc
69	LgTrip 3546+GS (ATG 3575)	MVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIMRI VRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVIL PYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDE RLITPDGS

70	LgTrip 3546+GS (ATG 3575)	atggtcttcacactcgacgatttcgttggggactgggaacagacagccgcctacaacctggaccaagtccttg aacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatcatgaggattgtccggag cggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggc ccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggca cactggtaatcgacggggttacgccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgtgt tcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgatc acccccgacggcagc
71	-V_LgBiT (ATG3618)	MFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRIV RSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILP YGTLVIDGVTPNMLNYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDE RLITPDGSMLFRVTINSHHHHHH
72	-V_LgBiT (ATG3618)	atgttcacactcgaagatttcgttggggactgggaacagacagccgcctacaacctggaccaagtccttgaa cagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccggagc ggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcc cagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcac actggtaatcgacggggttacgccgaacatgctgaactatttcggacggccgtatgaaggcatcgccgtgttc gacggcaaaaagatcactgtaacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgatcac ccccgacggctccatgctgttccgagtaaccatcaacagccatcatcaccatcaccactaa
73	-VF_LgBiT (ATG3619)	MTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRIVR SGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPY GTLVIDGVTPNMLNYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDER LITPDGSMLFRVTINSHHHHHH
74	-VF_LgBiT (ATG3619)	atgacactcgaagatttcgttggggactgggaacagacagccgcctacaacctggaccaagtccttgaacag ggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccggagcggtg aaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccaga tcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactg gtaatcgacggggttacgccgaacatgctgaactatttcggacggccgtatgaaggcatcgccgtgttcgac ggcaaaaagatcactgtaacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgatcacccc cgacggctccatgctgttccgagtaaccatcaacagccatcatcaccatcaccactaa
75	-VFT_LgBiT (ATG3620)	MLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRIVRS GENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYG TLVIDGVTPNMLNYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLI TPDGSMLFRVTINSHHHHHH
76	-VFT_LgBiT (ATG3620)	atgctcgaagatttcgttggggactgggaacagacagccgcctacaacctggaccaagtccttgaacaggg aggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccggagcggtgaa aatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatc gaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggt aatcgacggggttacgccgaacatgctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacgg caaaaagatcactgtaacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgatcacccccg acggctccatgctgttccgagtaaccatcaacagccatcatcaccatcaccactaa
77	-VFTL_LgBiT (ATG3621)	MEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRIVRSG ENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGT LVIDGVTPNMLNYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLIT PDGSMLFRVTINSHHHHHH

78	-VFTL_LgBiT (ATG3621)	atggaagatttcgttggggactgggaacagacagccgcctacaacctggaccaagtccttgaacagggagg tgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccggagcggtgaaaatg ccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaag aggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcg acggggttacgccgaacatgctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaa agatcactgtaacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgatcacccccgacggc tccatgctgttccgagtaaccatcaacagccatcatcaccatcaccactaa
79	(M)FKKIS-GSSG-LgBiT (ATG3632)	MFKKISGSSGVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIQRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVD DHHFKVILPYGTLVIDGVTPNMLNYFGRPYEGIAVFDGKKITVTGTL WNGNKIIDERLITPDGSMLFRVTINSHHHHHH
80	(M)FKKIS-GSSG-LgBiT (ATG3632)	atgttcaagaagattagcggctcgagcggtgtcttcacactcgaagatttcgttggggactgggaacagaca gccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtcc gtaactccgatccaaaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgt atgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatc atcactttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacatgctgaactatttc ggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaacg gcaacaaaattatcgacgagcgcctgatcacccccgacggctccatgctgttccgagtaaccatcaacagcc atcatcaccatcaccactaa
81	(M)KKIS-GSSG-LgBiT (ATG3633)	MKKISGSSGVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAV SVTPIQRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDD HHFKVILPYGTLVIDGVTPNMLNYFGRPYEGIAVFDGKKITVTGTLW NGNKIIDERLITPDGSMLFRVTINSHHHHHH
82	(M)KKIS-GSSG-LgBiT (ATG3633)	atgaagaagattagcggctcgagcggtgtcttcacactcgaagatttcgttggggactgggaacagacagcc gcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaa ctccgatccaaaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatga aggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatca ctttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacatgctgaactatttcggac ggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaacggcaa caaaattatcgacgagcgcctgatcacccccgacggctccatgctgttccgagtaaccatcaacagccatcat caccatcaccactaa
83	(M)KIS-GSSG-LgBiT (ATG3634)	MKISGSSGVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVS VTPIQRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDD HHFKVILPYGTLVIDGVTPNMLNYFGRPYEGIAVFDGKKITVTGTLW NGNKIIDERLITPDGSMLFRVTINSHHHHHH
84	(M)KIS-GSSG-LgBiT (ATG3634)	atgaagattagcggctcgagcggtgtcttcacactcgaagatttcgttggggactgggaacagacagccgcc tacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactc cgatccaaaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaag gtctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactt taaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacatgctgaactatttcggacgg ccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaacggcaacaa aattatcgacgagcgcctgatcacccccgacggctccatgctgttccgagtaaccatcaacagccatcatcac catcaccactaa

85	(M)IS-GSSG-LgBiT (ATG3635)	MISGSSGVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSV TPIQRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDH HFKVILPYGTLVIDGVTPNMLNYFGRPYEGIAVFDGKKITVTGTLWN GNKIIDERLITPDGSMLFRVTINSHHHHHH
86	(M)IS-GSSG-LgBiT (ATG3635)	atgattagcggctcgagcggtgtcttcacactcgaagatttcgttggggactgggaacagacagccgcctac aacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccga tccaaaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtct gagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaag gtgatcctgccctatggcacactggtaatcgacggggttacgccgaacatgctgaactatttcggacggccgt atgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaacggcaacaaaatt atcgacgagcgcctgatcacccccgacggctccatgctgttccgagtaaccatcaacagccatcatcaccat caccactaa
87	(M)S-GSSG-LgBiT (ATG3636)	MSGSSGVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSV TPIQRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDH HFKVILPYGTLVIDGVTPNMLNYFGRPYEGIAVFDGKKITVTGTLWN GNKIIDERLITPDGSMLFRVTINSHHHHHH
88	(M)S-GSSG-LgBiT (ATG3636)	atgagcggctcgagcggtgtcttcacactcgaagatttcgttggggactgggaacagacagccgcctacaac ctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatcc aaaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgag cgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtg atcctgccctatggcacactggtaatcgacggggttacgccgaacatgctgaactatttcggacggccgtatg aaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaacggcaacaaaattatc gacgagcgcctgatcacccccgacggctccatgctgttccgagtaaccatcaacagccatcatcaccatcac cactaa
89	LgTrip + GSM (ATG3722)	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPV DDHHFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTL WNGNKIIDERLITPDGSM
90	LgTrip + GSM (ATG3722)	atgaaacatcaccatcaccatcatgtcttcacactcgacgatttcgttggggactgggaacagacagccgcct acaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactcc gatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggt ctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcacttta aggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggc cgtatgaaggcatcgccgtgttcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaa aattatcgacgagcgcctgatcacccccgacggcagcatgtaa
91	LgTrip + GSML (ATG3723)	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPV DDHHFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTL WNGNKIIDERLITPDGSML
92	LgTrip + GSML (ATG3723)	atgaaacatcaccatcaccatcatgtcttcacactcgacgatttcgttggggactgggaacagacagccgcct acaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactcc gatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggt ctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcacttta aggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggc cgtatgaaggcatcgccgtgttcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaa aattatcgacgagcgcctgatcacccccgacggcagcatgctgtaa
93	LgTrip + GSMLF (ATG3724)	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPV DDHHFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTL WNGNKIIDERLITPDGSMLF

94	LgTrip + GSMLF (ATG3724)	atgaaacatcaccatcaccatcatgtcttcacactcgacgatttcgttggggactgggaacagacagccgcct acaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactcc gatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggt ctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcacttta aggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggc cgtatgaaggcatcgccgtgttcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaa aattatcgacgagcgcctgatcacccccgacggcagcatgctgttctaa
95	LgTrip – TPD (ATG3725)	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPV DDHHFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTL WNGNKIIDERLI
96	LgTrip – TPD (ATG3725)	atgaaacatcaccatcaccatcatgtcttcacactcgacgatttcgttggggactgggaacagacagccgcct acaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactcc gatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggt ctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcacttta aggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggc cgtatgaaggcatcgccgtgttcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaa aattatcgacgagcgcctgatctaa
97	LgTrip – ITPD (ATG3726)	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPV DDHHFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTL WNGNKIIDERL
98	LgTrip – ITPD (ATG3726)	atgaaacatcaccatcaccatcatgtcttcacactcgacgatttcgttggggactgggaacagacagccgcct acaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactcc gatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggt ctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcacttta aggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggc cgtatgaaggcatcgccgtgttcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaa aattatcgacgagcgcctgtaa
99	LgTrip – LITPD (ATG3727)	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPV DDHHFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTL WNGNKIIDER
100	LgTrip – LITPD (ATG3727)	atgaaacatcaccatcaccatcatgtcttcacactcgacgatttcgttggggactgggaacagacagccgcct acaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactcc gatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggt ctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcacttta aggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggc cgtatgaaggcatcgccgtgttcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaa aattatcgacgagcgctaa
101	FRB-15GS-AI-86 (ATG1634)	MVAILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERG PQTLKETSFNQAYGRDLMEAQEWCRKYMKSGNVKDLTQAWDLYY HVFRRISGGSGGGGSGGSSSGGAIVSGWRLFKKIS

102	FRB-15GS-AI-86 (ATG1634)	atggtggccatcctctggcatgagatgtggcatgaaggcctggaagaggcatctcgtttgtactttggggaaa ggaacgtgaaaggcatgtttgaggtgctggagcccttgcatgctatgatggaacggggcccccagactctg aaggaaacatcctttaatcaggcctatggtcgagatttaatggaggcccaagagtggtgcaggaagtacatg aaatcagggaatgtcaaggacctcacccaagcctgggacctctattatcatgtgttccgacgaatcagtggtg gttcaggtggtggcgggagcggtggctcgagcagcggtggagcgatcgtgagcggctggcggctgttcaa gaagattagctaa
103	FRB-15GS-AI-289 (ATG3586)	MVAILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERG PQTLKETSFNQAYGRDLMEAQEWCRKYMKSGNVKDLTQAWDLYY HVFRRISGGSGGGGSGGSSSGGAIVSVSGWRLFKKIS
104	FRB-15GS-AI-289 (ATG3586)	atggtggccatcctctggcatgagatgtggcatgaaggcctggaagaggcatctcgtttgtactttggggaaa ggaacgtgaaaggcatgtttgaggtgctggagcccttgcatgctatgatggaacggggcccccagactctg aaggaaacatcctttaatcaggcctatggtcgagatttaatggaggcccaagagtggtgcaggaagtacatg aaatcagggaatgtcaaggacctcacccaagcctgggacctctattatcatgtgttccgacgaatcagtggtg gttcaggtggtggcgggagcggtggctcgagcagcggtggagcgatcgttagcgttagcggctggcgcct gttcaagaagatcagctaa
105	FRB-15GS-AI-86-His6 (ATG3743)	MVAILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERG PQTLKETSFNQAYGRDLMEAQEWCRKYMKSGNVKDLTQAWDLYY HVFRRISGGSGGGGSGGSSSGGAIVSGWRLFKKISHHHHHH
106	FRB-15GS-AI-86-His6 (ATG3743)	atggtggccatcctctggcatgagatgtggcatgaaggcctggaagaggcatctcgtttgtactttggggaaa ggaacgtgaaaggcatgtttgaggtgctggagcccttgcatgctatgatggaacggggcccccagactctg aaggaaacatcctttaatcaggcctatggtcgagatttaatggaggcccaagagtggtgcaggaagtacatg aaatcagggaatgtcaaggacctcacccaagcctgggacctctattatcatgtgttccgacgaatcagtggtg gttcaggtggtggcgggagcggtggctcgagcagcggtggagcgatcgtgagcggctggcggctgttcaa gaagattagccatcatcaccatcaccactaa
107	FRB-15GS-AI-289-His6 (ATG3744)	MVAILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERG PQTLKETSFNQAYGRDLMEAQEWCRKYMKSGNVKDLTQAWDLYY HVFRRISGGSGGGGSGGSSSGGAIVSVSGWRLFKKISHHHHHH
108	FRB-15GS-AI-289-His6 (ATG3744)	atggtggccatcctctggcatgagatgtggcatgaaggcctggaagaggcatctcgtttgtactttggggaaa ggaacgtgaaaggcatgtttgaggtgctggagcccttgcatgctatgatggaacggggcccccagactctg aaggaaacatcctttaatcaggcctatggtcgagatttaatggaggcccaagagtggtgcaggaagtacatg aaatcagggaatgtcaaggacctcacccaagcctgggacctctattatcatgtgttccgacgaatcagtggtg gttcaggtggtggcgggagcggtggctcgagcagcggtggagcgatcgttagcgtgagcggctggcggc tgttcaagaagattagccatcatcaccatcaccactaa
109	His6-FRB-5GS-86 (ATG3760)	MKHHHHHHVAILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPL HAMMERGPQTLKETSFNQAYGRDLMEAQEWCRKYMKSGNVKDLT QAWDLYYHVFRRISGGSGGVSGWRLFKKIS
110	His6-FRB-5GS-86 (ATG3760)	atgaaacatcaccatcaccatcatgtggccatcctctggcatgagatgtggcatgaaggcctggaagaggca tctcgtttgtactttggggaaaggaacgtgaaaggcatgtttgaggtgctggagcccttgcatgctatgatgga acggggcccccagactctgaaggaaacatcctttaatcaggcctatggtcgagatttaatggaggcccaaga gtggtgcaggaagtacatgaaatcagggaatgtcaaggacctcacccaagcctgggacctctattatcatgtg ttccgacgaatcagtggtggttcaggtggtgtgagcggctggcggctgttcaagaagattagctaa
111	His6-FRB-10GS-86 (ATG3761)	MKHHHHHHVAILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPL HAMMERGPQTLKETSFNQAYGRDLMEAQEWCRKYMKSGNVKDLT QAWDLYYHVFRRISGGSGGGGSGGVSGWRLFKKIS

112	His6-FRB-10GS-86 (ATG3761)	atgaaacatcaccatcaccatcatgtggccatcctctggcatgagatgtggcatgaaggcctggaagaggca tctcgtttgtactttggggaaaggaacgtgaaaggcatgtttgaggtgctggagcccttgcatgctatgatgga acggggcccccagactctgaaggaaacatcctttaatcaggcctatggtcgagatttaatggaggcccaaga gtggtgcaggaagtacatgaaatcagggaatgtcaaggacctcacccaagcctgggacctctattatcatgtg ttccgacgaatcagtggtggttcaggtggtggcgggagcggtggcgtgagcggctggcggctgttcaagaa gattagctaa
113	His6-FRB-15GS-86 (ATG3762)	MKHHHHHHVAILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPL HAMMERGPQTLKETSFNQAYGRDLMEAQEWCRKYMKSGNVKDLT QAWDLYYHVFRRISGGSGGGGSGGSSSGGVSGWRLFKKIS
114	His6-FRB-15GS-86 (ATG3762)	atgaaacatcaccatcaccatcatgtggccatcctctggcatgagatgtggcatgaaggcctggaagaggca tctcgtttgtactttggggaaaggaacgtgaaaggcatgtttgaggtgctggagcccttgcatgctatgatgga acggggcccccagactctgaaggaaacatcctttaatcaggcctatggtcgagatttaatggaggcccaaga gtggtgcaggaagtacatgaaatcagggaatgtcaaggacctcacccaagcctgggacctctattatcatgtg ttccgacgaatcagtggtggttcaggtggtggcgggagcggtggctcgagcagcggtggagtgagcggct ggcggctgttcaagaagattagctaa
115	His6-FRB-5GS-289 (ATG3763)	MKHHHHHHVAILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPL HAMMERGPQTLKETSFNQAYGRDLMEAQEWCRKYMKSGNVKDLT QAWDLYYHVFRRISGGSGGVSVSGWRLFKKIS
116	His6-FRB-5GS-289 (ATG3763)	atgaaacatcaccatcaccatcatgtggccatcctctggcatgagatgtggcatgaaggcctggaagaggca tctcgtttgtactttggggaaaggaacgtgaaaggcatgtttgaggtgctggagcccttgcatgctatgatgga acggggcccccagactctgaaggaaacatcctttaatcaggcctatggtcgagatttaatggaggcccaaga gtggtgcaggaagtacatgaaatcagggaatgtcaaggacctcacccaagcctgggacctctattatcatgtg ttccgacgaatcagtggtggttcaggtggtgttagcgttagcggctggcgcctgttcaagaagatcagctaa
117	His6-FRB-10GS-289 (ATG3764)	MKHHHHHHVAILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPL HAMMERGPQTLKETSFNQAYGRDLMEAQEWCRKYMKSGNVKDLT QAWDLYYHVFRRISGGSGGGGSGGVSVSGWRLFKKIS
118	His6-FRB-10GS-289 (ATG3764)	atgaaacatcaccatcaccatcatgtggccatcctctggcatgagatgtggcatgaaggcctggaagaggca tctcgtttgtactttggggaaaggaacgtgaaaggcatgtttgaggtgctggagcccttgcatgctatgatgga acggggcccccagactctgaaggaaacatcctttaatcaggcctatggtcgagatttaatggaggcccaaga gtggtgcaggaagtacatgaaatcagggaatgtcaaggacctcacccaagcctgggacctctattatcatgtg ttccgacgaatcagtggtggttcaggtggtggcgggagcggtggcgttagcgttagcggctggcgcctgttc aagaagatcagctaa
119	His6-FRB-15GS-289 (ATG3765)	MKHHHHHHVAILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPL HAMMERGPQTLKETSFNQAYGRDLMEAQEWCRKYMKSGNVKDLT QAWDLYYHVFRRISGGSGGGGSGGSSSGGVSVSGWRLFKKIS
120	His6-FRB-15GS-289 (ATG3765)	atgaaacatcaccatcaccatcatgtggccatcctctggcatgagatgtggcatgaaggcctggaagaggca tctcgtttgtactttggggaaaggaacgtgaaaggcatgtttgaggtgctggagcccttgcatgctatgatgga acggggcccccagactctgaaggaaacatcctttaatcaggcctatggtcgagatttaatggaggcccaaga gtggtgcaggaagtacatgaaatcagggaatgtcaaggacctcacccaagcctgggacctctattatcatgtg ttccgacgaatcagtggtggttcaggtggtggcgggagcggtggctcgagcagcggtggagttagcgttag cggctggcgcctgttcaagaagatcagctaa

121	SmTrip9-FKBP fusion template (ATG780)	M— GSMLFRVTINS — SSSGGGGSGGGSSGGGVQVETISPGDGRTFPKRGQTCVVHYTG MLEDGKKFDSSRDRNKPFKFMLGKQEVIRGWEEGVAQMSVGQRAK LTISPDYAYGATGHPGIIPPHATLVFDVELLKLE
122	SmTrip9-FKBP fusion template (ATG780)	atgggctccatgctgttccgagtaaccatcaacagctcgagttcaggtggtggcgggagcggtggagggag cagcggtggaggagtgcaggtggaaaccatctccccaggagacgggcgcaccttccccaagcgcggcca gacctgcgtggtgcactacaccgggatgcttgaagatggaaagaaatttgattcctcccgggacagaaacaa gccctttaagtttatgctaggcaagcaggaggtgatccgaggctgggaagaaggggttgcccagatgagtgt gggtcagagagccaaactgactatatctccagattatgcctatggtgccactgggcacccaggcatcatccca ccacatgccactctcgtcttcgatgtggagcttctaaaactggaataa
123	FKBP-SmTrip9 fusion template (ATG777)	MGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSSRDRNKP FKFMLGKQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGII PPHATLVFDVELLKLEGGSGGGGSGGSSSGGAI— GSMLFRVTINS
124	FKBP-SmTrip9 fusion template (ATG777)	Atgggagtgcaggtggaaaccatctccccaggagacgggcgcaccttccccaagcgcggccagacctgc gtggtgcactacaccgggatgcttgaagatggaaagaaatttgattcctcccgggacagaaacaagccctta agtttatgctaggcaagcaggaggtgatccgaggctgggaagaaggggttgcccagatgagtgtgggtca gagagccaaactgactatatctccagattatgcctatggtgccactgggcacccaggcatcatcccaccacat gccactctcgtcttcgatgtggagcttctaaaactggaaggtggttcaggtggtggcgggagcggtggctcg agcagcggtggagcgatcggctccatgctgttccgagtaaccatcaacagc
125	LgBiT (ATG2623)	MVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRI VRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVIL PYGTLVIDGVTPNMLNYFGRPYEGIAVFDGKKITVTGTLWNGNKIID ERLITPDGSMLFRVTINSHHHHHH
126	LgBiT (ATG2623)	atggtcttcacactcgaagatttcgttggggactgggaacagacagccgcctacaacctggaccaagtccttg aacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccggag cggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggc ccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggca cactggtaatcgacggggttacgccgaacatgctgaactatttcggacggccgtatgaaggcatcgccgtgtt cgacggcaaaaagatcactgtaacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgatca cccccgacggctccatgctgttccgagtaaccatcaacagccatcatcaccatcaccactaa
127	pep78	NVSGWRLFKKISN
128	pep79	NVTGYRLFKKISN
129	pep80	VSGWRLFKKISN
130	pep81	SGWRLFKKISN
131	pep82	GWRLFKKISN
132	pep99	VTGYRLFEKIS
133	pep219	SGWRLFKKIS
134	pep225	VSGWRL
135	pep226	VSGWRLF
136	pep227	VSGWRLFK
137	pep228	VSGWRLFKK
138	pep229	VSGWRLFKKI
139	pep243	VSGWRLYKKIS
140	pep272	GSMLFRVTINSVSGWALFKKIS

141	pep274	GSMLFRVTINSVTGYRLFEEIL
142	pep287 (WT SmTrip9)+Cterm solubility tag	GSMLFRVTINSSSWKR
143	pep288	VSGVSGWRLFKKIS
144	pep290	VVSGWRLFKKIS
145	pep291	SSWKRSMLFRVTINS
146	pep292	SSWKRMLFRVTINS
147	pep293	SSWKRDGSMLFRVTINS
148	pep294	SSWKRPDGSMLFRVTINS
149	pep296	SSWKRSMLFRVTINSV
150	pep297	SSWKRMLFRVTINSV
151	pep298	SSWKRDGSMLFRVTINSV
152	pep299	SSWKRPDGSMLFRVTINSV
153	pep301	SSWKRSMLFRVTINSVS
154	pep302	SSWKRMLFRVTINSVS
155	pep303	SSWKRDGSMLFRVTINSVS
156	pep304	SSWKRPDGSMLFRVTINSVS
157	pep305	SSWKRGSMLFRVTIN
158	pep306	SSWKRGSMLFRVTI
159	pep307	SSWKRSMLFRVTIN
160	pep308	SSWKRMLFRVTIN
161	pep309	SSWKRDGSMLFRVTIN
162	pep310	SSWKRPDGSMLFRVTIN
163	pep311	SSWKRSMLFRVTI
164	pep312	SSWKRMLFRVTI
165	pep313	SSWKRDGSMLFRVTI
166	pep314	SSWKRPDGSMLFRVTI
167	pep316	VSGWRLFKKISVFTL
168	pep317	VSGWRLFKKISVFT
169	pep318	VSGWRLFKKISVF
170	pep319	VSGWRLFKKISV
171	pep320	VSGWRLCKKIS
172	pep326	VSGWRLFKKISGSMLFRVTINS
173	pep380	SSWKRLFRVTINS
174	pep383	SSWKRFRVTINS
175	pep386	SSWKRRVTINS
176	pep389	SSWKRTPDGSMLFRVTINS
177	pep392	SSWKRITPDGSMLFRVTINS

178	pep395	SSWKRLITPDGSMLFRVTINS
179	pep396	SSRGSMLFRVTINSWK
180	pep397	SKRGSMLFRVTINSWS
181	pep398	SWRGSMLFRVTINS
182	pep400	SSRGSMLFRVTIWK
183	pep401	SSWKRGSMLYRVTINS
184	pep402	SSWKRGSMLWRVTINS
185	pep403	SSWKRGSMLHRVTINS
186	pep404	SSWKRGSLLFRVTINS
187	pep405	SSWKRGSKLFRVTINS
188	pep406	SSWKRGSRLFRVTINS
189	pep407	SSWKRGSFLFRVTINS
190	pep408	SSWKRGSWLFRVTINS
191	pep409	SSWKRGSMLFRVSINS
192	pep410	SSWKRGSMLFRVQINS
193	pep411	SSWKRGSMLFRVNINS
194	SmTrip9-286 with cysteine	SSWKRGSMLFRVTINSC
195	HiBit with cysteine	CVSGWRLFKKIS
196	SmTrip9-286 with azide	SSWKRGSMLFRVTINSK(Aza)
197	HiBit with azide	(aza)KVSGWRLFKKIS
198	WT OgLuc dipeptide	GSLLFRVTINGVTGWRLCENILA
199	WT NanoLuc dipeptide	GSLLFRVTINVGVTGWRLCERILA
200	pep157	SVSGWRLFKKIS
201	pep158	NSVSGWRLFKKIS
202	pep206	GWRLFKKIS
203	HiBiT-His-LgTrip3546 (ATG 3745)	Atggtgagcggctggcggctgttcaagaagattagccaccatcaccatcaccatcatcacttcacactcgac gatttcgttggggactgggaacagacagccgcctacaacctggaccaagtccttgaacagggaggtgtgtc cagtttgctgcagaatctcgccgtgtccgtaactccgatcatgaggattgtccggagcggtgaaaatgccctg aagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtg tttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggg gttacgccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatc actaccacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgatcacccccgactaa
204	HiBiT-His-LgTrip3546 (ATG 3745)	MVSGWRLFKKISHHHHHHHHFTLDDFVGDWEQTAAYNLDQVLEQ GGVSSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQI EEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVF DGKKITTTGTLWNGNKIIDERLITPD

205	His-HiBiT-GSSG-LgTrip3546 (ATG 3746)	Atgaaacatcaccatcaccatcatgtgagcggctggcggctgttcaagaagattagcggcagctccggtttc acactcgacgatttcgttggggactgggaacagacagccgcctacaacctggaccaagtccttgaacaggg aggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatcatgaggattgtccggagcggtgaa aatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatc gaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggt aatcgacggggttacgccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacg gcaaaaagatcactaccacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgatcaccccc gactaa
206	His-HiBiT-GSSG-LgTrip3546 (ATG 3746)	MKHHHHHHVSGWRLFKKISGSSGFTLDDFVGDWEQTAAYNLDQVL EQGGVSSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQM AQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKLNYFGRPYEGI AVFDGKKITTTGTLWNGNKIIDERLITPD
207	FRB-15GS-86, no AI in linker (ATG3768)	Atggtggccatcctctggcatgagatgtggcatgaaggcctggaagaggcatctcgtttgtactttggggaa aggaacgtgaaaggcatgtttgaggtgctggagcccttgcatgctatgatggaacggggcccccagactct gaaggaaacatcctttaatcaggcctatggtcgagatttaatggaggcccaagagtggtgcaggaagtacat gaaatcagggaatgtcaaggacctcacccaagcctgggacctctattatcatgtgttccgacgaatcagtggt ggttcaggtggtggcgggagcggtggctcgagcagcggtggagtgagcggctggcggctgttcaagaag attagctaa
208	FRB-15GS-86, no AI in linker (ATG3768)	MVAILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERG PQTLKETSFNQAYGRDLMEAQEWCRKYMKSGNVKDLTQAWDLYY HVFRRISGGSGGGGSGGSSSGGVSGWRLFKKIS
209	FRB-15GS-289 (ATG3769)	Atggtggccatcctctggcatgagatgtggcatgaaggcctggaagaggcatctcgtttgtactttggggaa aggaacgtgaaaggcatgtttgaggtgctggagcccttgcatgctatgatggaacggggcccccagactct gaaggaaacatcctttaatcaggcctatggtcgagatttaatggaggcccaagagtggtgcaggaagtacat gaaatcagggaatgtcaaggacctcacccaagcctgggacctctattatcatgtgttccgacgaatcagtggt ggttcaggtggtggcgggagcggtggctcgagcagcggtggagttagcgttagcggctggcgcctgttca agaagatcagctaa
210	FRB-15GS-289 (ATG3769)	MVAILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERG PQTLKETSFNQAYGRDLMEAQEWCRKYMKSGNVKDLTQAWDLYY HVFRRISGGSGGGGSGGSSSGGVSVSGWRLFKKIS
211	FKBP-SmTrip9 fusion template, no AI in linker (ATG3770)	atgggagtgcaggtggaaaccatctccccaggagacgggcgcaccttccccaagcgcggccagacctgc gtggtgcactacaccgggatgcttgaagatggaaagaaatttgattcctcccgggacagaaacaagcccttta agtttatgctaggcaagcaggaggtgatccgaggctgggaagaaggggttgcccagatgagtgtgggtca gagagccaaactgactatatctccagattatgcctatggtgccactgggcacccaggcatcatcccaccacat gccactctcgtcttcgatgtggagcttctaaaactggaaggtggttcaggtggtggcgggagcggtggctcg agcagcggtgga
212	FKBP-SmTrip9 fusion template, no AI in linker (ATG3770)	MGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSSRDRNKP FKFMLGKQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGII PPHATLVFDVELLKLEGGSGGGGSGGSSSGG
213	295	GSMLFRVTINSV
214	300	GSMLFRVTINSVS
215	412	MLFRVTINSVSG
216	413	MLFRVTINSVSGW
217	415	MLFRVTINSVSGWK
218	416	MLFRVTINSVSGWR

219	418	GSMLFRVTINSVSG
220	419	GSMLFRVTINSVSGW
221	422	GSMLFRVTINSVSGWR
222	423	GSMLFRVTINSVSGWK
223	434	GSMLFRVTIWK
224	435	GSMLFRVTINSWK
225	477	MLFRVTINSWK
226	478	MLFRVTINSWS
227	479	MLFRVTIWS
228	480	MLFRVTIWK
229	481	MLFRVKINS
230	482	GSMLFRVTINSWS
231	483	GSMLFRVKINS
232	484	GSMLFRVTIWS
233	485	MLFRVNINS
234	486	MLFRVWINS
235	487	LLFRVKINS
236	488	FLFRVTINS
237	295	SSWKRGSMLFRVTINSV
238	300	SSWKRGSMLFRVTINSVS
239	412	SSWKRMLFRVTINSVSG
240	413	SSWKRMLFRVTINSVSGW
241	414	SSWKRMLFRVTINSVSGWR
242	415	SSWKRMLFRVTINSVSGWK
243	417	MLFRVTINSVSGWK
244	418	SSWKRGSMLFRVTINSVSG
245	419	SSWKRGSMLFRVTINSVSGW
246	420	SSWKRGSMLFRVTINSVSGWR
247	421	SSWKRGSMLFRVTINSVSGWK
248	424	SSWKRGSYLFRVTINS
249	425	SSWKRGSMLFRVKINS
250	426	SSWKRGSMLFRVRINS
251	427	SSWKRGSMLFRVWINS
252	428	SSKRGSMLFRVTIWSV
253	429	SSKRGSMLFRVTIWSVS
254	430	SSWRGSMLFRVTIKS
255	431	KRSSGSMLFRVTIWS
256	432	SSKRMLFRVTIWS
257	433	KRSSMLFRVTIWS

258	445	GSMKFRVTINSWK
259	450	GSMLFRKTINSWK
260	455	GSMLFRVTKNSWK
261	522	GKMLFRVTIWK
262	523	GSMKFRVTINSWK
263	524	GSMKFRVTIWK
264	525	GRMLFRVTINSWK
265	526	GRMLFRVTIWK
266	527	GSMRFRVTINSWK
267	528	GSMRFRVTIWK
268	529	GDMLFRVTINSWK
269	530	GDMLFRVTIWK
270	531	GSMDFRVTINSWK
271	532	GSMDFRVTIWK
272	533	GEMLFRVTINSWK
273	535	GSMEFRVTINSWK
274	536	GSMEFRVTIWK
275	538	GSMLFRVTIWKVK
276	539	GSMLFRVTIWSVK
277	540	GSMLFRVTIWSK
278	541	GSMLFRVTIWKWK
279	542	GSMLFRVTIWKK
280	245	GSMLFRVTINS
281	292.x	MLFRVTINS
282	297.x	MLFVTINSV
283	302.x	MLFRVTINSVS
284	305.x	GSMLFRVTIN
285	306.x	GSMLFRVTI
286	307.x	SMLFRVTIN
287	308.x	MLFRVTIN
288	312.x	MLFRVTI
289	399	SSKRGSMLFRVTIWS
290	273	GSMLFRVTINSGVSGWALFKKIS
291	264	GSMLFRVTINSGVSGWRLFKKIS
292	167	VSGWALFKKIS
293	331	GSMLFRVTINSVSGVSGWRLFKKIS
294	LgTrip 3546 (no His6)	MVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIMRI VRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVIL PYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDE RLITPD

295	LgTrip 3546 (no His6)	atggtcttcacactcgacgatttcgttggggactgggaacagacagccgcctacaacctggaccaagtccttg aacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatcatgaggattgtccggag cggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggc ccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggca cactggtaatcgacggggttacgccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgtgt tcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgatc acccccgac
296	LgTrip 2098 (no His6)	MVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIMRI VRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVIL PYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITVTGTLWNGNKIID ERLITPD
297	LgTrip 2098 (no His6)	atggtcttcacactcgaagatttcgttggggactgggaacagacagccgcctacaacctggaccaagtccttg aacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccggag cggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggc ccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggca cactggtaatcgacggggttacgccgaacatgctgaactatttcggacggccgtatgaaggcatcgccgtgtt cgacggcaaaaagatcactgtaacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgatca cccccgac
298	157	SVSGWRLFKKIS
299	158	NSVSGWRLFKKIS
300	206	GWRLFKKIS
301	264	GSMLFRVTINSGVSGWRLFKKIS
302	489	GSMLFRVTINSWK (N-term unblocked)
303	490	GSMLFRVTINSWK (C-term unblocked)
304	491	GSMLFRVTINSWK (Both unblocked)
305	492	GSMLFRVTINKWK
306	493	GSMLFRVTIKSWK
307	494	GSMLFRVTINRWK
308	495	GSMLFRVTIRSWK
309	496	GSMLFRVTINDWK
310	497	GSMLFRVTIDSWK
311	498	GSMLFRVTINEWK
312	499	GSMLFRVTIESWK
313	465	GSMRFRVTINSWK (Both termini unblocked)
314	466	GSMDFRVTINSWK (Both termini unblocked)
315	467	GSMEFRVTINSWK (Both termini unblocked)
316	468	GSMLFRRTINSWK (Both termini unblocked)
317	469	GSMLFRDTINSWK (Both termini unblocked)
318	470	GSMLFRETINSWK (Both termini unblocked)
319	472	GSMLFRVTDNSWK (Both termini unblocked)
320	473	GSMLFRVTENSWK (Both termini unblocked)
321	474	GSMKFRVTINSWK (Both termini unblocked)
322	475	GSMLFRKTINSWK (Both termini unblocked)

323	476	GSMLFRVTKNSWK (Both termini unblocked)
324	436	GSMLFRVTINS (N-term unblocked)
325	437	GSMLFRVSINS (N-term unblocked)
326	438	GSMLFRVNINS (N-term unblocked)
327	439	GSKLFRVTINS (N-term unblocked)
328	440	GSRLFRVTINS (N-term unblocked)
329	441	GSMWFRVTINS (N-term unblocked)
330	442	GSMSFRVTINS (N-term unblocked)
331	443	GSMNFRVTINS (N-term unblocked)
332	444	GSMKFRVTINS (N-term unblocked)
333	446	GSMLFRWTINS (N-term unblocked)
334	447	GSMLFRSTINS (N-term unblocked)
335	448	GSMLFRNTINS (N-term unblocked)
336	449	GSMLFRKTINS (N-term unblocked)
337	451	GSMLFRVTWNS (N-term unblocked)
338	452	GSMLFRVTSNS (N-term unblocked)
339	453	GSMLFRVTNNS (N-term unblocked)
340	454	GSMLFRVTKNS (N-term unblocked)
341	456	GSMLFRVTIKS (N-term unblocked)
342	Antares ATG 3802	MKHHHHHHVSKGEELIKENMRSKLYLEGSVNGHQFKCTHEGEGKP YEGKQTNRIKVVEGGPLPFAFDILATHFMYGSKVFIKYPADLPDYFK QSFPEGFTWERVMVFEDGGVLTATQDTSLQDGELIYNVKVRGVNFP ANGPVMQKKTLGWEPSTETMYPADGGLEGRCDKALKLVGGGHLH VNFKTTYKSKKPVKMPGVHYVDRRLERIKEADNETYVEQYEHAVA RYSNLGGGFTLEDFVGDWRQTAGYNLDQVLEQGGVSSLFQNLGVS VTPIQRIVLSGENGLKIDIHVIIPYEGLSGDQMGQIEKIFKVVYPVDDH HFKVILHYGTLVIDGVTPNMIDYFGRPYEGIAVFDGKKITVTGTLWN GNKIIDERLINPDGSLLFRVTINGVTGWRLCERILARHELIKENMRSK LYLEGSVNGHQFKCTHEGEGKPYEGKQTNRIKVVEGGPLPFAFDILA THFMYGSKVFIKYPADLPDYFKQSFPEGFTWERVMVFEDGGVLTAT QDTSLQDGELIYNVKVRGVNFPANGPVMQKKTLGWEPSTETMYPA DGGLEGRCDKALKLVGGGHLHVNFKTTYKSKKPVKMPGVHYVDR RLERIKEADNETYVEQYEHAVARYSNLGGGMDELYK

343	Antares ATG 3802	atgaaacatcaccatcaccatcatgtgagcaagggagaagaacttataaaagaaaacatgcggtctaaactgt acctcgagggctccgtcaatgggcaccagtttaagtgtacccacgagggtgagggaaagccctatgaggg gaagcagacaaaccgcatcaaggtcgtcgaaggggacccctcccgtttgcctttgatatcttggctactcac tttatgtacggaagcaaagttttcataaagtatcctgccgaccttcctgattattttaaacagtcatttcccgaggg tttcacatgggaaagggtcatggtgtttgaggatggaggcgtgctcactgcaactcaggacacctcactgca ggacggcgagctgatctacaatgtgaaggtccggggtgtaaacttccctgccaacgggcctgtaatgcaga agaagaccctgggatgggagccgtccaccgaaaccatgtaccctgctgatggtgggctggagggccgatg tgacaaggctctgaagctcgttggaggtggtcatttgcacgtaaatttcaagacaacttacaagagcaaaaaa cccgtaaaaatgcccggggttcattacgttgacagaaggcttgaacgcataaaggaagctgataacgagaca tacgtggagcagtacgagcacgccgttgcccggtactcaaacctgggggtggctttacactggaggatttt gtgggagattggagacagacagccggctacaatctggatcaggtgctggaacaaggaggagtgtcttctct gtttcagaatctgggagtgagcgtgacacctatccagaggatcgtgctgtctggcgagaatggactgaagat cgatattcacgtgatcatcccctacgaaggcctgtctggagaccagatgggccagattgagaagatcttcaaa gtggtgtatcctgtggacgatcaccacttcaaggtgatcctgcactacggcaccctggtgattgatggagtga cacctaacatgatcgactacttcggaagaccttacgagggaatcgccgtgttcgacggaaagaagatcaccg tgacaggaacactgtggaatggaaacaagatcatcgacgagcggctgatcaaccctgatggatctctgctgtt cagagtgaccatcaacggagtgacaggatggagactgtgcgagagaattctggctagacatgagctaatca aggaaaatatgagaagtaagctatacttagaggggtccgtcaacggtcaccagtttaaatgcactcatgaagg tgaggggaaaccttatgaaggtaagcagactaatcgaataaaagtggtcgagggcggtcctctgccattcgc tttcgatattctggccactcactttatgtatgggtctaaggtctttattaaatacccgctgatttgccagactacttt aaacagtccttccctgaaggattcacatgggagcgggtgatggtgttcgaggatggaggcgttcttactgcaa ctcaggatacttccttgcaagacggggaactgatctacaacgttaaggtccgcggcgtcaatttcccagccaa tggtccagtgatgcagaagaaaaccttggggtgggagccctcaacggagacaatgtaccctgcggacggc gggcttgagggtagatgtgataaggcattgaaactcgtcgggggcggccaccttcatgtgaatttcaagacta catataaaagtaaaaaaccagtcaagatgcctggagtgcactacgtggatcgtaggttggagaggataaaag aagccgacaacgaaacttatgtagagcaatatgagcacgccgtggctcgttattccaacttgggcggaggaa tggatgaactgtacaag
344	Antares (LgBiT) ATG 3803	MKHHHHHHVSKGEELIKENMRSKLYLEGSVNGHQFKCTHEGEGKP YEGKQTNRIKVVEGGPLPFAFDILATHFMYGSKVFIKYPADLPDYFK QSFPEGFTWERVMVFEDGGVLTATQDTSLQDGELIYNVKVRGVNFP ANGPVMQKKTLGWEPSTETMYPADGGLEGRCDKALKLVGGGHLH VNFKTTYKSKKPVKMPGVHYVDRRLERIKEADNETYVEQYEHAVA RYSNLGGGFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVS VTPIQRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDD HHFKVILPYGTLVIDGVTPNMLNYFGRPYEGIAVFDGKKITVTGTLW NGNKIIDERLITPDGSMLFRVTINSRHELIKENMRSKLYLEGSVNGHQ FKCTHEGEGKPYEGKQTNRIKVVEGGPLPFAFDILATHFMYGSKVFI KYPADLPDYFKQSFPEGFTWERVMVFEDGGVLTATQDTSLQDGELI YNVKVRGVNFPANGPVMQKKTLGWEPSTETMYPADGGLEGRCDK ALKLVGGGHLHVNFKTTYKSKKPVKMPGVHYVDRRLERIKEADNE TYVEQYEHAVARYSNLGGGMDELYK

345	Antares (LgBiT) ATG 3803	atgaaacatcaccatcaccatcatgtgagcaagggagaagaacttataaaagaaaacatgcggtctaaactgt acctcgagggctccgtcaatgggcaccagtttaagtgtacccacgagggtgagggaaagccctatgaggg gaagcagacaaaccgcatcaaggtcgtcgaagggggacccctcccgtttgcctttgatatcttggctactcac tttatgtacggaagcaaagttttcataaagtatcctgccgaccttcctgattattttaaacagtcatttcccgaggg tttcacatgggaaagggtcatggtgtttgaggatggaggcgtgctcactgcaactcaggacacctcactgca ggacggcgagctgatctacaatgtgaaggtccggggtgtaaacttccctgccaacgggcctgtaatgcaga agaagaccctgggatgggagccgtccaccgaaaccatgtaccctgctgatggtgggctggagggccgatg tgacaaggctctgaagctcgttggaggtggtcatttgcacgtaaatttcaagacaacttacaagagcaaaaaa cccgtaaaaatgcccggggttcattacgttgacagaaggcttgaacgcataaaggaagctgataacgagaca tacgtggagcagtacgagcacgccgttgcccggtactcaaacctgggggtggcttcacactcgaagatttc gttggggactgggaacagacagccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagttt gctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccggagcggtgaaaatgccctgaagat cgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaa ggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggggtta cgccgaacatgctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactg taacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgatcacccccgacggctccatgctg ttccgagtaaccatcaacagcagacatgagctaatcaaggaaaatatgagaagtaagctatacttagaggggt ccgtcaacggtcaccagtttaaatgcactcatgaaggtgaggggaaaccttatgaaggtaagcagactaatc gaataaaagtggtcgagggcggtcctctgccattcgctttcgatattctggccactcactttatgtatgggtctaa ggtctttattaaatacccccgctgatttgccagactactttaaacagtccttccctgaaggattcacatgggagcg ggtgatggtgttcgaggatggaggcgttcttactgcaactcaggatacttccttgcaagacggggaactgatc tacaacgttaaggtccgcggcgtcaatttcccagccaatggtccagtgatgcagaagaaaaccttggggtgg gagccctcaacggagacaatgtaccctgcggacggcgggcttgagggtagatgtgataaggcattgaaact cgtcgggggcggccaccttcatgtgaatttcaagactacatataaaagtaaaaaaccagtcaagatgcctgga gtgcactacgtggatcgtaggttggagaggataaaagaagccgacaacgaaacttatgtagagcaatatgag cacgccgtggctcgttattccaacttgggcggaggaatggatgaactgtacaag
346	Antares (LgTrip 3546) ATG 3804	MKHHHHHHVSKGEELIKENMRSKLYLEGSVNGHQFKCTHEGEGKP YEGKQTNRIKVVEGGPLPFAFDILATHFMYGSKVFIKYPADLPDYFK QSFPEGFTWERVMVFEDGGVLTATQDTSLQDGELIYNVKVRGVNFP ANGPVMQKKTLGWEPSTETMYPADGGLEGRCDKALKLVGGGHLH VNFKTTYKSKKPVKMPGVHYVDRRLERIKEADNETYVEQYEHAVA RYSNLGGGFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVS VTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDD HHFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTLW NGNKIIDERLITPDRHELIKENMRSKLYLEGSVNGHQFKCTHEGEGK PYEGKQTNRIKVVEGGPLPFAFDILATHFMYGSKVFIKYPADLPDYF KQSFPEGFTWERVMVFEDGGVLTATQDTSLQDGELIYNVKVRGVNF PANGPVMQKKTLGWEPSTETMYPADGGLEGRCDKALKLVGGGHL HVNFKTTYKSKKPVKMPGVHYVDRRLERIKEADNETYVEQYEHAV ARYSNLGGGMDELYK

347	Antares (LgTrip 3546) ATG 3804	atgaaacatcaccatcaccatcatgtgagcaagggagaagaacttataaaagaaaacatgcggtctaaactgt acctcgagggctccgtcaatgggcaccagtttaagtgtacccacgagggtgagggaaagccctatgaggg gaagcagacaaaccgcatcaaggtcgtcgaaggggacccctcccgtttgcctttgatatcttggctactcac tttatgtacggaagcaaagttttcataaagtatcctgccgaccttcctgattattttaaacagtcatttcccgaggg tttcacatgggaaagggtcatggtgtttgaggatggaggcgtgctcactgcaactcaggacacctcactgca ggacggcgagctgatctacaatgtgaaggtccggggtgtaaacttccctgccaacgggcctgtaatgcaga agaagaccctgggatgggagccgtccaccgaaaccatgtaccctgctgatggtgggctggagggccgatg tgacaaggctctgaagctcgttggaggtggtcatttgcacgtaaatttcaagacaacttacaagagcaaaaaa cccgtaaaaatgcccggggttcattacgttgacagaaggcttgaacgcataaaggaagctgataacgagaca tacgtggagcagtacgagcacgccgttgcccggtactcaaacctgggggtggcttcacactcgacgatttc gttggggactgggaacagacagccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagttt gctgcagaatctcgccgtgtccgtaactccgatcatgaggattgtccggagcggtgaaaatgccctgaagat cgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaa ggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggggtta cgccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcacta ccacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgatcacccccgacagacatgagct aatcaaggaaaatatgagaagtaagctatacttagaggggtccgtcaacggtcaccagtttaaatgcactcat gaaggtgaggggaaaccttatgaaggtaagcagactaatcgaataaaagtggtcgagggcggtcctctgcc attcgctttcgatattctggccactcactttatgtatgggtctaaggtctttattaaatacccccgctgatttgccaga ctactttaaacagtccttccctgaaggattcacatgggagcgggtgatggtgttcgaggatggaggcgttctta ctgcaactcaggatacttccttgcaagacggggaactgatctacaacgttaaggtccgcggcgtcaatttccc agccaatggtccagtgatgcagaagaaaaccttggggtgggagccctcaacggagacaatgtaccctgcg gacggcgggcttgagggtagatgtgataaggcattgaaactcgtcgggggcggccaccttcatgtgaatttc aagactacatataaaagtaaaaaaccagtcaagatgcctggagtgcactacgtggatcgtaggttggagagg ataaaagaagccgacaacgaaacttatgtagagcaatatgagcacgccgtggctcgttattccaacttgggc ggaggaatggatgaactgtacaag
348	ATG 3815	MKHHHHHHFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVS VTPIQRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDD HHFKVILPYGTLVIDGVTPNMLNYFGRPYEGIAVFDGKKITVTGTLW NGNKIIDERLITPDGSMLFRVTINSGGSGGSSGELIKENMRSKLYLEG SVNGHQFKCTHEGEGKPYEGKQTNRIKVVEGGPLPFAFDILATHFM YGSKVFIKYPADLPDYFKQSFPEGFTWERVMVFEDGGVLTATQDTS LQDGELIYNVKVRGVNFPANGPVMQKKTLGWEPSTETMYPADGGL EGRCDKALKLVGGGHLHVNFKTTYKSKKPVKMPGVHYVDRRLERI KEADNETYVEQYEHAVARYSNLGGGMDELYK

349	ATG 3815	atgaaacatcaccatcaccatcatttcacactcgaagatttcgttggggactgggaacagacagccgcctaca acctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgat ccaaaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctg agcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaagg tgatcctgccctatggcacactggtaatcgacggggttacgccgaacatgctgaactatttcggacggccgta tgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaacggcaacaaaattat cgacgagcgcctgatcacccccgacggctccatgctgttccgagtaaccatcaacagcggaggctcaggtg gatcctcaggtgagctaatcaaggaaaatatgagaagtaagctatacttagaggggtccgtcaacggtcacc agtttaaatgcactcatgaaggtgaggggaaaccttatgaaggtaagcagactaatcgaataaaagtggtcga gggcggtcctctgccattcgctttcgatattctggccactcactttatgtatgggtctaaggtctttattaaatacc ccgctgatttgccagactactttaaacagtccttccctgaaggattcacatgggagcgggtgatggtgttcgag gatggaggcgttcttactgcaactcaggatacttccttgcaagacggggaactgatctacaacgttaaggtcc gcggcgtcaatttcccagccaatggtccagtgatgcagaagaaaaccttggggtgggagccctcaacggag acaatgtaccctgcggacggcgggcttgagggtagatgtgataaggcattgaaactcgtcgggggcggcc accttcatgtgaatttcaagactacatataaaagtaaaaaaccagtcaagatgcctggagtgcactacgtggat cgtaggttggagaggataaaagaagccgacaacgaaacttatgtagagcaatatgagcacgccgtggctcg ttattccaacttgggcggaggaatggatgaactgtacaag
350	ATG 3816	MKHHHHHHFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVS VTPIQRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDD HHFKVILPYGTLVIDGVTPNMLNYFGRPYEGIAVFDGKKITVTGTLW NGNKIIDERLITPDGSMLFRVTINSRHELIKENMRSKLYLEGSVNGHQ FKCTHEGEGKPYEGKQTNRIKVVEGGPLPFAFDILATHFMYGSKVFI KYPADLPDYFKQSFPEGFTWERVMVFEDGGVLTATQDTSLQDGELI YNVKVRGVNFPANGPVMQKKTLGWEPSTETMYPADGGLEGRCDK ALKLVGGGHLHVNFKTTYKSKKPVKMPGVHYVDRRLERIKEADNE TYVEQYEHAVARYSNLGGGMDELYK
351	ATG 3816	Atgaaacatcaccatcaccatcatttcacactcgaagatttcgttggggactgggaacagacagccgcctac aacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccga tccaaaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtct gagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaag gtgatcctgccctatggcacactggtaatcgacggggttacgccgaacatgctgaactatttcggacggccgt atgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaacggcaacaaaatt atcgacgagcgcctgatcacccccgacggctccatgctgttccgagtaaccatcaacagcagacatgagcta atcaaggaaaatatgagaagtaagctatacttagaggggtccgtcaacggtcaccagtttaaatgcactcatg aaggtgaggggaaaccttatgaaggtaagcagactaatcgaataaaagtggtcgagggcggtcctctgcca ttcgctttcgatattctggccactcactttatgtatgggtctaaggtctttattaaataccccgctgatttgccagact actttaaacagtccttccctgaaggattcacatgggagcgggtgatggtgttcgaggatggaggcgttcttact gcaactcaggatacttccttgcaagacggggaactgatctacaacgttaaggtccgcggcgtcaatttcccag ccaatggtccagtgatgcagaagaaaaccttggggtgggagccctcaacggagacaatgtaccctgcgga cggcgggcttgagggtagatgtgataaggcattgaaactcgtcgggggcggccaccttcatgtgaatttcaa gactacatataaaagtaaaaaaccagtcaagatgcctggagtgcactacgtggatcgtaggttggagaggat aaaagaagccgacaacgaaacttatgtagagcaatatgagcacgccgtggctcgttattccaacttgggcgg aggaatggatgaactgtacaag

352	ATG 3817	MKHHHHHHFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAV SVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVD DHHFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTL WNGNKIIDERLITPDGGSGGSSGELIKENMRSKLYLEGSVNGHQFKC THEGEGKPYEGKQTNRIKVVEGGPLPFAFDILATHFMYGSKVFIKYP ADLPDYFKQSFPEGFTWERVMVFEDGGVLTATQDTSLQDGELIYNV KVRGVNFPANGPVMQKKTLGWEPSTETMYPADGGLEGRCDKALKL VGGGHLHVNFKTTYKSKKPVKMPGVHYVDRRLERIKEADNETYVE QYEHAVARYSNLGGGMDELYK
353	ATG 3817	Atgaaacatcaccatcaccatcatttcacactcgacgatttcgttggggactgggaacagacagccgcctac aacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccga tcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtct gagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaag gtgatcctgccctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggccg tatgaaggcatcgccgtgttcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaaaat tatcgacgagcgcctgatcacccccgacggaggctcaggtggatcctcaggtgagctaatcaaggaaaata tgagaagtaagctatacttagaggggtccgtcaacggtcaccagtttaaatgcactcatgaaggtgagggga aaccttatgaaggtaagcagactaatcgaataaaagtggtcgagggcggtcctctgccattcgctttcgatatt ctggccactcactttatgtatgggtctaaggtctttattaaataccccgctgatttgccagactactttaaacagtc cttccctgaaggattcacatgggagcgggtgatggtgttcgaggatggaggcgttcttactgcaactcaggat acttccttgcaagacggggaactgatctacaacgttaaggtccgcggcgtcaatttcccagccaatggtccag tgatgcagaagaaaaccttggggtgggagccctcaacggagacaatgtaccctgcggacggcgggcttga gggtagatgtgataaggcattgaaactcgtcgggggcggccaccttcatgtgaatttcaagactacatataaa agtaaaaaaccagtcaagatgcctggagtgcactacgtggatcgtaggttggagaggataaaagaagccga caacgaaacttatgtagagcaatatgagcacgccgtggctcgttattccaacttgggcggaggaatggatga actgtacaag
354	ATG 3818	MKHHHHHHFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAV SVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVD DHHFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTL WNGNKIIDERLITPDRHELIKENMRSKLYLEGSVNGHQFKCTHEGEG KPYEGKQTNRIKVVEGGPLPFAFDILATHFMYGSKVFIKYPADLPDY FKQSFPEGFTWERVMVFEDGGVLTATQDTSLQDGELIYNVKVRGVN FPANGPVMQKKTLGWEPSTETMYPADGGLEGRCDKALKLVGGGHL HVNFKTTYKSKKPVKMPGVHYVDRRLERIKEADNETYVEQYEHAV ARYSNLGGGMDELYK

355	ATG 3818	Atgaaacatcaccatcaccatcatttcacactcgacgatttcgttggggactgggaacagacagccgcctac aacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccga tcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtct gagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaag gtgatcctgccctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggccg tatgaaggcatcgccgtgttcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaaaat tatcgacgagcgcctgatcacccccgacagacatgagctaatcaaggaaaatatgagaagtaagctatactt agaggggtccgtcaacggtcaccagtttaaatgcactcatgaaggtgaggggaaaccttatgaaggtaagca gactaatcgaataaaagtggtcgagggcggtcctctgccattcgctttcgatattctggccactcactttatgtat gggtctaaggtctttattaaataccccgctgatttgccagactactttaaacagtccttccctgaaggattcacat gggagcgggtgatggtgttcgaggatggaggcgttcttactgcaactcaggatacttccttgcaagacgggg aactgatctacaacgttaaggtccgcggcgtcaatttcccagccaatggtccagtgatgcagaagaaaaccctt ggggtgggagccctcaacggagacaatgtaccctgcggacggcgggcttgagggtagatgtgataaggc attgaaactcgtcgggggcggccaccttcatgtgaatttcaagactacatataaaagtaaaaaaccagtcaag atgcctggagtgcactacgtggatcgtaggttggagaggataaaagaagccgacaacgaaacttatgtaga gcaatatgagcacgccgtggctcgttattccaacttgggcggaggaatggatgaactgtacaag
356	LgTrip 2899 (LgTrip 2098+Q42L)	MKHHHHHHVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPILRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVD DHHFKVILPYGTLVIDGVTPNMLNYFGRPYEGIAVFDGKKITVTGTL WNGNKIIDERLITPD
357	LgTrip 2899 (LgTrip 2098+Q42L)	atgaaacatcaccatcaccatcatgtcttcacactcgaagatttcgttggggactgggaacagaccgccgcct acaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactcc gatcctaaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggt ctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcacttta aggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacatgctgaactatttcggacggc cgtatgaaggcatcgccgtgttcgacggcaaaaagatcactgtaacagggaccctgtggaacggcaacaaa attatcgacgagcgcctgatcaccccgac
358	ATG-3930	atgAAACATCACCATCACCATCATgtcTTCACACTCGACGATTTCGTT GGGGACTGGGAACAGACAGCCGCCTACAACCTGGACCAAGTCCT TGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCGTGTC CGTAACTCCGATCATGAGGATTGTCCGGAGCGGTGAAAATGCCCT GAAGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGC CGACCAAATGGCCCAGATCGAAGAGGTGTTTAAGGTGGTGTACC CTGTGGATGATCATCACTTTAAGGTGATCCTGCCCTATGGCACAC TGGTAATCGACGGGGTTACGCCGAACAAGCTGAACTATTTCGGAC GGCCGTATGAAGGCATCGCCGTGTTCGACGGCTAA
359	ATG-3930	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPV DDHHFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVFDG

360	SmTrip9-15GS-ProteinG (ATG 4002)	gggagctccGGTGGTGGCGGGAGCGGAGGTGGAGGctcgAGCGGTATG ACGTATAAGTTAATCCTTAATGGTAAAACATTGAAAGGCGAGAC AACTACTGAAGCTGTTGATGCTGCTACTGCAGAAAAAGTCTTCAA ACAATACGCTAACGACAACGGTGTTGACGGTGAATGGACTTACG ACGATGCGACGAAAACCTTTACGGTCACCGAAAAACCAGAAGTG ATCGATGCGTCTGAATTAACACCAGCCGTGACAACTTACAAACTT GTTATTAATGGTAAAACATTGAAAGGCGAAACAACTACTGAGGC TGTTGATGCTGCTACTGCAGAGAAGGTGTTCAAACAATATGCGAA TGACAACGGTGTTGACGGTGAGTGGACTTACGACGATGCGACTA AGACCTTTACAGTTACTGAAAAACCAGAAGTGATCGATGCGTCTG AGTTAACACCAGCCGTGACAACTTACAAACTTGTTATTAATGGTA AAACATTGAAAGGCGAAACAACTACTAAAGCAGTAGACGCAGAA ACTGCGGAGAAGGCCTTCAAACAATACGCTAACGACAACGGTGT TGATGGTGTTTGGACTTATGATGATGCCACAAAAACCTTTACGGT AACTGAGCATCATCACCATCACCACTAA
361	SmTrip9-15GS-ProteinG (ATG 4002)	GSSGGGGSGGGGSSGMTYKLILNGKTLKGETTTEAVDAATAEKVFK QYANDNGVDGEWTYDDATKTFTVTEKPEVIDASELTPAVTTYKLVI NGKTLKGETTTEAVDAATAEKVFKQYANDNGVDGEWTYDDATKT FTVTEKPEVIDASELTPAVTTYKLVINGKTLKGETTTKAVDAETAEK AFKQYANDNGVDGVWTYDDATKTFTVTEHHHHHH
362	ATG-3929	atgAAACATCACCATCACCATCATgtcTTCACACTCGACGATTTCGTT GGGGACTGGGAACAGACAGCCGCCTACAACCTGGACCAAGTCCT TGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCGTGTC CGTAACTCCGATCATGAGGATTGTCCGGAGCGGTGAAAATGCCCT GAAGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGC CGACCAAATGGCCCAGATCGAAGAGGTGTTTAAGGTGGTGTACC CTGTGGATGATCATCACTTTAAGGTGATCCTGCCCTATGGCACAC TGGTAATCGACGGGGTTACGCCGAACAAGCTGAACTATTTCGGAT AA
363	ATG-3929	Mkhhhhhhvftlddfvgdweqtaaynldqvleqggvssllqnlavsvtpimrivrsgenalkidihviip yeglsadqmaqieevfkvvypvddhhfkvilpygtlvidgvtpnklnyfg
364	ATG-3930	atgAAACATCACCATCACCATCATgtcTTCACACTCGACGATTTCGTT GGGGACTGGGAACAGACAGCCGCCTACAACCTGGACCAAGTCCT TGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCGTGTC CGTAACTCCGATCATGAGGATTGTCCGGAGCGGTGAAAATGCCCT GAAGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGC CGACCAAATGGCCCAGATCGAAGAGGTGTTTAAGGTGGTGTACC CTGTGGATGATCATCACTTTAAGGTGATCCTGCCCTATGGCACAC TGGTAATCGACGGGGTTACGCCGAACAAGCTGAACTATTTCGGAC GGCCGTATGAAGGCATCGCCGTGTTCGACGGCTAA

365	ATG-3930	Mkhhhhhhvftlddfvgdweqtaaynldqvleqggvssllqnlavsvtpimrivrsgenalkidihviip yeglsadqmaqieevfkvvypvddhhfkvilpygtlvidgvtpnklnyfgrpyegiavfdg
366	ATG-3931	atgAAACATCACCATCACCATCATgtcTTCACACTCGACGATTTCGTT GGGGACTGGGAACAGACAGCCGCCTACAACCTGGACCAAGTCCT TGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCGTGTC CGTAACTCCGATCATGAGGATTGTCCGGAGCGGTGAAAATGCCCT GAAGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGC CGACCAAATGGCCCAGATCGAAGAGGTGTTTAAGGTGGTGTACC CTGTGGATGATCATCACTTTAAGGTGATCCTGCCCTATGGCACAC TGGTAATCGACGGGGTTACGCCGAACAAGCTGAACTATTTCGGAC GGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACT ACCACAGGGACCCTGTAA
367	ATG-3931	Mkhhhhhhvftlddfvgdweqtaaynldqvleqggvssllqnlavsvtpimrivrsgenalkidihviip yeglsadqmaqieevfkvvypvddhhfkvilpygtlvidgvtpnklnyfgrpyegiavfdgkkitttgtl
368	ATG-3932	atgAAACATCACCATCACCATCATgtcTTCACACTCGACGATTTCGTT GGGGACTGGGAACAGACAGCCGCCTACAACCTGGACCAAGTCCT TGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCGTGTC CGTAACTCCGATCATGAGGATTGTCCGGAGCGGTGAAAATGCCCT GAAGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGC CGACCAAATGGCCCAGATCGAAGAGGTGTTTAAGGTGGTGTACC CTGTGGATGATCATCACTTTAAGGTGATCCTGCCCTATGGCACAC TGGTAATCGACGGGGTTACGCCGAACAAGCTGAACTATTTCGGAC GGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACT ACCACAGGGACCCTGTGGAACGGCTAA
369	ATG-3932	Mkhhhhhhvftlddfvgdweqtaaynldqvleqggvssllqnlavsvtpimrivrsgenalkidihviip yeglsadqmaqieevfkvvypvddhhfkvilpygtlvidgvtpnklnyfgrpyegiavfdgkkitttgtl wng
370	ATG-4808	Atggtttccgtgagcggctggcggctgttcaagaagattagcttcacactcgacgatttcgttggggactggg aacagacagccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcg ccgtgtccgtaactccgatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcat catcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgt ggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacaagctg aactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactaccacagggaccctg tggaacggcaacaaaattatcgacgagcgcctgatcacccccgactaa

371	ATG-4808	Mvsvsgwrlfkkisftlddfvgdweqtaaynldqvleqggvssllqnlavsvtpimrivrsgenalkidi hviipyeglsadqmaqieevfkvvypvddhhfkvilpygtlvidgvtpnklnyfgrpyegiavfdgkkit ttgtlwngnkiiderlitpd
372	ATG-4809	Atggtttccgtgagcggctggcggctgttcaagaagattagcggcagctccggtttcacactcgacgatttcg ttggggactgggaacagacagccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttg ctgcagaatctcgccgtgtccgtaactccgatcatgaggattgtccggagcggtgaaaatgccctgaagatc gacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaag gtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggggttac gccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactac cacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgatcacccccgactaa
373	ATG-4809	MVSVSGWRLFKKISGSSGFTLDDFVGDWEQTAAYNLDQVLEQGGV SSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEV FKVVYPVDDHHFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVFDGK KITTTGTLWNGNKIIDERLITPD
374	ATG-4810	Atggtttccgtgagcggctggcggctgttcaagaagattagcggctcgagcggtggctcgagcggtttcac actcgacgatttcgttggggactgggaacagacagccgcctacaacctggaccaagtccttgaacagggag gtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatcatgaggattgtccggagcggtgaaaat gccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaa gaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatc gacggggttacgccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaa aaagatcactaccacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgatcacccccgact aa
375	ATG-4810	MVSVSGWRLFKKISGSSGGSSGFTLDDFVGDWEQTAAYNLDQVLEQ GGVSSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQI EEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVF DGKKITTTGTLWNGNKIIDERLITPD
376	ATG-4811	Atggtttccgtgagcggctggcggctgttcaagaagattagcggctcgagcggtggctcgagcggtggctc gagcggtttcacactcgacgatttcgttggggactgggaacagacagccgcctacaacctggaccaagtcct tgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatcatgaggattgtccgg agcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatg gcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatgg cacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgt gttcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaaaattatcgacgagcgcctga tcacccccgactaa

377	ATG-4811	MVSVSGWRLFKKISGSSGGSSGGSSGFTLDDFVGDWEQTAAYNLDQ VLEQGGVSSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQ MAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKLNYFGRPYEG IAVFDGKKITTTGTLWNGNKIIDERLITPD
378	ATG-4812	Atggtttccgtgagcggctggcggctgttcaagaagattagcggctcgagcggtggctcgagcggtggctc gagcggtggctcgagcggtttcacactcgacgatttcgttggggactgggaacagacagccgcctacaacc tggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatcatg aggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagc gccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtga tcctgccctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggccgtatga aggcatcgccgtgttcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaaaattatcg acgagcgcctgatcacccccgactaa
379	ATG-4812	MVSVSGWRLFKKISGSSGGSSGGSSGGSSGFTLDDFVGDWEQTAAY NLDQVLEQGGVSSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEG LSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKLNYF GRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPD
380	ATG-4813	Atggtttccgtgagcggctggcggctgttcaagaagattagcggctcgagcggtggctcgagcggtggctc gagcggtggctcgagcggtggctcgagcggtttcacactcgacgatttcgttggggactgggaacagacag ccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgt aactccgatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtat gaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcat cactttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcg gacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactaccacagggaccctgtggaacgg caacaaaattatcgacgagcgcctgatcacccccgactaa
381	ATG-4813	MVSVSGWRLFKKISGSSGGSSGGSSGGSSGGSSGFTLDDFVGDWEQ TAAYNLDQVLEQGGVSSLLQNLAVSVTPIMRIVRSGENALKIDIHVII PYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKL NYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPD
382	ATG-4814	Atggtgagcggctggcggctgttcaagaagattagcggctcgagcggtggctcgagcggtggctcgagc ggtggctcgagcggtggctcgagcggtttcacactcgacgatttcgttggggactgggaacagacagccgc ctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaact ccgatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaag gtctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactt taaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcggacg gccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactaccacagggaccctgtggaacggcaac aaaattatcgacgagcgcctgatcacccccgactaa

383	ATG-4814	MVSGWRLFKKISGSSGGSSGGSSGGSSGGSSGFTLDDFVGDWEQTA AYNLDQVLEQGGVSSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPY EGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKLN YFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPD
384	ATG-4815	Atggtcttcacactcgacgatttcgttggggactgggaacagacagccgcctacaacctggaccaagtcctt gaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatcatgaggattgtccgga gcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatgg cccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggc acactggtaatcgacggggttacgccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgtg ttcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgat caccccgacgtttccgtgagcggctggcggctgttcaagaagattagctaa
385	ATG-4815	MVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIMRI VRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVIL PYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDE RLITPDVSVSGWRLFKKIS
386	ATG-4816	Atggtcttcacactcgacgatttcgttggggactgggaacagacagccgcctacaacctggaccaagtcctt gaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatcatgaggattgtccgga gcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatgg cccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggc acactggtaatcgacggggttacgccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgtg ttcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgat caccccgacggctcgagcggtgtttccgtgagcggctggcggctgttcaagaagattagctaa
387	ATG-4816	MVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIMRI VRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVIL PYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDE RLITPDGSSGVSVSGWRLFKKIS
388	ATG-4817	Atggtcttcacactcgacgatttcgttggggactgggaacagacagccgcctacaacctggaccaagtcctt gaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatcatgaggattgtccgga gcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatgg cccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggc acactggtaatcgacggggttacgccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgtg ttcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgat caccccgacggctcgagcggtggctcgagcggtgtttccgtgagcggctggcggctgttcaagaagatta gctaa

389	ATG-4817	MVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIMRI VRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVIL PYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDE RLITPDGSSGGSSGVSVSGWRLFKKIS
390	ATG-4818	Atggtcttcacactcgacgatttcgttggggactgggaacagacagccgcctacaacctggaccaagtcctt gaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatcatgaggattgtccgga gcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatgg cccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggc acactggtaatcgacggggttacgccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgtg ttcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgat caccccccgacggctcgagcggtggctcgagcggtgtgagcggctggcggctgttcaagaagattagctaa
391	ATG-4818	MVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIMRI VRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVIL PYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDE RLITPDGSSGGSSGVSVSGWRLFKKIS
392	ATG-4819	Atggtttccgtgagcggctggcggctgttcaagaagattagcttcacactcgacgatttcgttggggactggg aacagacagccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcg ccgtgtccgtaactccgatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcat catcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgt ggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacaagctg aactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactaccacagggaccctg tggaacggcaacaaaattatcgacgagcgcctgatcacccccgaccatcaccatcaccatcattaa
393	ATG-4819	MVSVSGWRLFKKISFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLL QNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKV VYPVDDHHFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKIT TTGTLWNGNKIIDERLITPDHHHHHH
394	ATG-4820	Atggtttccgtgagcggctggcggctgttcaagaagattagcggcagctccggtttcacactcgacgatttcg ttggggactgggaacagacagccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttg ctgcagaatctcgccgtgtccgtaactccgatcatgaggattgtccggagcggtgaaaatgccctgaagatc gacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaag gtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggggttac gccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactac cacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgatcacccccgaccatcaccatcacc atcattaa

395	ATG-4820	MVSVSGWRLFKKISGSSGFTLDDFVGDWEQTAAYNLDQVLEQGGV SSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEV FKVVYPVDDHHFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVFDGK KITTTGTLWNGNKIIDERLITPDHHHHHH
396	ATG-4821	Atggtttccgtgagcggctggcggctgttcaagaagattagcggctcgagcggtggctcgagcggtttcac actcgacgatttcgttggggactgggaacagacagccgcctacaacctggaccaagtccttgaacagggag gtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatcatgaggattgtccggagcggtgaaaat gccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaa gaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatc gacggggttacgccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaa aaagatcactaccacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgatcacccccgacc atcaccatcaccatcattaa
397	ATG-4821	MVSVSGWRLFKKISGSSGGSSGFTLDDFVGDWEQTAAYNLDQVLEQ GGVSSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQI EEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVF DGKKITTTGTLWNGNKIIDERLITPDHHHHHH
398	ATG-4822	Atggtttccgtgagcggctggcggctgttcaagaagattagcggctcgagcggtggctcgagcggtggctc gagcggtttcacactcgacgatttcgttggggactgggaacagacagccgcctacaacctggaccaagtcct tgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatcatgaggattgtccgg agcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatg gcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatgg cacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgt gttcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaaaattatcgacgagcgcctga tcacccccgaccatcaccatcaccatcattaa
399	ATG-4822	MVSVSGWRLFKKISGSSGGSSGGSSGFTLDDFVGDWEQTAAYNLDQ VLEQGGVSSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQ MAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKLNYFGRPYEG IAVFDGKKITTTGTLWNGNKIIDERLITPDHHHHHH
400	ATG-4823	Atggtttccgtgagcggctggcggctgttcaagaagattagcggctcgagcggtggctcgagcggtggctc gagcggtggctcgagcggtttcacactcgacgatttcgttggggactgggaacagacagccgcctacaacc tggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatcatg aggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagc gccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtga tcctgccctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggccgtatga aggcatcgccgtgttcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaaaattatcg acgagcgcctgatcacccccgaccatcaccatcaccatcattaa

401	ATG-4823	MVSVSGWRLFKKISGSSGGSSGGSSGGSSGFTLDDFVGDWEQTAAY NLDQVLEQGGVSSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEG LSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKLNYF GRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPDHHHHHH
402	ATG-4824	Atggtgagcggctggcggctgttcaagaagattagcggctcgagcggtggctcgagcggtggctcgagc ggtggctcgagcggtggctcgagcggtttcacactcgacgatttcgttggggactgggaacagacagccgc ctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaact ccgatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaag gtctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactt taaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcggacg gccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactaccacagggaccctgtggaacggcaac aaaattatcgacgagcgcctgatcacccccgaccatcaccatcaccatcattaa
403	ATG-4824	MVSGWRLFKKISGSSGGSSGGSSGGSSGGSSGFTLDDFVGDWEQTA AYNLDQVLEQGGVSSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPY EGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKLN YFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPDHHHHHH
404	ATG-4825	Atggtttccgtgagcggctggcggctgttcaagaagattagcggctcgagcggtggctcgagcggtggctc gagcggtggctcgagcggtggctcgagcggtttcacactcgacgatttcgttggggactgggaacagacag ccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgt aactccgatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtat gaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcat cactttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcg gacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactaccacagggaccctgtggaacgg caacaaaattatcgacgagcgcctgatcacccccgaccatcaccatcaccatcattaa
405	ATG-4825	MVSVSGWRLFKKISGSSGGSSGGSSGGSSGGSSGFTLDDFVGDWEQ TAAYNLDQVLEQGGVSSLLQNLAVSVTPIMRIVRSGENALKIDIHVII PYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKL NYFGRPYEGIAVFDGKKITTTGTLWNGNKIIDERLITPDHHHHHH
406	ATG-4826	Atgaaacatcaccatcaccatcatgtcttcacactcgacgatttcgttggggactgggaacagacagccgcct acaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactcc gatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggt ctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcacttta aggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggc cgtatgaaggcatcgccgtgttcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaa aattatcgacgagcgcctgatcacccccgacgtttccgtgagcggctggcggctgttcaagaagattagctaa

407	ATG-4826	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPV DDHHFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTL WNGNKIIDERLITPDVSVSGWRLFKKIS
408	ATG-4827	Atgaaacatcaccatcaccatcatgtcttcacactcgacgatttcgttggggactgggaacagacagccgcct acaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactcc gatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggt ctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcacttta aggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggc cgtatgaaggcatcgccgtgttcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaa aattatcgacgagcgcctgatcacccccgacggctcgagcggtgtttccgtgagcggctggcggctgttcaa gaagattagctaa
409	ATG-4827	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPV DDHHFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTL WNGNKIIDERLITPDGSSGVSVSGWRLFKKIS
410	ATG-4828	Atgaaacatcaccatcaccatcatgtcttcacactcgacgatttcgttggggactgggaacagacagccgcct acaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactcc gatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggt ctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcacttta aggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggc cgtatgaaggcatcgccgtgttcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaa aattatcgacgagcgcctgatcacccccgacggctcgagcggtggctcgagcggtgtgagcggctggcgg ctgttcaagaagattagctaa
411	ATG-4828	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPV DDHHFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTL WNGNKIIDERLITPDGSSGGSSGVSGWRLFKKIS
412	ATG-4829	Atgaaacatcaccatcaccatcatgtcttcacactcgacgatttcgttggggactgggaacagacagccgcct acaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactcc gatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggt ctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcacttta aggtgatcctgccctatggcacactggtaatcgacggggttacgccgaacaagctgaactatttcggacggc cgtatgaaggcatcgccgtgttcgacggcaaaaagatcactaccacagggaccctgtggaacggcaacaa aattatcgacgagcgcctgatcacccccgacggctcgagcggtggctcgagcggtgtttccgtgagcggct ggcggctgttcaagaagattagctaa

413	ATG-4829	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPV DDHHFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTL WNGNKIIDERLITPDGSSGGSSGVSVSGWRLFKKIS
414	ATG-2623	atggtcttcacactcgaagatttcgttggggactgggaacagacagccgcctacaacctggaccaagtccttg aacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccggag cggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggc ccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggca cactggtaatcgacggggttacgccgaacatgctgaactatttcggacggccgtatgaaggcatcgccgtgtt cgacggcaaaaagatcactgtaacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgatca cccccgacggctccatgctgttccgagtaaccatcaacagccatcatcaccatcaccactaa
415	ATG-2623	MVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRI VRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVIL PYGTLVIDGVTPNMLNYFGRPYEGIAVFDGKKITVTGTLWNGNKIID ERLITPDGSMLFRVTINSHHHHHH
416	ATG-3745	atggtgagcggctggcggctgttcaagaagattagccaccatcaccatcaccatcatcacttcacactcgacg atttcgttggggactgggaacagacagccgcctacaacctggaccaagtccttgaacagggaggtgtgtcca gtttgctgcagaatctcgccgtgtccgtaactccgatcatgaggattgtccggagcggtgaaaatgccctgaa gatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgttt aaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggggt tacgccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcac taccacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgatcacccccgactaa
417	ATG-3745	MVSGWRLFKKISHHHHHHHHHFTLDDFVGDWEQTAAYNLDQVLEQ GGVSSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQI EEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVF DGKKITTTGTLWNGNKIIDERLITPD
418	ATG-3746	atgaaacatcaccatcaccatcatgtgagcggctggcggctgttcaagaagattagcggcagctccggtttca cactcgacgatttcgttggggactgggaacagacagccgcctacaacctggaccaagtccttgaacaggga ggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatcatgaggattgtccggagcggtgaaa atgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcg aagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaa tcgacggggttacgccgaacaagctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacggc aaaaagatcactaccacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgatcacccccga ctaa

419	ATG-3746	MKHHHHHHVSGWRLFKKISGSSGFTLDDFVGDWEQTAAYNLDQVL EQGGVSSLLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQM AQIEEVFKVVYPVDDHHFKVILPYGTLVIDGVTPNKLNYFGRPYEGI AVFDGKKITTTGTLWNGNKIIDERLITPD
420	ATG-4632	atggtgagcggctggcggctgttcaagaagattagcggcagctccggtttcacactcgacgatttcgttggg gactgggaacagacagccgcctacaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgca gaatctcgccgtgtccgtaactccgatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatc catgtcatcatcccgtatgaaggtctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgt accctgtggatgatcatcactttaaggtgatcctgccctatggcacactggtaatcgacggggttacgccgaa caagctgaactatttcggacggccgtatgaaggcatcgccgtgttcgacggcaaaaagatcactaccacagg gaccctgtggaacggcaacaaaattatcgacgagcgcctgatcacccccgaccatcaccatcaccatcatta a
421	ATG-4632	MVSGWRLFKKISGSSGFTLDDFVGDWEQTAAYNLDQVLEQGGVSS LLQNLAVSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFK VVYPVDDHHFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKI TTTGTLWNGNKIIDERLITPDHHHHHH
422	ATG-2757	atggtcttcacactcgaagatttcgttggggactgggaacagacagccgcctacaacctggaccaagtccttg aacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccggag cggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggc ccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggca cactggtaatcgacggggttacgccgaacatgctgaactatttcggacggccgtatgaaggcatcgccgtgtt cgacggcaaaaagatcactgtaacagggaccctgtggaacgagaacaaaattatcgacgagcgcctgatca cccccgacggctccatgctgttccgagtaaccatcaacagccatcatcaccatcaccactaa
423	ATG-2757	MVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRI VRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVIL PYGTLVIDGVTPNMLNYFGRPYEGIAVFDGKKITVTGTLWNENKIID ERLITPDGSMLFRVTINSHHHHHH
424	ATG-2760	atggtcttcacactcgaagatttcgttggggactgggaacagacagccgcctacaacctggaccaagtccttg aacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggattgtccggag cggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatggc ccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggca cactggtaatcgacggggttacgccgaacatgctgaactatttcggacggccgtatgaaggcatcgccgtgtt cgacggcaaaaagatcactgtaacagggaccctgtggaacggcgttaaaattatcgacgagcgcctgatca cccccgacggctccatgctgttccgagtaaccatcaacagccatcatcaccatcaccactaa

425	ATG-2760	MVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQRI VRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVIL PYGTLVIDGVTPNMLNYFGRPYEGIAVFDGKKITVTGTLWNGVKIID ERLITPDGSMLFRVTINSHHHHHH
426	ATG-3882	atggtcttcacactcgaagatttcgttggggactgggaacagacagccgcctacaacctggaccaagtccttg aacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggatggtccgga gcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatgg cccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggc acactggtaatcgacggggttacgccgaacatgctgaactatttcggacggccgtatgaaggcatcgccgtg ttcgacggcaaaaagatcactgtaacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgat caccccgacggctccatgctgttccgagtaaccatcaacagccatcatcaccatcaccactaa
427	ATG-3882	MVFTLEDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQR MVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKV ILPYGTLVIDGVTPNMLNYFGRPYEGIAVFDGKKITVTGTLWNGNKII DERLITPDGSMLFRVTINSHHHHHH
428	ATG-3901	atggtcttcacactcgaagatttcgttggggactggaagcagacagccgcctacaacctggaccaagtccttg aacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggatggtccgga gcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatgg cccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggc acactggtaatcgacggggttacgccgaacatgctgaactatttcggacggccgtatgaaggcatcgccgtg ttcgacggcaaaaagatcactgtaacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgat caccccgacggctccatgctgttccgagtaaccatcaacagccatcatcaccatcaccactaa
429	ATG-3901	MVFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQR MVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKV ILPYGTLVIDGVTPNMLNYFGRPYEGIAVFDGKKITVTGTLWNGNKII DERLITPDGSMLFRVTINSHHHHHH
430	ATG-3945	atggtcttcacactcgaagatttcgttggggactggaagcagacagccgcctacaacctggaccaagtccttg aacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggatggtccgga gcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatgg cccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggc acactggtaatcgacggggttacgccgaacatgctgaactatttcggacggccgtatgaaggcatcgccgtg ttcgacggcaaaaagatcactgtaacagggaccctgtggaacgacgtcaaaattatcgacgagcgcctgatc acccccgacggctccatgctgttccgagtaaccatcaacagccatcatcaccatcaccactaa

431	ATG-3945	MVFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQR MVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKV ILPYGTLVIDGVTPNMLNYFGRPYEGIAVFDGKKITVTGTLWNDVKII DERLITPDGSMLFRVTINSHHHHHH
432	ATG-3984	atggtcttcacactcgaagatttcgttggggactggaagcagacagccgcctacaacctggaccaagtccttg aacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggatggtccgga gcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatgg cccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggc acactggtaatcgacggggttacgccgaacatgctgaactatttcggacggccgtatgaaggcatcgccgtg ttcgacggcaaaaagatcactgtaacagggaccctgtggaacgacgtcaaaattatcgacgagcgcctgatc acccccgacggctccatgtccttccgagtaaccatcaacagccatcatcaccatcaccactaa
433	ATG-3984	MVFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQR MVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKV ILPYGTLVIDGVTPNMLNYFGRPYEGIAVFDGKKITVTGTLWNDVKII DERLITPDGSMSFRVTINSHHHHHH
434	ATG-4147	atggtcttcacactcgaagatttcgttggggactggaagcagacagccgcctacaacctggaccaagtccttg aacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggatggtccgga gcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatgg cccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggc acactggtaatcgacggggttacgccgaacatgctgaactatttcggacggccgtatgaaggcatcgccgtg ttcgacggcaaaaagatcactgtaacagggaccctgtggaacggcaacaaaattatcgacgagcgcctgat caccccgacggctccatgtccttccgagtaaccatcaacagccatcatcaccatcaccactaa
435	ATG-4147	MVFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQR MVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKV ILPYGTLVIDGVTPNMLNYFGRPYEGIAVFDGKKITVTGTLWNGNKII DERLITPDGSMSFRVTINSHHHHHH
436	ATG-4166	atggtcttcacactcgaagatttcgttggggactggaagcagacagccgcctacaacctggaccaagtccttg aacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactccgatccaaaggatggtccgga gcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggtctgagcgccgaccaaatgg cccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcactttaaggtgatcctgccctatggc acactggtaatcgacggggttacgccgaacatgctgaactatttcggacggccgtatgaaggcatcgccgtg ttcgacggcaaaaagatcactgtaacagggaccctgtggaacggcgtcaaaattatcgacgagcgcctgatc acccccgacggctccatgtccttccgagtaaccatcaacagccatcatcaccatcaccactaa

437	ATG-4166	MVFTLEDFVGDWKQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIQR MVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKV ILPYGTLVIDGVTPNMLNYFGRPYEGIAVFDGKKITVTGTLWNGVKII DERLITPDGSMSFRVTINSHHHHHH
438	ATG-5037	ATGAAACATCACCATCACCATCATGTCTTCACACTCGACGATTTC GTTGGGGACTGGGAACAGACAGCCGCCTACAACCTGGACCAAGT CCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCGT GTCCGTAACTCCGATCATGAGGATTGTCCGGAGCGGTGAAAATGC CCTGAAGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAG CGCCGACCAAATGGCCCAGATCGAAGAGGTGTTTAAGGTGGTGT ACCCTGTGGATGATCATCACTTTAAGGTGATCCTGCCCTATGGCA CACTGGTAATCGACGGGGTTACGCCGAACAAGCTGAACTATTTCG GACACCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATC ACTACCACAGGGACCCTGTGGAACGGCAACAAAATTATCGACGA GCGCCTGATCACCCCCGACTAA
439	ATG-5037	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPV DDHHFKVILPYGTLVIDGVTPNKLNYFGHPYEGIAVFDGKKITTTGT LWNGNKIIDERLITPD
440	ATG-5038	ATGAAACATCACCATCACCATCATGTCTTCACACTCGACGATTTC GTTGGGGACTGGGAACAGACAGCCGCCTACAACCTGGACCAAGT CCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCGT GTCCGTAACTCCGATCATGAGGATTGTCCGGAGCGGTGAAAATGC CCTGAAGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAG CGCCGACCAAATGGCCCAGATCGAAGAGGTGTTTAAGGTGGTGT ACCCTGTGGATGATCATCACTTTAAGGTGATCCTGCCCTATGGCA CACTGGTAATCGACGGGGTTACGCCGAACAAGCTGAACTATTTCG GACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCGAGAAGATC ACTACCACAGGGACCCTGTGGAACGGCAACAAAATTATCGACGA GCGCCTGATCACCCCCGACTAA
441	ATG-5038	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPV DDHHFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVFDGEKITTTGTL WNGNKIIDERLITPD

442	ATG-5039	ATGAAACATCACCATCACCATCATGTCTTCACACTCGACGATTTC GTTGGGGACTGGGAACAGACAGCCGCCTACAACCTGGACCAAGT CCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCGT GTCCGTAACTCCGATCATGAGGATTGTCCGGAGCGGTGAAAATGC CCTGAAGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAG CGCCGACCAAATGGCCCAGATCGAAGAGGTGTTTAAGGTGGTGT ACCCTGTGGATGATCATCACTTTAAGGTGATCCTGCCCTATGGCA CACTGGTAATCGACGGGGTTACGCCGAACAAGCTGAACTATTTCG GACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATC ACTACCACAGGGACCCTGCCTAACGGCAACAAAATTATCGACGA GCGCCTGATCACCCCCGACTAA
443	ATG-5039	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPV DDHHFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTL PNGNKIIDERLITPD
444	ATG-5040	ATGAAACATCACCATCACCATCATGTCTTCACACTCGACGATTTC GTTGGGGACTGGGAACAGACAGCCGCCTACAACCTGGACCAAGT CCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCGT GTCCGTAACTCCGATCATGAGGATTGTCCGGAGCGGTGAAAATGC CCTGAAGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAG CGCCGACCAAATGGCCCAGATCGAAGAGGTGTTTAAGGTGGTGT ACCCTGTGGATGATCATCACTTTAAGGTGATCCTGCCCTATGGCA CACTGGTAATCGACGGGGTTACGCCGAACAAGCTGAACTATTTCG GACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATC ACTACCACAGGGACCCTGTGGAACGGCAACAAAATTATCGACGA GCGCCTGATCGATCCCGACTAA
445	ATG-5040	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPV DDHHFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTL WNGNKIIDERLIDPD
446	ATG-5041	ATGAAACATCACCATCACCATCATGTCTTCACACTCGACGATTTC GTTGGGGACTGGGAACAGACAGCCGCCTACAACCTGGACCAAGT CCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCGT GTCCGTAACTCCGATCATGAGGATTGTCCGGAGCGGTGAAAATGC CCTGAAGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAG CGCCGACCAAATGGCCCAGATCGAAGAGGTGTTTAAGGTGGTGT ACCCTGTGGATGATCATCACTTTAAGGTGATCCTGCCCTATGGCA CACTGGTAATCGACGGGGTTACGCCGAACAAGCTGAACTATTTCG GACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATC ACTACCACAGGGACCCTGTGGAACGGCAACAAAATTATCGACGA GCGCCTGATCACCGATGACTAA

447	ATG-5041	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPV DDHHFKVILPYGTLVIDGVTPNKLNYFGRPYEGIAVFDGKKITTTGTL WNGNKIIDERLITDD
448	ATG-5135	ATGAAACATCACCATCACCATCATGTCTTCACACTCGACGATTTC GTTGGGGACTGGGAACAGACAGCCGCCTACAACCTGGACCAAGT CCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCGT GTCCGTAACTCCGATCATGAGGATTGTCCGGAGCGGTGAAAATGC CCTGAAGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAG CGCCGACCAAATGGCCCAGATCGAAGAGGTGTTTAAGGTGGTGT ACCCTGTGGATGATCATCACTTTAAGGTGATCCTGCCCTATGGCA CACTGGTAATCGACGGGGTTACGCCGAACAAGCTGAACTATTTCG GACACCCGTATGAAGGCATCGCCGTGTTCGACGGCGAGAAGATC ACTACCACAGGGACCCTGTGGAACGGCAACAAAATTATCGACGA GCGCCTGATCACCCCCGACTAA
449	ATG-5135	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPV DDHHFKVILPYGTLVIDGVTPNKLNYFGHPYEGIAVFDGEKITTTGTL WNGNKIIDERLITPD
450	ATG-5146 (LgTrip 5146)	ATGAAACATCACCATCACCATCATGTCTTCACACTCGACGATTTC GTTGGGGACTGGGAACAGACAGCCGCCTACAACCTGGACCAAGT CCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCGT GTCCGTAACTCCGATCATGAGGATTGTCCGGAGCGGTGAAAATGC CCTGAAGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAG CGCCGACCAAATGGCCCAGATCGAAGAGGTGTTTAAGGTGGTGT ACCCTGTGGATGATCATCACTTTAAGGTGATCCTGCCCTATGGCA CACTGGTAATCGACGGGGTTACGCCGAACAAGCTGAACTATTTCG GACACCCGTATGAAGGCATCGCCGTGTTCGACGGCGAGAAGATC ACTACCACAGGGACCCTGTGGAACGGCAACAAAATTATCGACGA GCGCCTGATCGATCCCGACTAA
451	ATG-5146 (LgTrip 5146)	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPV DDHHFKVILPYGTLVIDGVTPNKLNYFGHPYEGIAVFDGEKITTTGTL WNGNKIIDERLIDPD

452	ATG-5158	ATGAAACATCACCATCACCATCATGTCTTCACACTCGACGATTTC GTTGGGGACTGGGAACAGACAGCCGCCTACAACCTGGACCAAGT CCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCGT GTCCGTAACTCCGATCATGAGGATTGTCCGGAGCGGTGAAAATGC CCTGAAGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAG CGCCGACCAAATGGCCCAGATCGAAGAGGTGTTTAAGGTGGTGT ACCCTGTGGATGATCATCACTTTAAGGTGATCCTGCCCTATGGCA CACTGGTAATCGACGGGGTTACGCCGAACAAGCTGAACTATTTCG GACACCCGTATGAAGGCATCGCCGTGTTCGACGGCGAGAAGATC ACTACCACAGGGACCCTGTGGAACGGCAACAAAATTATCGACGA GCGCCTGATCGATGATGACTAA
453	ATG-5158	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPV DDHHFKVILPYGTLVIDGVTPNKLNYFGHPYEGIAVFDGEKITTTGTL WNGNKIIDERLIDDD
454	ATG-5260	ATGAAACATCACCATCACCATCATGATTTCACACTCGACGATTTC GTTGGGGACTGGGAACAGACAGCCGCCTACAACCTGGACCAAGT CCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCGT GTCCGTAACTCCGATCATGAGGATTGTCCGGAGCGGTGAAAATGC CCTGAAGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAG CGCCGACCAAATGGCCCAGATCGAAGAGGTGTTTAAGGTGGTGT ACCCTGTGGATGATCATCACTTTAAGGTGATCCTGCCCATCGGCA CACTGGTAATCGACGGGGTTACGCCGAACAAGCTGAACTATTTCG GACACCCGTATGAAGGCATCGCCGTGTTCGACGGCGAGAAGATC ACTACCACAGGGACCCTGTGGAACGGCAACAAAATTATCGACGA GCGCCTGATCGATCCCGACTAA
455	ATG-5260	MKHHHHHHDFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPV DDHHFKVILPIGTLVIDGVTPNKLNYFGHPYEGIAVFDGEKITTTGTL WNGNKIIDERLIDPD
456	ATG-5266	ATGAAACATCACCATCACCATCATGTCTTCACACTCGACGATTTC GTTGGGGACTGGGAACAGACAGCCGCCTACAACCTGGACCAAGT CCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCGT GTCCGTAACTCCGATCATGAGGATTGTCCGGAGCGGTGAAAATGC CCTGAAGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAG CGCCGACCAAATGGCCCAGATCGAAGAGGTGTTTAAGGTGGTGT ACCCTGTGGATGATCATCACTTTAAGGTGATCCTGCCCATCGGCA CACTGGTAATCGACGGGGAGACGCCGAACAAGCTGAACTATTTC GGACACCCGTATGAAGGCATCGCCGTGTTCGACGGCGAGAAGAT CACTACCACAGGGACCCTGTGGAACGGCAACAAAATTATCGACG AGCGCCTGATCGATCCCGACTAA

457	ATG-5266	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPV DDHHFKVILPIGTLVIDGETPNKLNYFGHPYEGIAVFDGEKITTTGTL WNGNKIIDERLIDPD
458	ATG-5267	ATGAAACATCACCATCACCATCATGTCTTCACACTCGACGATTTC GTTGGGGACTGGGAACAGACAGCCGCCTACAACCTGGACCAAGT CCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCGT GTCCGTAACTCCGATCATGAGGATTGTCCGGAGCGGTGAAAATGC CCTGAAGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAG CGCCGACCAAATGGCCCAGATCGAAGAGGTGTTTAAGGTGGTGT ACCCTGTGGATGATCATCACTTTAAGGTGATCCTGCCCATCGGCA CACTGGTAATCGACGGGGTTACGCCGAACAAGCTGAACTATTTCG GACACCCGTATGAAGGCATCGCCGATTTCGACGGCGAGAAGATC ACTACCACAGGGACCCTGTGGAACGGCAACAAAATTATCGACGA GCGCCTGATCGATCCCGACTAA
459	ATG-5267	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPV DDHHFKVILPIGTLVIDGVTPNKLNYFGHPYEGIADFDGEKITTTGTL WNGNKIIDERLIDPD
460	ATG-5278	ATGAAACATCACCATCACCATCATGTCTTCACACTCGACGATTTC GTTGGGGACTGGGAACAGACAGCCGCCTACAACCTGGACCAAGT CCTTGAACAGGGAGGTGTGTCCAGTTTGCTGCAGAATCTCGCCGT GTCCGTAACTCCGATCATGAGGATTGTCCGGAGCGGTGAAAATGC CCTGAAGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAG CGCCGACCAAATGGCCCAGATCGAAGAGGTGTTTAAGGTGGTGT ACCCTGTGGATGATCATCACTTTAAGGTGATCCTGCCCATCGGCA CACTGGTAATCGACGGGGAGACGCCGAACAAGCTGAACTATTTC GGACACCCGTATGAAGGCATCGCCGATTTCGACGGCGAGAAGAT CACTACCACAGGGACCCTGTGGAACGGCAACAAAATTATCGACG AGCGCCTGATCGATCCCGACTAA
461	ATG-5278	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPV DDHHFKVILPIGTLVIDGETPNKLNYFGHPYEGIADFDGEKITTTGTL WNGNKIIDERLIDPD

462	ATG-4794	atgaaacatcaccatcaccatcatgtcttcacactcgacgatttcgttggggactgggaacagacagccgcct acaacctggaccaagtccttgaacagggaggtgtgtccagtttgctgcagaatctcgccgtgtccgtaactcc gatcatgaggattgtccggagcggtgaaaatgccctgaagatcgacatccatgtcatcatcccgtatgaaggt ctgagcgccgaccaaatggcccagatcgaagaggtgtttaaggtggtgtaccctgtggatgatcatcacttta aggtgatcctgccctatggcacactggtaatcgac
463	ATG-4794	MKHHHHHHVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLA VSVTPIMRIVRSGENALKIDIHVIIPYEGLSADQMAQIEEVFKVVYPV DDHHFKVILPYGTLVID
720	HALOTAG	MAEIGTGFPFDPHYVEVLGERMHYVDVGPRDGTPVLFLHGNPTSSY VWRNIIPHVAPTHRCIAPDLIGMGKSDKPDLGYFFDDHVRFMDAFIE ALGLEEVVLVIHDWGSALGFHWAKRNPERVKGIAFMEFIRPIPTWDE WPEFARETFQAFRTTDVGRKLIIDQNVFIEGTLPMGVVRPLTEVEMD HYREPFLNPVDREPLWRFPNELPIAGEPANIVALVEEYMDWLHQSPV PKLLFWGTPGVLIPPAEAARLAKSLPNCKAVDIGPGLNLLQEDNPDLI GSEIARWLSTLEISG
721	ATG3998 [6xHis-TNFa(sol)-VS-HiBiT]	atgaaacatcaccatcaccatcatgtcagatcatcttctcgaaccccgagtgacaagcctgtagcccatgttgt agcaaaccctcaagctgaggggcagctccagtggctgaaccgccgggccaatgccctcctggccaatggc gtggagctgagagataaccagctggtggtgccatcagagggcctgtacctcatctactcccaggtcctcttca agggccaaggctgcccctccacccatgtgctcctcacccacaccatcagccgcatcgccgtctcctaccaga ccaaggtcaacctcctctctgccatcaagagcccctgccagagggagaccccagaggggctgaggccaa gccctggtatgagcccatctatctgggaggggtcttccagctggagaagggtgaccgactcagcgctgaga tcaatcggcccgactatctcgactttgccgagtctgggcaggtctactttgggatcattgccctgtcgagttcag gtggtggcgggagcggtggagggagcagcggtggagtttccgtgagcggctggcggctgttcaagaaga ttagctaa
722	ATG3998 [6xHis-TNFa(sol)-VS-HiBiT]	MKHHHHHHVRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALL ANGVELRDNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAV SYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGDRL SAEINRPDYLDFAESGQVYFGIIALSSSGGGGSGGGSSGGVSVSGWR LFKKIS.
723	ATG4002 [smTrip9(521)-15GS-protein G-6xHis]	ATGGgcaagatgctgttccgagtaaccatcaacagctggaaggggagctccGGTGGTGGCGG GAGCGGAGGTGGAGGctcgAGCGGTATGACGTATAAGTTAATCCTT AATGGTAAAACATTGAAAGGCGAGACAACTACTGAAGCTGTTGA TGCTGCTACTGCAGAAAAAGTCTTCAAACAATACGCTAACGACA ACGGTGTTGACGGTGAATGGACTTACGACGATGCGACGAAAACC TTTACGGTCACCGAAAAACCAGAAGTGATCGATGCGTCTGAATTA ACACCAGCCGTGACAACTTACAAACTTGTTATTAATGGTAAAACA TTGAAAGGCGAAACAACTACTGAGGCTGTTGATGCTGCTACTGCA GAGAAGGTGTTCAAACAATATGCGAATGACAACGGTGTTGACGG TGAGTGGACTTACGACGATGCGACTAAGACCTTTACAGTTACTGA AAAACCAGAAGTGATCGATGCGTCTGAGTTAACACCAGCCGTGA CAACTTACAAACTTGTTATTAATGGTAAAACATTGAAAGGCGAAA CAACTACTAAAGCAGTAGACGCAGAAACTGCGGAGAAGGCCTTC AAACAATACGCTAACGACAACGGTGTTGATGGTGTTTGGACTTAT

		GATGATGCCACAAAAACCTTTACGGTAACTGAGCATCATCACCATCACCACTAA
724	ATG4002 [smTrip9(521)-15GS-protein G-6xHis]	MGKMLFRVTINSWKGSSGGGGSGGGGSSGMTYKLILNGKTLKGETTTEAVDAATAEKVFKQYANDNGVDGEWTYDDATKTFTVTEKPEVIDASELTPAVTTYKLVINGKTLKGETTTEAVDAATAEKVFKQYANDNGVDGEWTYDDATKTFTVTEKPEVIDASELTPAVTTYKLVINGKTLKGETTTKAVDAETAEKAFKQYANDNGVDGVWTYDDATKTFTVTEHHHHHH.
725	ATG4496 SmTrip9(743)-15GS-G	atggacaagatgctgttccgagtaaccatcaacaagtggaaggggagctccggtggtggcgggagcggaggtggaggctcgagcggtatgacgtataagttaatccttaatggtaaaacattgaaaggcgagacaactactgaagctgttgatgctgctactgcagaaaaagtcttcaaacaatacgctaacgacaacggtgttgacggtgaatggacttacgacgatgcgacgaaaaccttacggtcaccgaaaaaccagaagtgatcgatgcgtctgaattaacaccagccgtgacaacttacaaacttgttattaatggtaaaacattgaaaggcgaaacaactactgaggctgttgatgctgctactgcagagaaggtgttcaaacaatatgcgaatgacaacggtgttgacggtgagtggacttacgacgatgcgactaagacctttacagttactgaaaaaccagaagtgatcgatgcgtctgagttaacaccagccgtgacaacttacaaacttgttattaatggtaaaacattgaaaggcgaaacaactactaaagcagtagacgcagaaactgcggagaaggccttcaaacaatacgctaacgacaacggtgttgatggtgtttggacttatgatgatgccacaaaaacctttacggtaactgagcatcatcaccatcaccac
726	ATG4496 SmTrip9(743)-15GS-G	MDKMLFRVTINKWKGSSGGGGSGGGGSSGMTYKLILNGKTLKGETTTEAVDAATAEKVFKQYANDNGVDGEWTYDDATKTFTVTEKPEVIDASELTPAVTTYKLVINGKTLKGETTTEAVDAATAEKVFKQYANDNGVDGEWTYDDATKTFTVTEKPEVIDASELTPAVTTYKLVINGKTLKGETTTKAVDAETAEKAFKQYANDNGVDGVWTYDDATKTFTVTEHHHHHH
727	ATG4558 SmTrip9(759)-15GS-G	atggacaagctcctgttcacggtaaccatcgagaagtataaggggagctccggtggtggcgggagcggaggtggaggctcgagcggtatgacgtataagttaatccttaatggtaaaacattgaaaggcgagacaactactgaagctgttgatgctgctactgcagaaaaagtcttcaaacaatacgctaacgacaacggtgttgacggtgaatggacttacgacgatgcgacgaaaaccttacggtcaccgaaaaaccagaagtgatcgatgcgtctgaattaacaccagccgtgacaacttacaaacttgttattaatggtaaaacattgaaaggcgaaacaactactgaggctgttgatgctgctactgcagagaaggtgttcaaacaatatgcgaatgacaacggtgttgacggtgagtggacttacgacgatgcgactaagacctttacagttactgaaaaaccagaagtgatcgatgcgtctgagttaacaccagccgtgacaacttacaaacttgttattaatggtaaaacattgaaaggcgaaacaactactaaagcagtagacgcagaaactgcggagaaggccttcaaacaatacgctaacgacaacggtgttgatggtgtttggacttatgatgatgccacaaaaacctttacggtaactgagcatcatcaccatcaccac
728	ATG4558 SmTrip9(759)-15GS-G	MDKLLFTVTIEKYKGSSGGGGSGGGGSSGMTYKLILNGKTLKGETTTEAVDAATAEKVFKQYANDNGVDGEWTYDDATKTFTVTEKPEVIDASELTPAVTTYKLVINGKTLKGETTTEAVDAATAEKVFKQYANDNGVDGEWTYDDATKTFTVTEKPEVIDASELTPAVTTYKLVINGKTLKGETTTKAVDAETAEKAFKQYANDNGVDGVWTYDDATKTFTVTEHHHHHH

729	ATG4551 SmTrip9(760)-15GS-G	atgaagaagatgctgttccgagtaaccatccagaagtggaaggggagctccggtggtggcgggagcgga ggtggaggctcgagcggtatgacgtataagttaatccttaatggtaaaacattgaaaggcgagacaactactg aagctgttgatgctgctactgcagaaaaagtcttcaaacaatacgctaacgacaacggtgttgacggtgaatg gacttacgacgatgcgacgaaaaccttacggtcaccgaaaaaccagaagtgatcgatgcgtctgaattaac accagccgtgacaacttacaaacttgttattaatggtaaaacattgaaaggcgaaacaactactgaggctgttg atgctgctactgcagagaaggtgttcaaacaatatgcgaatgacaacggtgttgacggtgagtggacttacga cgatgcgactaagacctttacagttactgaaaaaccagaagtgatcgatgcgtctgagttaacaccagccgtg acaacttacaaacttgttattaatggtaaaacattgaaaggcgaaacaactactaaagcagtagacgcagaaa ctgcggagaaggccttcaaacaatacgctaacgacaacggtgttgatggtgtttggacttatgatgatgccac aaaaaccttacggtaactgagcatcatcaccatcaccac
730	ATG4551 SmTrip9(760)-15GS-G	MKKMLFRVTIQKWKGSSGGGGSGGGGSSGMTYKLILNGKTLKGET TTEAVDAATAEKVFKQYANDNGVDGEWTYDDATKTFTVTEKPEVI DASELTPAVTTYKLVINGKTLKGETTTEAVDAATAEKVFKQYANDN GVDGEWTYDDATKTFTVTEKPEVIDASELTPAVTTYKLVINGKTLK GETTTKAVDAETAEKAFKQYANDNGVDGVWTYDDATKTFTVTEH HHHHH

[0591] Table 3. Exemplary peptide sequences.

Pep ID	SEQ ID NO.	Sequence
521 (SmTrip9 Pep521)	16	GKMLFRVTINSWK
289 (SmTrip10 Pep289; VSHiBiT)	17	VSVSGWRLFKKIS
691 (SmTrip10 Pep691; HW-0977)	18	VSGWRLFRRIS
692 (SmTrip10 Pep692; HW-1053)	19	VSVSGWRLFRRIS
693 (SmTrip9 Pep693; HW-0984 (SulfoSE-PEG3); HW-1042 (SulfoSE-PEG6))	20	GRMLFRVTINSWR
743 (SmTrip9 Pep743)	21	GKMLFRVTINKWK
759 (SmTrip9 Pep759)	22	DKLLFTVTIEKYK
760 (SmTrip9 Pep760)	23	KKMLFRVTIQKWK
895 (SmTrip9 Pep895; HW-1010 (SulfoSE-PEG3); HW-1043 (SulfoSE-PEG6))	24	GRLLFVVVIERYR
929 (SmTrip9 Pep929; HW-1055 (SulfoSE-PEG3); HW-1052 (SulfoSE-PEG6))	25	RRMLFRVTIQRWR

937 (SmTrip9 Pep937; HW-0987)	26	VSGWRLFRRISC
938 (SmTrip9 Pep938; HW-0992 (TAMRA); HW-1050 (SA))	27	GRMLFRVTINSWRC
86	464	VSGWRLFKKIS
229	465	VSGWRLFKKI
543	466	WNGNKIIDERLITPD
544	467	KKITTTGTLWNGR
545	468	RPYEGIAVFDGK
591	469	GKMLFRVTIWKVSVSGWRLFKKIS
592	470	GKMLFRVTIWKVSGWRLFKKIS
593	471	GSMKFRVTINSWKVSVSGWRLFKKIS
594	472	GSMKFRVTINSWKVSGWRLFKKIS
595	473	GSMKFRVTINSWKNVTGYRLFKKISN
596	474	GSMKFRVTINSWKVTGYRLFEKIS
597	475	GSMKFRVTIWKVSVSGWRLFKKIS
598	476	GSMKFRVTIWKVSGWRLFKKIS
599	477	GRMLFRVTINSWKVSVSGWRLFKKIS
600	478	GRMLFRVTINSWKVSGWRLFKKIS
601	479	GRMLFRVTIWKVSVSGWRLFKKIS
602	480	GRMLFRVTIWKVSGWRLFKKIS
603	481	GSMLFRVTINSVSVSGWRLFKKIS
604	482	GSMLFKVTINSVSGWRLFKKIS
605	483	GSMLFQVTINSVSGWRLFKKIS
606	484	GSMLFEVTINSVSGWRLFKKIS
607	485	GSMLFNVTINSVSGWRLFKKIS
608	486	GRPYEGIAVFDGKKITTTGTL
609	487	GSMKFRVTINSWKVTGYRLFEKES
610	488	GSMKFRVTINSWKVEGYRLFEKIS
611	489	KKITTTGTLWNGNKIIDERLITPD
612	490	WNGNKIIDERLITPDGSMLFRVTINS
671	491	GKMLFRVTIQKWK
668	492	GKMLFRVTIGKWK
727	493	GKMLFRVTIGRWK

669	494	GKMLFRVTIGNWK
674	495	GKMLFRVTIQNWK
702	496	GKMLFRVTIDKWK
703	497	GKMLFRVTIEKWK
705	498	EKMLFRVTIESWK
724	499	EKLLFRVTIESWK
725	500	EKLLFRVTIESYK
730	501	GKMLFRVTIERWK
731	502	GKMLFRVTIDRWK
738	503	DKMLFRVTIQKWK
739	504	DKMLFRVTIGKWK
848	505	DKMLFRVTIGRWK
740	506	DKMLFRVTIGNWK
741	507	DKMLFRVTIQNWK
732	508	DKMLFRVTIDKWK
742	509	DKMLFRVTIEKWK
735	510	DKMLFRVTIERWK
733	511	DKMLFRVTIDRWK
798	512	RPYEGIAVFDGKKITVTGTLWNGNKIIDER LITPD
849	513	EKMLFRVTIQKWK
708	514	EKMLFRVTIGKWK
709	515	EKMLFRVTIGRWK
775	516	DKMLFTVTIQKVSGWRLFKKIS
788	517	DKLLFTVTIEKVSGWRLFKKIS
789	518	DKLLFTVTIEKWKVSGWRLFKKIS
790	519	DKLLFTVTIEKYKVSGWRLFKKIS
792	520	DKLLFTVTIEKYKVSVSGWRLFKKIS
795	521	KKMLFRVTIQKVSGWRLFKKIS
797	522	KKMLFRVTIQKWKVSVSGWRLFKKIS
796	523	KKMLFRVTIQKWKVSGWRLFKKIS
804	524	DKLLFTVTIGKVSGWRLFKKIS
805	525	DKLLFTVTIGKYKVSGWRLFKKIS
806	526	DKLLFTVTIGKYKVSVSGWRLFKKIS
807	527	DKLLFTVTIGKWKVSVSGWRLFKKIS

808	528	DKLLFTVTIQKVSGWRLFKKIS
813	529	KKMLFTVTIQKVSGWRLFKKIS
816	530	KKLLFRVTIQKVSGWRLFKKIS
825	531	DKLLFTVTIEKVSGWRLFKKI
826	532	DKLLFTVTIEKYKVSVSGWRLFKKI
827	533	DRLLFTVTIERVSGWRLFKKIS
831	534	EKLLFTVTIEKVSGWRLFKKIS
832	535	KKLLFTVTIGKVSGWRLFKKIS
833	536	GSMRFRVTINSWRVTGYRLFERES
834	537	GSMKFRVTINSVTGYRLFEKES
844	538	KKITTTGTLWNGNKIID
845	539	ERLITPDGSMLFRVTINSVSGWRLFKKIS
846	540	GRPYEGIAVDFGKKITTTGTLWNGNKIIDE RLITPDGSMLFRVTINSVSGWRLFKKIS
847	541	GVTPNKLNYFGRPYEGIAVDFGKKITTTGT LWNGNKIIDERLITPDGSMLFRVTINSVSG WRLFKKIS
850	542	EKMLFRVTIGNWK
851	543	EKMLFRVTIQNWK
706	544	EKMLFRVTIDKWK
707	545	EKMLFRVTIEKWK
737	546	EKMLFRVTIERWK
736	547	EKMLFRVTIDRWK
852	548	KKMLFRVTIGKWK
853	549	KKMLFRVTIGRWK
854	550	KKMLFRVTIGNWK
855	551	KKMLFRVTIQNWK
856	552	KKMLFRVTIDKWK
857	553	KKMLFRVTIEKWK
858	554	KKMLFRVTIERWK
859	555	KKMLFRVTIDRWK
860	556	RKMLFRVTIQKWK
861	557	RKMLFRVTIGKWK
862	558	RKMLFRVTIGRWK
863	559	RKMLFRVTIGNWK
864	560	RKMLFRVTIQNWK

865	561	RKMLFRVTIDKWK
866	562	RKMLFRVTIEKWK
867	563	RKMLFRVTIERWK
868	564	RKMLFRVTIDRWK
656	565	EQMLFRVTINSWK
869	566	SRMLFRVTINSWK
533	567	GEMLFRVTINSWK
690	568	GKMKFRVTINSWK
678	569	GKMLFRVKINSWK
679	570	GKMLFRVRINSWK
681	571	GKMLFRVDINSWK
663	572	GKMLFRVTIDSWK
714	573	EKMLFKVTIQKWK
870	574	EKMLFTVTIQKWK
871	575	EKMLFKVTIDKWK
872	576	EKMLFTVTIDKWK
873	577	EKMLFKVTIGRWK
744	578	DKMLFKVTIQKWK
745	579	DKMLFTVTIQKWK
874	580	DKMLFKVTIDKWK
875	581	DKMLFTVTIDKWK
876	582	GKMLFKVTIEKWK
877	583	GKMLFTVTIEKWK
748	584	DKMLFKVTIGKWK
749	585	DKMLFTVTIGKWK
878	586	DKMLFKVTIGNWK
879	587	DKMLFKVTIQNWK
781	588	GKMLFKVTINKWK
782	589	GKMLFTVTINKWK
752	590	DKMLFKVTIEKWK
753	591	DKMLFTVTIEKWK
750	592	DKLLFKVTIGKWK
786	593	DKMLFTVTINKWK
756	594	DKLLFTVTIQKWK
757	595	DKLLFTVTIQKYK

758	596	DKLLFTVTIEKWK
793	597	DKLLFTVTIGKWK
794	598	DKLLFTVTIGKYK
799	599	DKLLFTVTINKWK
800	600	DKLLFTVTINKYK
780	601	GKMLFRVTINS
765	602	DKMLFTVTIQK
779	603	DKMLFKVTIQK
820	604	DKLLFTVTIGK
819	605	DKMLFTVTIGK
822	606	DKMLFTVTIEK
821	607	DKLLFTVTIEK
627	608	*DKMLFRVTINSWK
628	609	*EKMLFRVTINSWK
629	610	*RKMLFRVTINSWK
630	611	*KKMLFRVTINSWK
631	612	*HKMLFRVTINSWK
632	613	*GLMLFRVTINSWK
633	614	*GQMLFRVTINSWK
634	615	*GTMLFRVTINSWK
635	616	*GKLLFRVTINSWK
636	617	*GKMLFKVTINSWK
637	618	*GKMLFRVTIQSWK
638	619	*GKMLFRVTIDSWK
639	620	*GKMLFRVTIGSWK
640	621	*GKMLFRVTINTWK
641	622	*GKMLFRVTINNWK
642	623	*GKMLFRVTINQWK
643	624	*GKMLFRVTINPWK
644	625	*GKMLFRVTINKWK
645	626	*GKMLFRVTINSWQ
646	627	*GKMLFRVTINSWN
647	628	*GKMLFRVTINSWT
648	629	*GKMLFRVTINSWH
649	630	*GKMLFRVTINSWP

650	631	*GKMLFRVTINSWR
677	632	GKMKFRVTIDSWK
680	633	GKMLFRVEINSWK
682	634	GKMLFRVQINSWK
683	635	GKMKFRVKINSWK
684	636	GKMKFRVRINSWK
685	637	GKMKFRVEINSWK
686	638	GKMKFRVDINSWK
687	639	GKMKFRVQINSWK
688	640	GKMKFRVNINSWK
689	641	GKMKFRVSINSWK
613	642	GKMLFRVNINSWK
614	643	GKMLFRVSINSWK
615	644	GKMLFRVWINSWK
616	645	GKMSFRVTINSWK
617	646	GKMWFRVTINSWK
618	647	GKMNFRVTINSWK
619	648	GSMLFRVTINSYK
620	649	GKMLFRVTINSYK
621	650	GKMLFRVTIKSWK
622	651	GKMLFRVTIESWK
716	652	GKMKFRVTIQSWK
717	653	GKMKFRVTIESWK
718	654	GKMKFRVTIKSWK
719	655	GKMKFRVTIRSWK
651	656	RLMLFRVTINSWK
652	657	RQMLFRVTINSWK
653	658	KLMLFRVTINSWK
654	659	KQMLFRVTINSWK
655	660	ELMLFRVTINSWK
657	661	DLMLFRVTINSWK
658	662	DQMLFRVTINSWK
659	663	DKMLFRVTINSWK
660	664	EKMLFRVTINSWK
661	665	RKMLFRVTINSWK

662	666	KKMLFRVTINSWK
665	667	GKMLFRVTIGSWK
667	668	GKMLFRVTINKWK
670	669	GKMLFRVTISKWK
671	670	GKMLFRVTIQKWK
672	671	GKMLFRVTITKWK
673	672	GKMLFRVTIKKWK
675	673	GKMLFKVTINSWK
676	674	RLMLFRVTIGKWK
701	675	GKMLFRVTINRWK
710	676	EKMLFTVTIGKWK
711	677	EKLLFTVTIGKWK
712	678	EKMLFTVTIGRWK
720	679	EKMLFTVTIEKWK
722	680	DKMLFRVTIESWK
726	681	EKLLFRVTIGKYK
746	682	DKLLFKVTIQKWK
747	683	DKLLFKVTIQKYK
751	684	DKLLFKVTIGKYK
754	685	DKLLFKVTIEKWK
755	686	DKLLFKVTIEKYK
761	687	KKLLFRVTIQKWK
762	688	DRMLFRVTIQRWR
766	689	ERMLFRVTIGRWR
768	690	GRMLFRVTINRWR
770	691	DRMLFRVTIERWR
783	692	DKMLFKVTIQKYK
784	693	DKMLFRVTINKWK
785	694	DKMLFKVTIEKYK
787	695	DKMLFKVTINKWK
693	696	GRMLFRVTINSWR
895	697	GRLLFVVVIERYR
937	698	VSGWRLFRRISC
938	699	GRMLFRVTINSWRC
939	700	GRLLFTVTIERYRC

840	701	GKLLFVVVIEKYK
900	702	GKLLFVTIEKVSGWRLFKKIS
*Terminus unblocked		

[0592] Table 4. Exemplary luciferase base sequences.

Pep ID	SEQ ID NO.	Sequence
LgTrip 3546 – WT strand 9 – HiBiT	703	MVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIMRIVRSGENA LKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVID**GVTPNK LNYFGRPYEGIAVFDG**KKITTTGTL**WNGNKIIDERLITPD**GSMLFRVTINS**VSG WRLFKKIS**
LgTrip 3546 – WT strand 9 – SmBiT	704	MVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIMRIVRSGENA LKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVID**GVTPNK LNYFGRPYEGIAVFDG**KKITTTGTL**WNGNKIIDERLITPD**GSMLFRVTINS**VTG YRLFEEIL**
LgTrip 3546 (1-5)	705	MVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIMRIVRSGENA LKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVID
LgTrip 3546 (1-6)	706	MVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIMRIVRSGENA LKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVID**GVTPNK LNYFGRPYEGIAVFDG**
LgTrip 3546 (1-7)	707	MVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIMRIVRSGENA LKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVID**GVTPNK LNYFGRPYEGIAVFDG**KKITTTGTL
LgTrip 3546 (1-8)	708	MVFTLDDFVGDWEQTAAYNLDQVLEQGGVSSLLQNLAVSVTPIMRIVRSGENA LKIDIHVIIPYEGLSADQMAQIEEVFKVVYPVDDHHFKVILPYGTLVID**GVTPNK LNYFGRPYEGIAVFDG**KKITTTGTL**WNGNKIIDERLITPD**
LgTrip 3546 (strands 6-8) – WT strand 9 – HiBiT	709	**GVTPNKLNYFGRPYEGIAVFDG**KKITTTGTL**WNGNKIIDERLITPD**GSMLFRV TINS**VSGWRLFKKIS**
LgTrip 3546 (strands 7-8) – WT strand 9 – HiBiT	710	KKITTTGTL**WNGNKIIDERLITPD**GSMLFRVTINS**VSGWRLFKKIS**
LgTrip 3546 (strand 8) –	711	**WNGNKIIDERLITPD**GSMLFRVTINS**VSGWRLFKKIS**

WT strand 9 – HiBiT		
WT strand 9 – HiBiT	712	GSMLFRVTINS**VSGWRLFKKIS**
LgTrip 3546 (strands 6-8) – WT strand 9 – SmBiT	713	**GVTPNKLNYFGRPYEGIAVFDG**KKITTTGTL**WNGNKIIDERLITPD**GSMLFRVTINS**VTGYRLFEEIL**
LgTrip 3546 (strands 7-8) – WT strand 9 – SmBiT	714	KKITTTGTL**WNGNKIIDERLITPD**GSMLFRVTINS**VTGYRLFEEIL**
LgTrip 3546 (strand 8) – WT strand 9 – SmBiT	715	**WNGNKIIDERLITPD**GSMLFRVTINS**VTGYRLFEEIL**
WT strand 9 – SmBiT	716	GSMLFRVTINS**VTGYRLFEEIL**
β6-like	717	**GVTPNKLNYFGRPYEGIAVFDG**
β7-like	718	KKITTTGTL
β8-like	719	**WNGNKIIDERLITPD**
ATG3998 [6xHis-TNFa(sol)-VS-HiBiT]	721	atgaaacatcaccatcaccatcatgtcagatcatcttctcgaaccccgagtgacaagcctgtagcccatgttgtagcaaaccctcaagctgaggggcagctccagtggctgaaccgccgggccaatgccctcctggccaatggcgtggagctgagagataaccagctggtggtgccatcagagggcctgtacctcatctactcccaggtcctcttcaagggccaaggctgcccctccacccatgtgctcctcacccacaccatcagccgcatcgccgtctcctaccagaccaaggtcaacctcctctctgccatcaagagcccctgccagagggagacccagaggggctgaggccaagccctggtatgagcccatctatctgggaggggtcttccagctggagaaggtgaccgactcagcgctgagatcaatcggcccgactatctcgactttgccgagtctgggcaggtctactttgggatcattgccctgtcgagttcaggtggtggcgggagcggtggagggagcagcggtggagtttccgtgagcggctggcggctgttcaagaagattagctaa
ATG3998 [6xHis-TNFa(sol)-VS-HiBiT]	722	MKHHHHHHVRSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRETPEGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLDFAESGQVYFGIIALSSSGGGGSGGGSSGGVSVSGWRLFKKIS.
ATG4002 [smTrip9(521)-15GS-protein G-6xHis]	723	ATGGgcaagatgctgttccgagtaaccatcaacagctggaaggggagctccGGTGGTGGCGGGAGCGGAGGTGGAGGctcgAGCGGTATGACGTATAAGTTAATCCTTAATGGTAAAACATTGAAAGGCGAGACAACTACTGAAGCTGTTGATGCTGCTACTGCAGAAAAAGTCTTCAAACAATACGCTAACGACAACGGTGTTGACGGTGAATGGACTTACGACGATGCGACGAAAACCTTTACGGTCACCGAAAAACCAGAAGTGATCGATGCGTCTGAATTAACACCAGCCGTGACAACTTACAAACTTGTTATTAATGGTAAAACATTGAAAGGCGAAACAACTACTGAGGCTGTTGATGCTGCTACTGCAGAGAAGGTGTTCAAACAATATGCGAATGACAACGGTGTTGACGGTGAGTGGACTTACGACGATGCGACTAAGACCTTTACAGTTACTGAAAAACCAGAAGTGATCGATGCGTCTGAGTTAACACCAGCCGTGACAACTTACAAACTTGTTATTAATGGTAAAACATTGAAAGGCGAAACAACTACTAAAGCAGTAGACGCAGAAACTGCGGAGAAGGCCTTCAAACAATACGCTAACGACAACGGTGTTGATGGTGT

		TTGGACTTATGATGATGCCACAAAAACCTTTACGGTAACTGAGCATCATCAC CATCACCACTAA
ATG4002 [smTrip9(52 1)-15GS-protein G-6xHis]	724	MGKMLFRVTINSWKGSSGGGGSGGGGSSGMTYKLILNGKTLKGETTTEAVDA ATAEKVFKQYANDNGVDGEWTYDDATKTFTVTEKPEVIDASELTPAVTTYKL VINGKTLKGETTTEAVDAATAEKVFKQYANDNGVDGEWTYDDATKTFTVTEK PEVIDASELTPAVTTYKLVINGKTLKGETTTKAVDAETAEKAFKQYANDNGVD GVWTYDDATKTFTVTEHHHHHH.

CLAIMS

What is claimed is:

1. A composition comprising:

a luminogenic substrate; and

a target analyte binding agent comprising a target analyte binding element and one of a polypeptide component of a bioluminescent complex, or a peptide component of a bioluminescent complex.

2. The composition of claim 1, wherein the polypeptide component of the target analyte binding agent comprises:

at least 60% sequence identity with SEQ ID NO: 5;

at least 60% sequence identity with SEQ ID NO: 9; or

at least 60% sequence identity with SEQ ID NO: 12.

3. The composition of claim 1, wherein the peptide component of the target analyte binding agent comprises:

at least 60% sequence identity with SEQ ID NO: 10;

at least 60% sequence identity with SEQ ID NO: 11;

at least 60% sequence identity with SEQ ID NO: 13; or

at least 60% sequence identity with SEQ ID NO: 14.

4. The composition of any of claims 1 to 3, further comprising a complementary peptide or polypeptide component of the bioluminescent complex;

wherein the target analyte binding agent and the complementary peptide or polypeptide component of the bioluminescent complex form a bioluminescent analyte detection complex in the presence of a target analyte.

5. The composition of claim 4, wherein the composition comprising the luminogenic substrate and the target analyte binding agent are combined in a dried formulation and the

complementary peptide or polypeptide component of the bioluminescent complex comprises a liquid formulation;

wherein the liquid formulation is added to the dried formulation and forms the bioluminescent analyte detection complex in the presence of the target analyte upon rehydration.

6. The composition of claim 4, wherein the composition comprising the luminogenic substrate, the target analyte binding agent, and the complementary peptide or polypeptide component of the bioluminescent complex are combined in a dried formulation;

wherein the dried formulation forms the bioluminescent analyte detection complex in the presence of the target analyte upon rehydration.

7. The composition of any of claims 1 to 6, wherein the complementary peptide or polypeptide component comprises a second target analyte binding element that forms the bioluminescent analyte detection complex in the presence of the target analyte.

8. The composition of any of claims 1 to 7, wherein the polypeptide component of the target analyte binding agent comprises at least 60% sequence identity with SEQ ID NO: 6, and wherein the complementary peptide or polypeptide component of the bioluminescent complex comprises at least 60% sequence identity with SEQ ID NO: 10.

9. The composition of any of claims 1 to 7, wherein the polypeptide component of the target analyte binding agent comprises at least 60% sequence identity with SEQ ID NO: 6, and wherein the complementary peptide or polypeptide component of the bioluminescent complex comprises at least 60% sequence identity with SEQ ID NO: 14.

10. A composition comprising a dried formulation comprising:

(a) a first target analyte binding agent comprising a first target analyte binding element and a polypeptide component having at least 60% sequence identity with SEQ ID NO: 9; and

(b) a second target analyte binding agent comprising a second target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 10.

11. The composition of claim 10, wherein the dried formulation further comprises a luminogenic substrate.

12. The composition of claim 10 or 11, further comprising a liquid formulation comprising the target analyte.

13. A composition comprising a dried formulation comprising:

(a) a first target analyte binding agent comprising a first target analyte binding element and a polypeptide component having at least 60% sequence identity with SEQ ID NO: 12; and

(b) a second target analyte binding agent comprising a second target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 14.

14. The composition of claim 13, wherein the dried formulation further comprises a luminogenic substrate.

15. The composition of claim 13 or 14, further comprising a liquid formulation comprising the target analyte.

16. A composition comprising a dried formulation comprising:

(a) a first target analyte binding agent comprising a first target analyte binding element and a peptide component having at least 60% sequence identity with SEQ ID NO: 13;

(b) a second target analyte binding agent comprising a second target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 15; and

(c) a complementary polypeptide component having at least 60% sequence identity with SEQ ID NO: 12.

17. The composition of claim 16, wherein the dried formulation further comprises a luminogenic substrate.

18. The composition of claim 16 or 17, further comprising a liquid formulation comprising the target analyte.

19. A composition comprising:

(a) a dried formulation comprising a first target analyte binding agent comprising a target analyte binding element and a polypeptide component having at least 60% sequence identity with SEQ ID NO: 9; and

(b) a liquid formulation comprising a second target analyte binding agent comprising a target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 10 or SEQ ID NO: 11.

20. A composition comprising:

(a) a dried formulation comprising a first target analyte binding agent comprising a target analyte binding element and a peptide component having at least 60% sequence identity with SEQ ID NO: 10 or SEQ ID NO: 11; and

(b) a liquid formulation comprising a second target analyte binding agent comprising a target analyte binding element and a complementary polypeptide component having at least 60% sequence identity with SEQ ID NO: 9.

21. A composition comprising:

(a) a dried formulation comprising a first target analyte binding agent comprising a target analyte binding element and a polypeptide component having at least 60% sequence identity with SEQ ID NO: 12; and

(b) a liquid formulation comprising a second target analyte binding agent comprising a target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 14.

22. The compositions of claims 19 to 21, wherein the dried formulation further comprises a luminogenic substrate.

23. The compositions of claims 19 to 21, wherein the liquid formulation further comprises a luminogenic substrate.

24. The compositions of claims 19 to 21, wherein the liquid formulation further comprises a sample comprising a target analyte, and wherein a bioluminescent analyte detection complex forms upon combining the dried formulation and the liquid formulation in the presence of the target analyte.

25. The composition of any of claims 1 to 4, further comprising a second complementary peptide or polypeptide component of the bioluminescent complex,

wherein the target analyte binding agent, the first complementary peptide or polypeptide component of the bioluminescent complex, and the second complementary peptide or polypeptide component of the bioluminescent complex form a bioluminescent analyte detection complex in the presence of a target analyte.

26. The composition of claim 25, wherein the composition comprising the target analyte binding agent comprises a dried formulation, and wherein the first complementary peptide or polypeptide component and the second complementary peptide or polypeptide of the bioluminescent complex comprise a liquid formulation;

wherein the liquid formulation is added to the dried formulation and forms the bioluminescent analyte detection complex in the presence of the target analyte upon rehydration.

27. The composition of claim 25, wherein the composition comprising the target analyte binding agent, and either the first or the second complementary peptide or polypeptide component are combined in a dried formulation, and wherein the first or the second complementary peptide or polypeptide component that is not present in the dried formulation comprises a liquid formulation;

wherein the liquid formulation is added to the dried formulation and forms the bioluminescent analyte detection complex in the presence of the target analyte upon rehydration.

28. The composition of claim 25, wherein the target analyte binding agent, the first complementary peptide or polypeptide component, and the second complementary peptide or polypeptide component are combined in a dried formulation that forms the bioluminescent analyte detection complex in the presence of the target analyte upon rehydration.

29. The compositions of claims 25 to 27, wherein the dried formulation further comprises a luminogenic substrate.

30. The compositions of claims 25 to 27, wherein the liquid formulation further comprises a luminogenic substrate.

31. The compositions of claims 25 to 27, wherein the liquid formulation further comprises a sample comprising a target analyte, and wherein a bioluminescent analyte detection complex forms upon combining the dried formulation and the liquid formulation in the presence of the target analyte.

32. The composition of any of claims 25 to 31, wherein either the first or the second complementary peptide or polypeptide component comprises a second target analyte binding element that forms the bioluminescent analyte detection complex in the presence of the target analyte upon rehydration.

33. The composition of any of claims 25 to 32, wherein the polypeptide component of the target analyte binding agent comprises at least 60% sequence identity with SEQ ID NO: 12, and wherein either the first or the second complementary peptide or polypeptide component of the bioluminescent complex comprises at least 60% sequence identity with either SEQ ID NO: 13 or SEQ ID NO: 15.

34. A composition comprising:

(a) a dried formulation comprising a first target analyte binding agent comprising a target analyte binding element and a polypeptide component having at least 60% sequence identity with SEQ ID NO: 12; and

(b) a liquid formulation comprising a second target analyte binding agent comprising a target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 13 or SEQ ID NO: 15; and

a second complementary peptide component having at least 60% sequence identity with SEQ ID NO: 13 or SEQ ID NO: 15.

35. A composition comprising:

(a) a dried formulation comprising a first target analyte binding agent comprising a target analyte binding element and a polypeptide component having at least 60% sequence identity with SEQ ID NO: 12, and a second target analyte binding agent comprising a target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 13 or SEQ ID NO: 15; and

(b) a liquid formulation comprising a second complementary peptide component having at least 60% sequence identity with SEQ ID NO: 13 or SEQ ID NO: 15.

36. A composition comprising:

(a) a dried formulation comprising a first target analyte binding agent comprising a target analyte binding element and a polypeptide component having at least 60% sequence identity with SEQ ID NO: 12, and complementary peptide component having at least 60% sequence identity with SEQ ID NO: 13 or SEQ ID NO: 15; and

(b) a liquid formulation comprising a second target analyte binding agent comprising a target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 13 or SEQ ID NO: 15.

37. A composition comprising:

(a) a dried formulation comprising a first target analyte binding agent comprising a target analyte binding element and a peptide component having at least 60% sequence identity with SEQ ID NO: 13, and a second target analyte binding agent comprising a target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 15; and

(b) a liquid formulation comprising a complementary polypeptide component having at least 60% sequence identity with SEQ ID NO: 12.

38. A composition comprising:

(a) a dried formulation comprising a complementary polypeptide component having at least 60% sequence identity with SEQ ID NO: 12; and

(b) a liquid formulation comprising a first target analyte binding agent comprising a target analyte binding element and a peptide component having at least 60% sequence identity with SEQ ID NO: 13, and a second target analyte binding agent comprising a target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 15.

39. A composition comprising a dried formulation comprising a first target analyte binding agent comprising a target analyte binding element and a peptide component having at least 60% sequence identity with SEQ ID NO: 13, a second target analyte binding agent comprising a target analyte binding element and a complementary peptide component having at least 60% sequence identity with SEQ ID NO: 15, and a complementary polypeptide component having at least 60% sequence identity with SEQ ID NO: 12.

40. The compositions of claims 34 to 39, wherein the dried formulation further comprises a luminogenic substrate.

41. The compositions of claims 34 to 39, wherein the liquid formulation further comprises a luminogenic substrate.

42. The compositions of claims 34 to 39, wherein the liquid formulation further comprises a sample comprising a target analyte, and wherein a bioluminescent analyte detection complex forms upon combining the dried formulation and the liquid formulation in the presence of the target analyte.

43. The composition of any of claims 1 to 42, wherein a bioluminescent signal produced in the presence of the luminogenic substrate is substantially increased when the target analyte binding agent contacts one or more of the complementary peptide or polypeptide components of the bioluminescent complex, as compared to a bioluminescent signal produced by the target analyte binding agent and the luminogenic substrate alone.

44. The composition of any of claims 1 to 43, wherein the target analyte is a target antibody.

45. The composition of any of claims 1 to 44, wherein the target analyte binding agent comprises an element that binds non-specifically to antibodies.

46. The composition of any of claims 1 to 44, wherein the target analyte binding agent comprises an element that binds specifically to an antibody.

47. The composition of claim 44, wherein the target antibody is an antibody against a pathogen, toxin, or therapeutic biologic.

48. The composition of any of claims 1 to 47, wherein a target analyte binding element is selected from the group consisting of an antibody, a polyclonal antibody, a monoclonal antibody, a recombinant antibody, an antibody fragment, protein A, an Ig binding domain of protein A, protein G, an Ig binding domain of protein G, protein A/G, an Ig binding domain of protein A/G, protein L, a Ig binding domain of protein L, protein M, an Ig binding domain of protein M, an oligonucleotide probe, a peptide nucleic acid, a DARPin, an aptamer, an affimer, a protein domain, and a purified protein.

49. The composition of any of claims 1 to 48, wherein the luminogenic substrate is selected from coelenterazine, coelenterazine-h, coelenterazine-h-h, furimazine, and other coelenterazine analogs or derivatives.

50. The composition of any of claims 1 to 49, further comprising a polymer.

51. The composition of claim 50, wherein the polymer is a naturally-occurring biopolymer.

52. The composition of claim 51, wherein the naturally-occurring biopolymer is selected from pullulan, trehalose, maltose, cellulose, dextran, and a combination of any thereof.

53. The composition of claim 51, wherein the naturally-occurring biopolymer is pullulan.

54. The composition of claim 50, wherein the polymer is a cyclic saccharide polymer or a derivative thereof.

55. The composition of claim 54, wherein the polymer is hydroxypropyl β-cyclodextrin.

56. The composition of claim 50, wherein the polymer is a synthetic polymer.

57. The composition of claim 56, wherein the synthetic polymer is selected from polystyrene, poly(meth)acrylate, and a combination of any thereof.

58. The composition of claim 56, wherein the synthetic polymer is a block copolymer comprising at least one poly(propylene oxide) block and at least one poly(ethylene oxide) block.

59. The composition of claim 58, wherein the synthetic polymer is poloxamer 188.

60. The composition of any of claims 1-59, wherein the composition further comprises a buffer, a surfactant, a reducing agent, a salt, a radical scavenger, a chelating agent, a protein, or any combination thereof.

61. The composition of claim 60, wherein the is surfactant selected from polysorbate 20, polysorbate 40, and polysorbate 80.

62. The composition of any of claims 1 to 61, wherein the composition is used in conjunction with an analyte detection platform to detect an analyte in a sample.

63. The composition of claim 62, wherein the sample is selected from blood, serum, plasma, urine, stool, cerebral spinal fluid, interstitial fluid, saliva, a tissue sample, a water sample, a soil sample, a plant sample, a food sample, a beverage sample, an oil, and an industrial fluid sample.

64. A method of detecting an analyte in a sample comprising combining any of the compositions of claims 1 to 63 with a sample comprising a target analyte.

65. The method of claim 64, wherein detecting the target analyte in the sample comprises detecting a bioluminescent signal generated from an analyte detection complex.

66. The method of claim 65, further comprising quantifying a bioluminescent signal generated from the analyte detection complex.

67. The method of claim 66, wherein the bioluminescent signal generated from the analyte detection complex is proportional to the concentration of the analyte.

68. The composition of any of claims 1 to 67, wherein one or more of the components of the composition exhibits enhanced stability within the composition compared to the component in solution alone.

69. A lateral flow detection system comprising:

an analytical membrane comprising a detection region and a control region, wherein the detection region comprises a first target analyte binding agent immobilized to the detection region;

a conjugate pad comprising a second target analyte binding agent; and

a sample pad; and

wherein the first target analyte binding agent and the second target analyte binding agent form a bioluminescent analyte detection complex in the at least one detection region when a target analyte is detected in a sample.

70. The system of claim 69, wherein the first target analyte binding agent comprises a target analyte binding element and is non-luminescent, and wherein the second target analyte binding agent comprises a target analyte binding element and a bioluminescent polypeptide.

71. The system of claim 70, wherein the bioluminescent polypeptide has at least 60% sequence identity with SEQ ID NO: 5.

72. The system of claim 69, wherein the first target analyte binding agent comprises a target analyte binding element and a polypeptide component of a bioluminescent complex, and the second target analyte binding agent comprises a target analyte binding element and a peptide component of a bioluminescent complex, wherein a bioluminescent signal produced in the presence of a luminogenic substrate is substantially increased when the first target analyte binding agent contacts the second target analyte binding agent, as compared to a bioluminescent signal produced by the first target analyte binding agent and the luminogenic substrate alone.

73. The system of claim 69, wherein the first target analyte binding agent comprises a target analyte binding element and a peptide component of a bioluminescent complex, and the second target analyte binding agent comprises a target analyte binding element and a polypeptide component of a bioluminescent complex, wherein a bioluminescent signal produced in the presence of a luminogenic substrate is substantially increased when the first target analyte binding agent contacts the second target analyte binding agent, as compared to a bioluminescent signal produced by the first target analyte binding agent and the luminogenic substrate alone.

74. The system of claim 72 or claim 73, wherein the polypeptide component of a bioluminescent complex has at least 60% sequence identity with SEQ ID NO: 6, and wherein the peptide component of a bioluminescent complex has at least 60% sequence identity with SEQ ID NO: 10.

75. The system of claim 72 or claim 73, wherein the polypeptide component of a bioluminescent complex has at least 60% sequence identity with SEQ ID NO: 12, and wherein

the peptide component of a bioluminescent complex has at least 60% sequence identity with SEQ ID NO: 14.

76. The system of claim 69, wherein the first target analyte binding agent comprises a target analyte binding element and a first peptide component of a tripartite bioluminescent complex, and the second target analyte binding agent comprises a target analyte binding element and a second peptide component of the tripartite bioluminescent complex, wherein a bioluminescent signal produced in the presence of a luminogenic substrate is substantially increased when the first target analyte binding agent contacts the second target analyte binding agent and a polypeptide component of the tripartite bioluminescent complex, as compared to a bioluminescent signal produced by (i) the first target analyte binding agent, the second target analyte binding agent, and/or the polypeptide component and (ii) the luminogenic substrate alone.

77. The system of claim 76, wherein the first peptide component of a tripartite bioluminescent complex has at least 60% sequence identity with SEQ ID NO: 11, wherein the first peptide component of a tripartite bioluminescent complex has at least 60% sequence identity with SEQ ID NO: 13, and wherein the polypeptide component of a tripartite bioluminescent complex has at least 60% sequence identity with SEQ ID NO: 12.

78. The system of any of claims 69-77, wherein the target analyte is a target antibody.

79. The system of claim 78, wherein the first target analyte binding agent comprises an element that binds non-specifically to antibodies.

80. The system of claim 79, wherein the second target analyte binding agent comprises an element that binds specifically to the target antibody.

81. The system of claim 78, wherein the target antibody is an antibody against a pathogen, toxin, or therapeutic biologic.

82. The system of any of claims 69 to 77, wherein a target analyte binding element is selected from the group consisting of an antibody, a polyclonal antibody, a monoclonal antibody, a recombinant antibody, an antibody fragment, protein A, an Ig binding domain of protein A, protein G, an Ig binding domain of protein G, protein A/G, an Ig binding domain of protein A/G, protein L, a Ig binding domain of protein L, protein M, an Ig binding domain of protein M, an oligonucleotide probe, a peptide nucleic acid, a DARPin, an aptamer, an affimer, a protein domain, and a purified protein.

83. The system of any of claims 69 to 82, further comprising a luminogenic substrate.

84. The system of claim 83, wherein the luminogenic substrate is selected from coelenterazine, coelenterazine-h, coelenterazine-h-h, furimazine, and other coelenterazine analogs or derivatives.

85. The system of any of claims 69 to 84, wherein the luminogenic substrate is applied to the system as part of a composition comprising the luminogenic substrate and a polymer selected from pullulan, trehalose, maltose, cellulose, dextran, polystyrene, poly(meth)acrylate, and a combination of any thereof.

86. The system of any of claims 69 to 84, wherein the composition is applied to at least one of the sample pad, the conjugation pad, the detection region, and the control region.

87. The system of any of claims 69 to 86, wherein the analytical membrane comprises a plurality of detection regions with each detection region comprising a distinct target analyte binding agent comprising distinct target analyte binding elements.

88. The system of any of claims 69 to 87, wherein the system further comprises a device for detecting or quantifying bioluminescent signals from the analyte detection complex.

89. A conjugate pad comprising at least one target analyte binding agent, wherein the at least one target analyte binding agent comprises a target analyte binding element and one of:

a bioluminescent polypeptide comprising at least 60% sequence identity with SEQ ID NO: 5;

a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 9;

a peptide comprising at least 60% sequence identity with SEQ ID NO: 10;

a peptide comprising at least 60% sequence identity with SEQ ID NO: 11;

a peptide comprising at least 60% sequence identity with SEQ ID NO: 13;

a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 12;

a peptide comprising at least 60% sequence identity with SEQ ID NO: 14; or

a fluorophore capable of being activated by energy transfer from an Oplophorus luciferase.

90. The conjugate pad of claim 89, the target analyte binding agent comprises a target analyte binding element and one of:

a bioluminescent polypeptide of SEQ ID NO: 5;

a polypeptide of SEQ ID NO: 9;

a peptide of SEQ ID NO: 10;

a peptide of SEQ ID NO: 11;

a peptide of SEQ ID NO: 13;

a polypeptide of SEQ ID NO: 12;

a peptide of SEQ ID NO: 14; or

a fluorophore capable of being activated by energy transfer from an Oplophorus luciferase.

91. The conjugate pad of claim 89 or 90, further comprising a luminogenic substrate.

92. The conjugate pad of claim 91, wherein the luminogenic substrate is selected from coelenterazine, coelenterazine-h, coelenterazine-h-h, furimazine, and other coelenterazine analogs or derivatives.

93. The conjugate pad of claim 91, wherein the luminogenic substrate contained on or within the conjugate pad as part of a composition comprising the luminogenic substrate and a polymer

selected from pullulan, trehalose, maltose, cellulose, dextran, polystyrene, poly(meth)acrylate, and a combination of any thereof.

94. The conjugate pad of claim 91, wherein the luminogenic substrate is contained on or within the conjugate pad as part of a composition comprising the luminogenic substrate and a substance that reduces autoluminescence.

95. The conjugate pad of claim 94, wherein the substance that reduces autoluminescence is ATT, derivatives of ATT, or thiourea.

96. An analytical membrane comprising a detection region and a control region, wherein the detection region comprises at least one target analyte binding agent immobilized to the detection region, and wherein the at least one target analyte binding agent comprises a target analyte binding element and one of:

a bioluminescent polypeptide comprising at least 60% sequence identity with SEQ ID NO: 5;

a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 9;

a peptide comprising at least 60% sequence identity with SEQ ID NO: 10;

a peptide comprising at least 60% sequence identity with SEQ ID NO: 11;

a peptide comprising at least 60% sequence identity with SEQ ID NO: 13;

a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 12;

a peptide comprising at least 60% sequence identity with SEQ ID NO: 14; or

a fluorophore capable of being activated by energy transfer from an Oplophorus luciferase.

97. The analytical membrane of claim 96, the target analyte binding agent comprises a target analyte binding element and one of:

a bioluminescent polypeptide of SEQ ID NO: 5;

a polypeptide of SEQ ID NO: 9;

a peptide of SEQ ID NO: 10;

a peptide of SEQ ID NO: 11;

a peptide of SEQ ID NO: 13;

a polypeptide of SEQ ID NO: 12;

a peptide of SEQ ID NO: 14; or

a fluorophore capable of being activated by energy transfer from an Oplophorus luciferase.

98. The analytical membrane of claim 96, further comprising a plurality of detection regions with each detection region comprising a distinct target analyte binding agent comprising distinct target analyte binding elements.

99. The analytical membrane of claim 97 or 98, further comprising a luminogenic substrate.

100. The analytical membrane of claim 97, wherein the luminogenic substrate is selected from coelenterazine, coelenterazine-h, coelenterazine-h-h, furimazine, and other coelenterazine analogs or derivatives.

101. The analytical membrane of claim 99, wherein the luminogenic substrate is reversibly conjugated to the conjugate pad as part of a composition comprising the luminogenic substrate and a polymer selected from pullulan, trehalose, maltose, cellulose, dextran, polystyrene, poly(meth)acrylate, and a combination of any thereof.

102. The analytical membrane of claim 99, wherein the luminogenic substrate is contained on or within the conjugate pad as part of a composition comprising the luminogenic substrate and a substance that reduces autoluminescence.

103. The analytical membrane of claim 99, wherein the substance that reduces autoluminescence is ATT, derivatives of ATT, or thiourea.

104. A solid phase detection platform comprising a detection region, wherein the detection region comprises at least one target analyte binding agent conjugated to the detection region, and wherein the at least one target analyte binding agent comprises a target analyte binding element and one of:

a bioluminescent polypeptide comprising at least 60% sequence identity with SEQ ID NO: 5;

a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 9;

a peptide comprising at least 60% sequence identity with SEQ ID NO: 10;

a peptide comprising at least 60% sequence identity with SEQ ID NO: 11;

a peptide comprising at least 60% sequence identity with SEQ ID NO: 13;

a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 12;

a peptide comprising at least 60% sequence identity with SEQ ID NO: 14; or

a fluorophore capable of being activated by energy transfer from an Oplophorus luciferase.

105. The detection platform of claim 104, wherein the target analyte binding agent comprises a target analyte binding element and one of:

a bioluminescent polypeptide of SEQ ID NO: 5;

a polypeptide of SEQ ID NO: 9;

a peptide of SEQ ID NO: 10;

a peptide of SEQ ID NO: 11;

a peptide of SEQ ID NO: 13;

a polypeptide of SEQ ID NO: 12;

a peptide of SEQ ID NO: 14; or

a fluorophore capable of being activated by energy transfer from an Oplophorus luciferase.

106. The detection platform of claim 104, wherein the detection platform comprises:

a first target analyte binding agent comprising a target analyte binding element and a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 6 conjugated to the detection region; and

a second target analyte binding agent comprising a target analyte binding element and a peptide comprising at least 60% sequence identity with SEQ ID NO: 10 applied to the detection region.

107. The detection platform of claim 106, wherein the detection platform comprises:

a first target analyte binding agent comprising a target analyte binding element and a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 10 conjugated to the detection region; and

a second target analyte binding agent comprising a target analyte binding element and a peptide comprising at least 60% sequence identity with SEQ ID NO: 6 applied to the detection region.

108. The detection platform of claim 106, wherein the detection platform comprises:

a first target analyte binding agent comprising a target analyte binding element and a peptide comprising at least 60% sequence identity with SEQ ID NO: 11 conjugated to the detection region;

a second target analyte binding agent comprising a target analyte binding element and a peptide comprising at least 60% sequence identity with SEQ ID NO: 13 applied to the detection region; and

a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 12 applied to the detection region.

109. The detection platform of claim 106, wherein the detection platform comprises:

a first target analyte binding agent comprising a target analyte binding element and a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 6 conjugated to the detection region; and

a second target analyte binding agent comprising a target analyte binding element and a polypeptide comprising at least 60% sequence identity with ID NO: 14 applied to the detection region.

110. The detection platform of claim 106, wherein the detection platform comprises:

a first target analyte binding agent comprising a target analyte binding element and a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 14 conjugated to the detection region; and

a second target analyte binding agent comprising a target analyte binding element and a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 6 applied to the detection region.

111. The detection platform of claim 106, wherein the detection platform comprises:

a first target analyte binding agent comprising a target analyte binding element and a bioluminescent polypeptide at least 60% sequence identity with SEQ ID NO: 5 conjugated to the detection region; and

a second target analyte binding agent comprising a target analyte binding element and a fluorophore capable of being activated by energy transfer from the bioluminescent polypeptide applied to the detection region.

112. The detection platform of claim 106, wherein the detection platform comprises:

a first target analyte binding agent comprising a target analyte binding element and a bioluminescent polypeptide at least 60% sequence identity with SEQ ID NO: 5 applied to the detection region; and

a second target analyte binding agent comprising a target analyte binding element and a fluorophore capable of being activated by energy transfer from the bioluminescent polypeptide conjugated to the detection region.

113. The detection platform of claim 106, further comprising a plurality of detection regions with each detection region comprising a distinct target analyte binding agent comprising distinct target analyte binding elements.

114. The detection platform of claim 106, further comprising a control region.

115. The detection platform of claim 106, further comprising a luminogenic substrate.

116. The detection platform of claim 115, wherein the luminogenic substrate is selected from coelenterazine, coelenterazine-h, coelenterazine-h-h, furimazine, and other coelenterazine analogs or derivatives.

117. The detection platform of claim 115, wherein the luminogenic substrate is reversibly conjugated to the conjugate pad as part of a composition comprising the luminogenic substrate and a polymer selected from pullulan, trehalose, maltose, cellulose, dextran, polystyrene, poly(meth)acrylate, and a combination of any thereof.

118. The detection platform of claim 115, wherein the luminogenic substrate is contained on or within the conjugate pad as part of a composition comprising the luminogenic substrate and a substance that reduces autoluminescence.

119. The analytical membrane of claim 118, wherein the substance that reduces autoluminescence is ATT, derivatives of ATT, or thiourea.

120. A solution phase detection platform comprising at least one detection receptacle and a lyophilized tablet (lyocake), wherein the lyocake comprises a target analyte binding agent comprising a target analyte binding element and one of:

a bioluminescent polypeptide comprising at least 60% sequence identity with SEQ ID NO: 5;

a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 9;

a peptide comprising at least 60% sequence identity with SEQ ID NO: 10;

a peptide comprising at least 60% sequence identity with SEQ ID NO: 11;

a peptide comprising at least 60% sequence identity with SEQ ID NO: 13;

a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 12;

a peptide comprising at least 60% sequence identity with SEQ ID NO: 14; or

a fluorophore capable of being activated by energy transfer from an Oplophorus luciferase.

121. The detection platform of claim 120, wherein the target analyte binding agent comprises a target analyte binding element and one of:

a bioluminescent polypeptide of SEQ ID NO: 5;

a polypeptide of SEQ ID NO: 9;

a peptide of SEQ ID NO: 10;

a peptide of SEQ ID NO: 11;

a peptide of SEQ ID NO: 13;

a polypeptide of SEQ ID NO: 12;

a peptide of SEQ ID NO: 14; or

a fluorophore capable of being activated by energy transfer from an Oplophorus luciferase.

122. The detection platform of claim 120, wherein the lyocake comprises:

a first target analyte binding agent comprising a target analyte binding element and a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 6; and

a second target analyte binding agent comprising a target analyte binding element and a peptide comprising at least 60% sequence identity with SEQ ID NO: 10.

123. The detection platform of claim 120, wherein the lyocake comprises:

a first target analyte binding agent comprising a target analyte binding element and a peptide comprising at least 60% sequence identity with SEQ ID NO: 11;

a second target analyte binding agent comprising a target analyte binding element and a peptide comprising at least 60% sequence identity with SEQ ID NO: 13; and

a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 12.

124. The detection platform of claim 120, wherein the lyocake comprises:

a first target analyte binding agent comprising a target analyte binding element and a polypeptide comprising at least 60% sequence identity with SEQ ID NO: 6; and

a second target analyte binding agent comprising a target analyte binding element and a polypeptide comprising at least 60% sequence identity with ID NO: 14.

125. The detection platform of claim 120, wherein the lyocake comprises:

a first target analyte binding agent comprising a target analyte binding element and a bioluminescent polypeptide at least 60% sequence identity with SEQ ID NO: 5; and

a second target analyte binding agent comprising a target analyte binding element and a fluorophore capable of being activated by energy transfer from the bioluminescent polypeptide.

126. The detection platform of claim 120, wherein the detection platform comprises a 96-well microtiter plate comprising a plurality of detection receptacles, and at least two distinct target analyte binding agents comprising distinct target analyte binding elements.

127. The detection platform of claim 120, wherein the lyocake comprises a luminogenic substrate.

128. The detection platform of claim 127, wherein the luminogenic substrate is selected from coelenterazine, coelenterazine-h, coelenterazine-h-h, furimazine, and other coelenterazine analogs or derivatives.

129. The detection platform of claim 127, wherein the lyocake comprises a luminogenic substrate and a polymer selected from pullulan, trehalose, maltose, cellulose, dextran, polystyrene, poly(meth)acrylate, and a combination of any thereof.

130. The detection platform of claim 127, wherein the luminogenic substrate is contained on or within the conjugate pad as part of a composition comprising the luminogenic substrate and a substance that reduces autoluminescence.

131. The analytical membrane of claim 120, wherein the substance that reduces autoluminescence is ATT, derivatives of ATT, or thiourea.

132. The detection platform of claim 120, further comprising at least one sample.

133. The detection platform of claim 132, wherein the sample is selected from blood, serum, plasma, urine, stool, cerebral spinal fluid, interstitial fluid, saliva, a tissue sample, a water sample, a soil sample, a plant sample, a food sample, a beverage sample, an oil, and an industrial fluid sample.

134. A method of detecting an analyte in a sample using the lateral flow assay system of claim 1, the method comprising:

applying a sample to the sample pad;

facilitating flow of the sample from the sample pad to the conjugate pad, and then from the conjugate pad to the detection region and the control region on the analytical membrane;

wherein the first target analyte binding agent, the second target analyte binding agent, and the target analyte form the analyte detection complex in the at least one detection region when the target analyte is detected in the sample.

135. The method of claim 134, wherein the sample is selected from blood, serum, plasma, urine, stool, cerebral spinal fluid, interstitial fluid, tissue, and saliva.

136. The method of claim 134, wherein the sample is selected from a water sample, a soil sample, a plant sample, a food sample, a beverage sample, an oil, and an industrial fluid sample.

137. The method of claim 134, wherein detecting the target analyte in the sample comprises detecting a bioluminescent signal generated from the analyte detection complex.

138. The method of claim 134, wherein the method further comprises quantifying a bioluminescent signal generated from the analyte detection complex.

139. The method of claim 134, wherein the method further comprises diagnosing a subject from which the sample was obtained as having or not having a disease based on the detection of the analyte.

140. A method of detecting an analyte in a sample using the solid phase detection platform of claim 34, the method comprising:

exposing a sample to the detection region and control region;

wherein the at least one target analyte binding agent and the at least one target analyte form an analyte detection complex in the at least one detection region when the target analyte is detected in the sample.

141. The method of claim 140, wherein the sample is selected from blood, serum, plasma, urine, stool, cerebral spinal fluid, interstitial fluid, tissue, and saliva.

142. The method of claim 140, wherein the sample is selected from a water sample, a soil sample, a plant sample, a food sample, a beverage sample, an oil, and an industrial fluid sample.

143. The method of claim 140, wherein detecting the target analyte in the sample comprises detecting a bioluminescent signal generated from the analyte detection complex.

144. The method of claim 140, wherein the method further comprises quantifying a bioluminescent signal generated from the analyte detection complex.

145. The method of claim 140, wherein the method further comprises diagnosing a subject from which the sample was obtained as having or not having a disease based on the detection of the analyte.

146. A method of producing a substrate for use in a bioluminescent assay, the method comprising:

applying a solution onto a substrate, the solution comprising at least one target analyte binding agent comprising a target analyte binding element and one of a polypeptide component of a bioluminescent complex or a peptide component of a bioluminescent complex; and

drying the substrate containing the solution.

147. The method of claim 146, wherein the solution further comprises a complementary peptide or polypeptide component of the bioluminescent complex; wherein the target analyte binding agent and the complementary peptide or polypeptide component of the bioluminescent complex form a bioluminescent analyte detection complex in the presence of a target analyte.

148. The method of claim 146 or claim 147, wherein the solution comprises a protein buffer and at least one excipient.

149. The method of any of claims 146 to 148, wherein the solution comprises a luminogenic substrate.

150. The method of any of claims 146 to 149, wherein the substrate is W-903 paper, FTA paper, FTA Elute paper, FTA DMPK paper, Ahlstrom A-226 paper, M-TFN paper, FTA paper, FP705 paper, Bode DNA collection paper, nitrocellulose paper, nylon paper, cellulose paper, Dacron paper, cotton paper, and polyester papers, or combinations thereof.

151. The method of any of claims 146 to 150, wherein the substrate is a mesh comprising plastic, nylon, metal, or combinations thereof.

152. The method of any of claims 146 to 151, wherein drying the substrate containing the solution comprises drying at a temperature from about 30°C to 40°C for a period of time between about 30 mins and 2 hours.

153. The method of any of claims 146 to 152, wherein drying the substrate containing the solution comprises lyophilizing and/or freezing the substrate.

154. The method of any of claims 146 to 153, further comprising drying the at least one target analyte binding agent and/or the complementary peptide or polypeptide component of the bioluminescent complex onto a first substrate, and drying the luminogenic substrate onto a second substrate.

155. The method of any of claims 146 to 154, wherein a bioluminescent signal is generated upon exposure of the substrate containing the solution to the target analyte, and wherein the bioluminescent signal is proportional to the concentration of the target analyte.

156. The method of any of claims 146 to 155, wherein the at least one target analyte binding agent and/or the complementary peptide or polypeptide component of the bioluminescent complex exhibit(s) enhanced stability when dried on the substrate.

157. A composition comprising:

a luminogenic substrate;

a target analyte binding agent comprising a target analyte binding element and a polypeptide component of a bioluminescent complex; and

a complementary polypeptide component of the bioluminescent complex;

wherein the target analyte binding agent and the complementary polypeptide component of the bioluminescent complex are capable of forming a bioluminescent analyte detection complex in the presence of a target analyte.

158. The composition of claim 157, wherein the composition further comprises a second target analyte binding agent comprising a second target analyte binding element and a second polypeptide component of a bioluminescent complex.

159. The composition of claim 157 or 158, wherein the first and second target analyte binding agents bind separate portions of the same target analyte.

160. The composition of any of claims 157 to 159, wherein the first and second polypeptide components of the bioluminescent complex bind the complementary polypeptide component of the bioluminescent complex to form a bioluminescent analyte detection complex in the presence of the target analyte.

161. The composition of any of claims 157 to 160, wherein the first and the second polypeptide components are linked to a modified dehalogenase capable of forming a covalent bond with a haloalkane substrate.

162. The composition of any of claims 157 to 161, wherein the first and the second target analyte binding elements comprise a haloalkane substrate.

163. The composition of any of claims 157 to 162, wherein the first or second polypeptide components of the first and second target analyte binding agents comprise:

at least 60% sequence identity with SEQ ID NO: 10;

at least 60% sequence identity with SEQ ID NO: 11;

at least 60% sequence identity with SEQ ID NO: 13; or

at least 60% sequence identity with SEQ ID NO: 15.

164. The composition of any of claims 157 to 163, wherein the complementary polypeptide component comprises:

at least 60% sequence identity with SEQ ID NO: 6;

at least 60% sequence identity with SEQ ID NO: 9; or

at least 60% sequence identity with SEQ ID NO: 12.

165. The composition of any of claims 157 to 164, wherein the target analyte binding element is selected from the group consisting of an antibody, a polyclonal antibody, a monoclonal antibody, a recombinant antibody, an antibody fragment, protein A, an Ig binding domain of protein A, protein G, an Ig binding domain of protein G, protein A/G, an Ig binding domain of protein A/G, protein L, a Ig binding domain of protein L, protein M, an Ig binding domain of protein M, an oligonucleotide probe, a peptide nucleic acid, a DARPin, an aptamer, an affimer, a protein domain, and a purified protein.

166. The composition of any of claims 157 to 165, wherein the target analyte is an antibody, and wherein the target analyte binding element of the first target analyte binding agent comprises antigen recognized by the antibody, and wherein the target analyte binding element of the second target analyte binding agent comprises an Fc binding region.

167. The composition of any of claims 157 to 166, wherein the first and/or second target analyte binding agents further comprise a fluorophore coupled to the first and/or second polypeptide components of the bioluminescent complex.

168. The composition of any of claims 157 to 167, wherein one or more components of the composition is in the form of a lyophilized tablet (lyocake) capable of forming a bioluminescent complex when reconstituted in a solution to detect and/or quantify the target analyte.

169. The composition of any of claims 157 to 168, wherein the composition comprises a solution-phase detection platform capable of detecting and/or quantifying the target analyte.

170. The composition of any of claims 157 to 169, wherein the polypeptide components and the luminogenic substrate are in the form of a lyophilized tablet (lyocake) capable of forming a bioluminescent complex when reconstituted in a solution to detect and/or quantify the target analyte.

171. A method of detecting an analyte in a sample comprising combining any of the compositions of claims 157 to 170 with a sample comprising a target analyte.

172. The method of claim 171, wherein detecting the target analyte in the sample comprises detecting a bioluminescent signal generated from an analyte detection complex.

173. The method of claim 172, further comprising quantifying a bioluminescent signal generated from the analyte detection complex.

174. The method of claim 173, wherein the bioluminescent signal generated from the analyte detection complex is proportional to the concentration of the analyte.

175. The composition of claim 157, wherein the luminogenic substrate is selected from coelenterazine, coelenterazine-h, coelenterazine-h-h, furimazine, and other coelenterazine analogs or derivatives.

176. The composition of claim 170, wherein the lyocake further comprises a substance to reduce autoluminescence.

177. The composition of claim 176, wherein the substance to reduce autoluminescence is ATT, a derivative or analog of ATT, or thiourea.

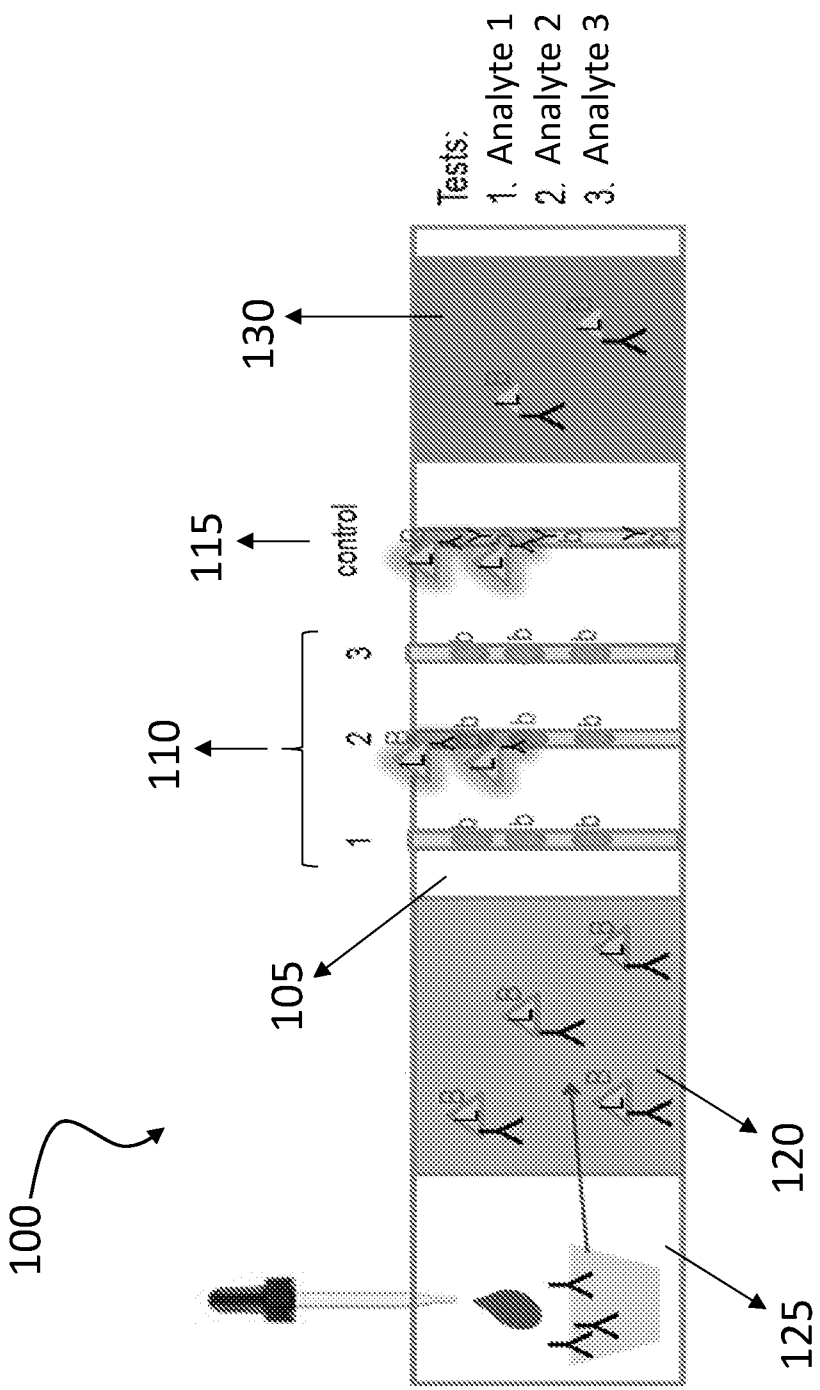

Tests:
1. Analyte 1
2. Analyte 2
3. Analyte 3
130
115
control
110
1
2
3
105
100
120
125

FIG. 1

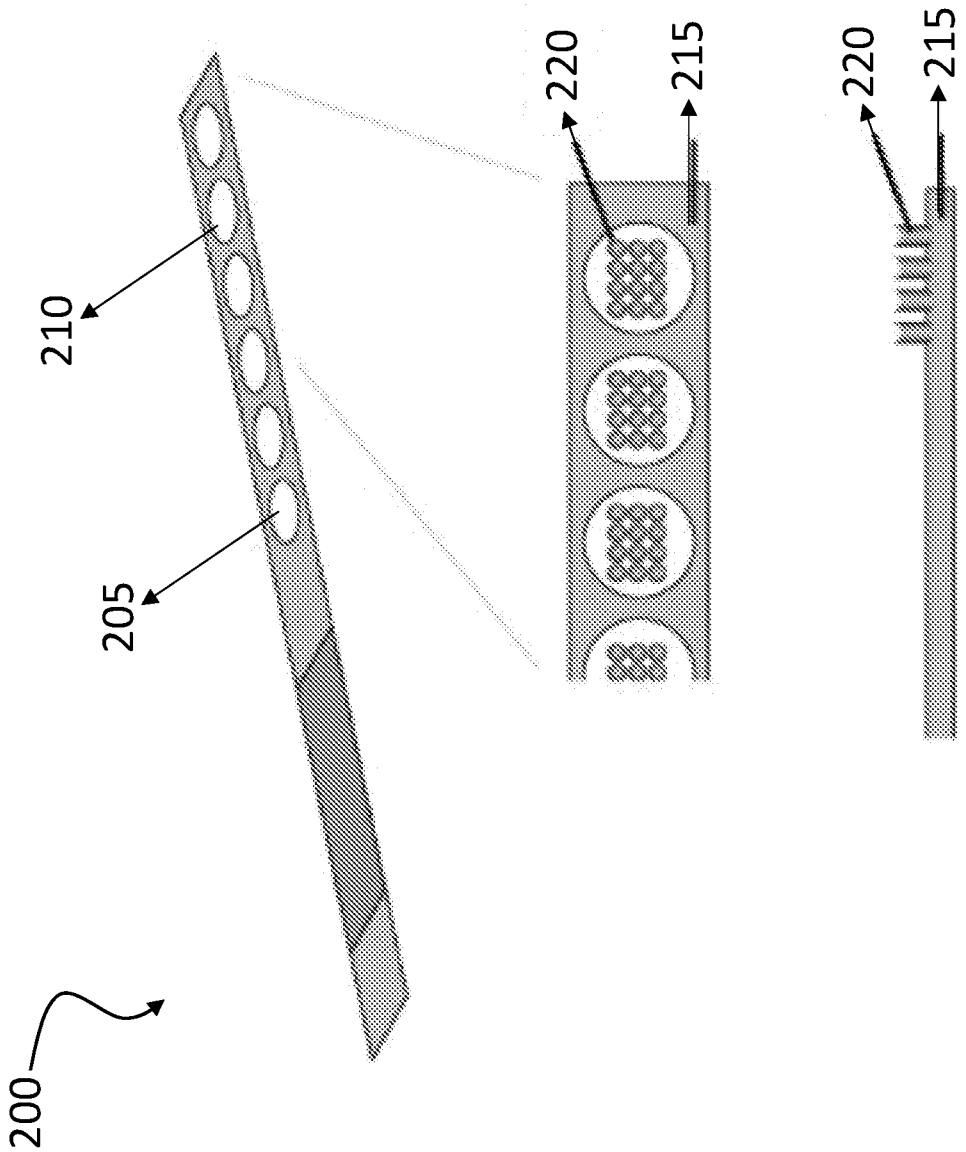
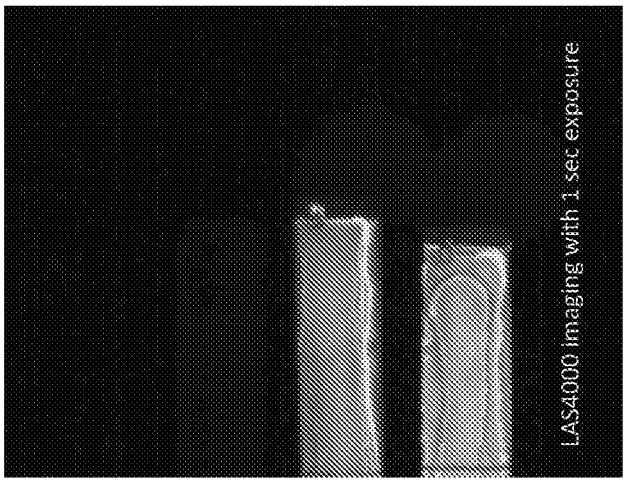


FIG. 2


LAS4000 imaging with 1 sec exposure

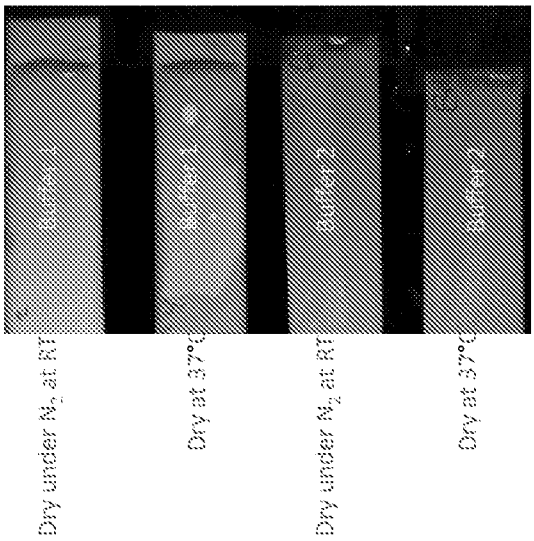

Dry under N_2 at RT
Dry at 37°C
Dry under N_2 at RT
Dry at 37°C

FIG. 3

Millipore nitrocellulose Hi-Flow Plus HFC07502
Whatman grade 541
Whatman 903 Protein Saver Snap Apart Cards
LAS4000 w/ 30 sec exposure
LAS4000 w/5 min exposure
buffer
Furimazine
HiBiT
LgBiT
All reagents placed into solid matrix, shipped at 4C and tested at 24hrs

FIG. 4

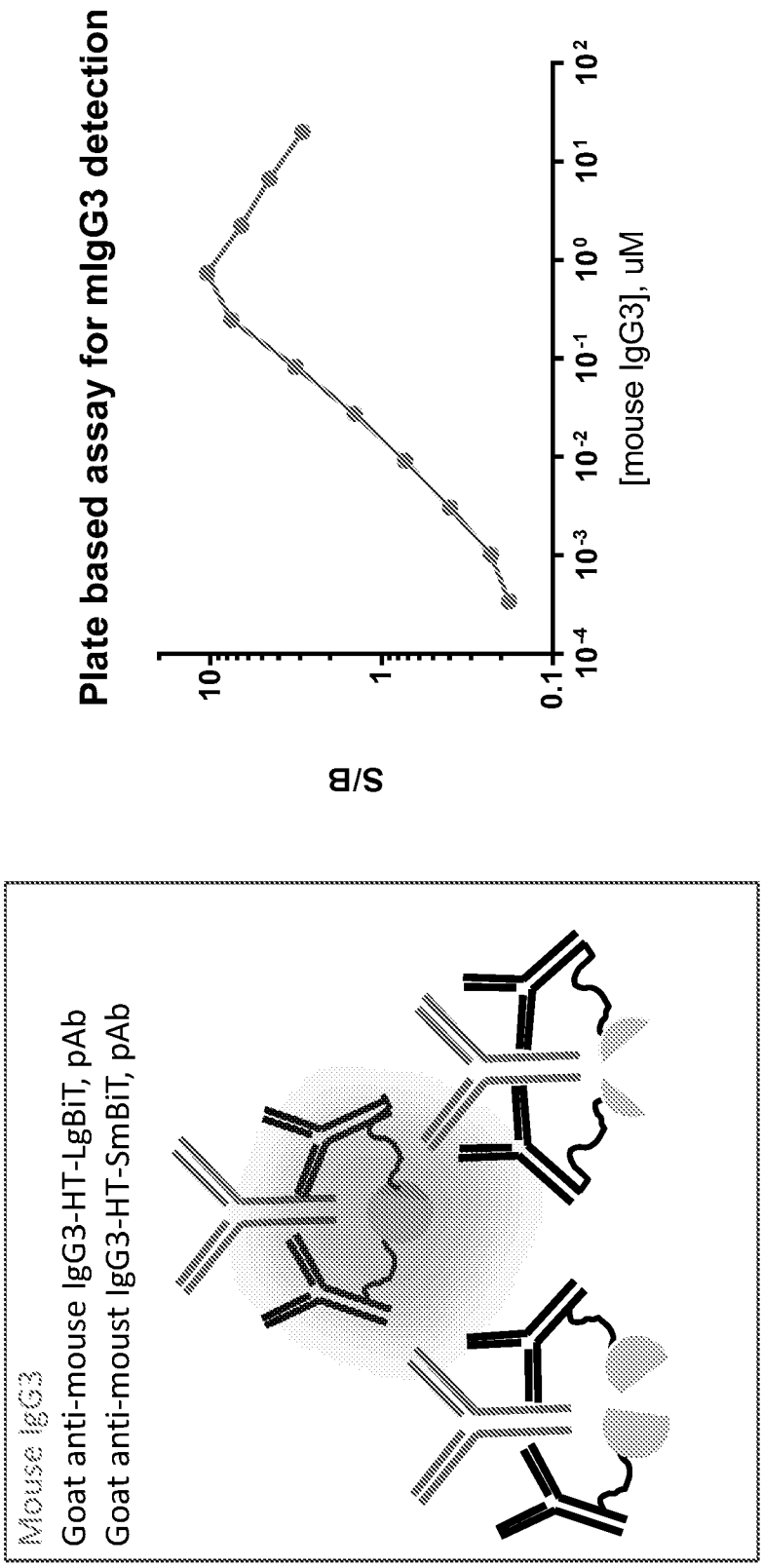


Plate based assay for mIgG3 detection
S/B
10
1
0.1
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
[mouse IgG3], uM
Mouse IgG3
Goat anti-mouse IgG3-HT-LgBiT, pAb
Goat anti-moust IgG3-HT-SmBiT, pAb

FIG. 5

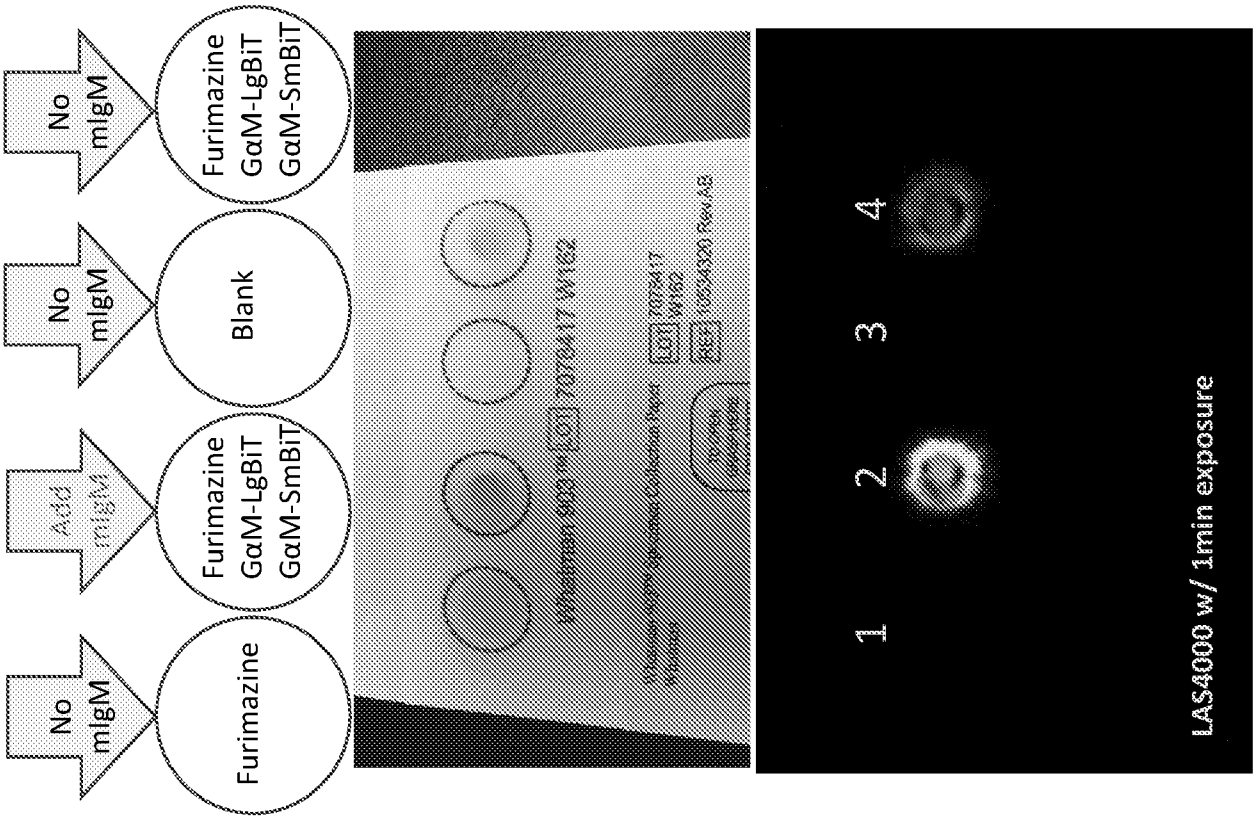

No mIgM
Furimazine
Add mIgM
Furimazine GαM-LgBiT GαM-SmBiT
No mIgM
Blank
No mIgM
Furimazine GαM-LgBiT GαM-SmBiT
LAS4000 w/ 1min exposure
1
2
3
4

FIG. 6

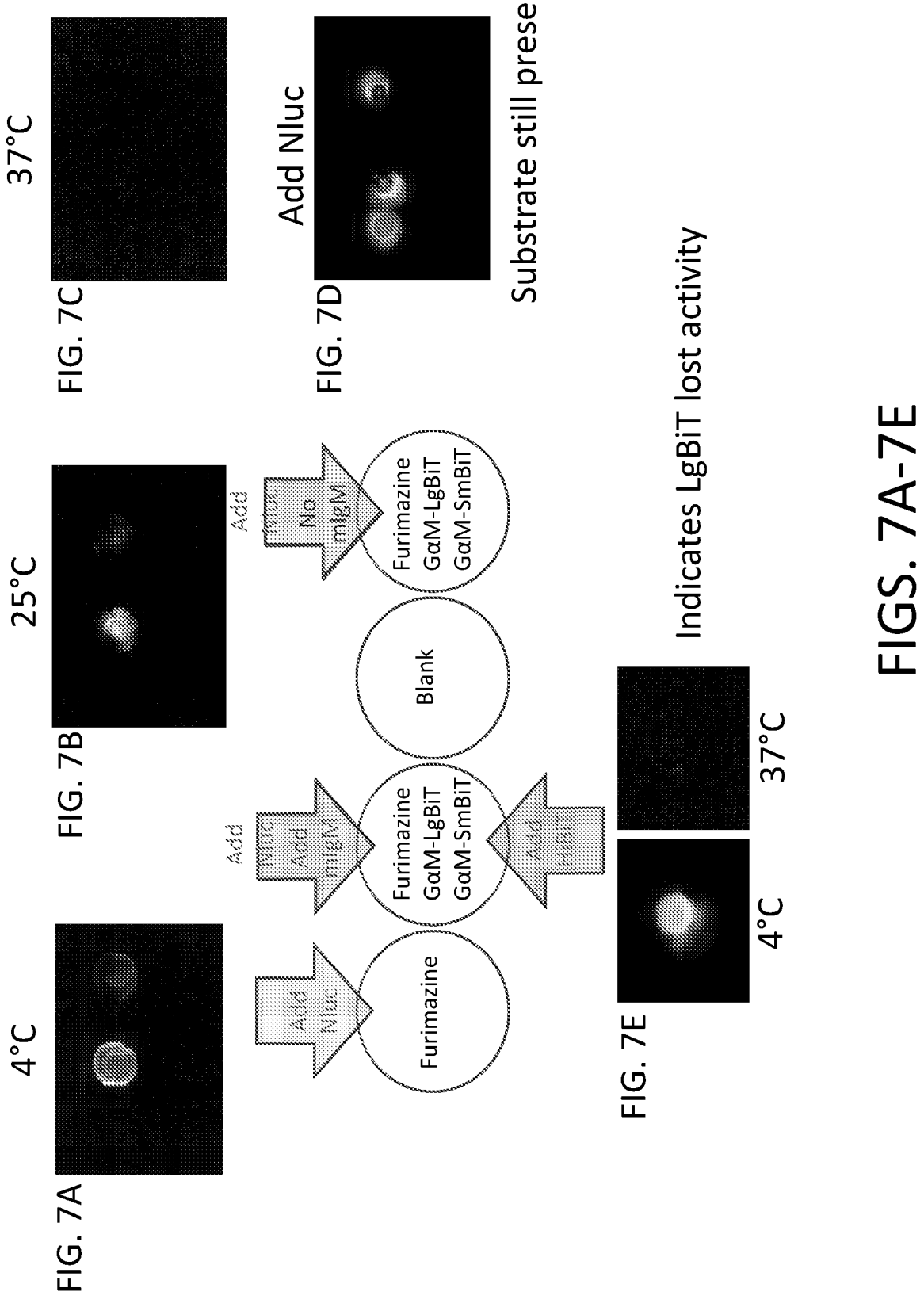


FIG. 7A FIG. 7B FIG. 7C FIG. 7D FIG. 7E

FIGS. 7A-7E

FIG. 8A

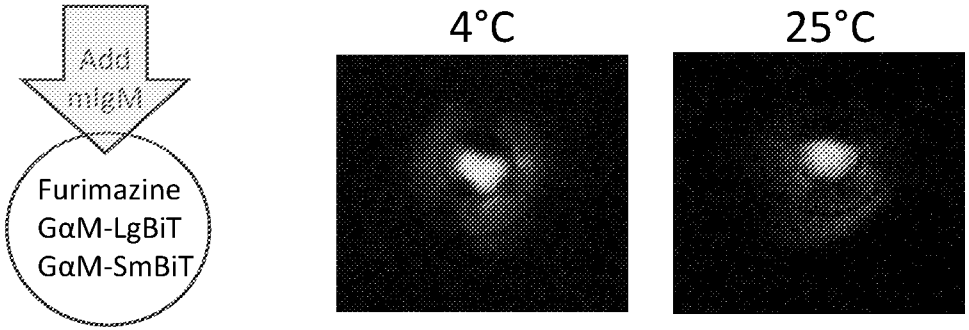
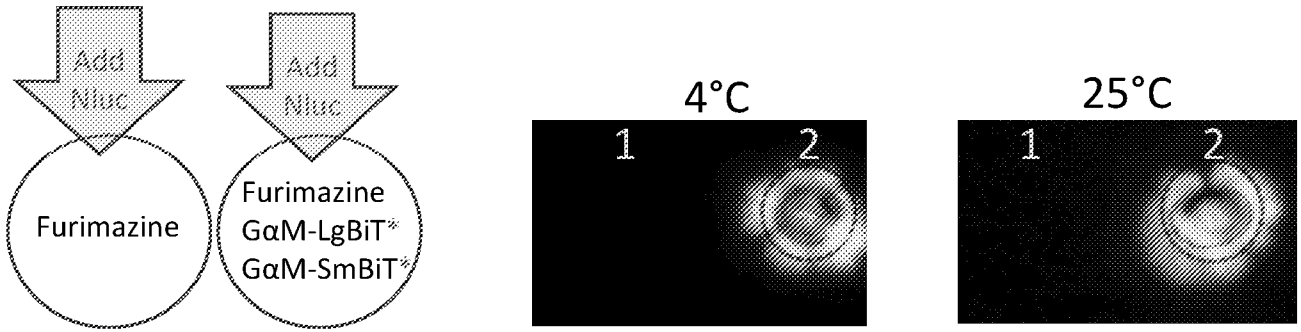


FIG. 8B



*Conjugation buffer: 20mM Na_3PO_4, 5% w/v BSA, 0.25% v/vtween20, 10% w/v sucrose

FIGS. 8A-8B

FIG. 9A

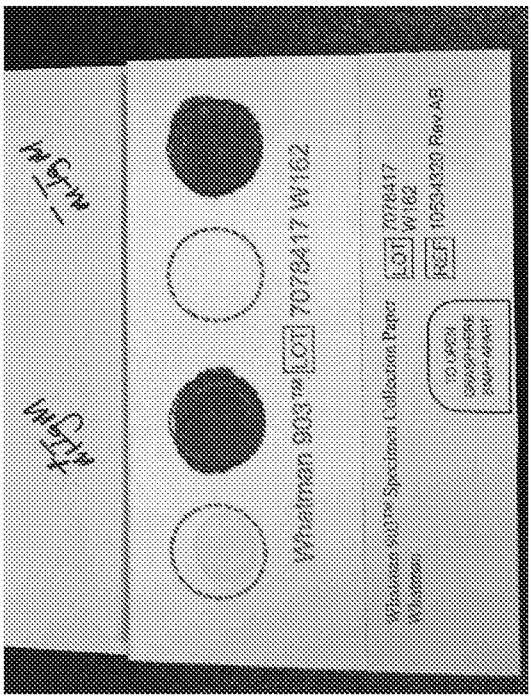


FIG. 9B

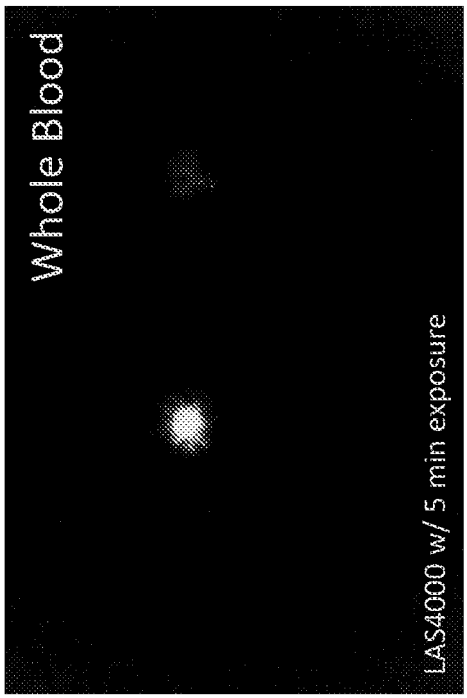
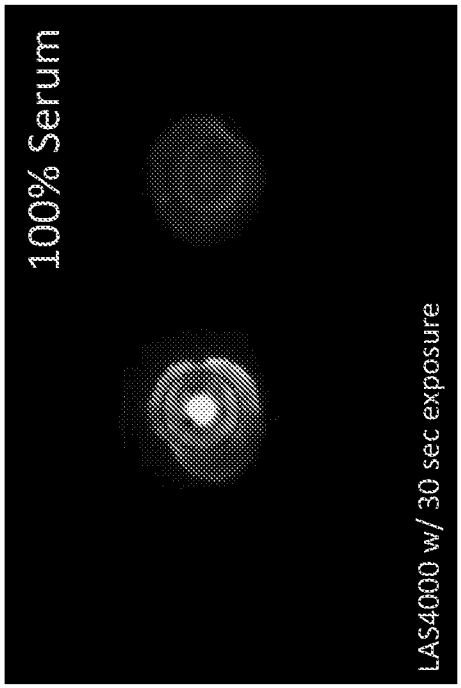
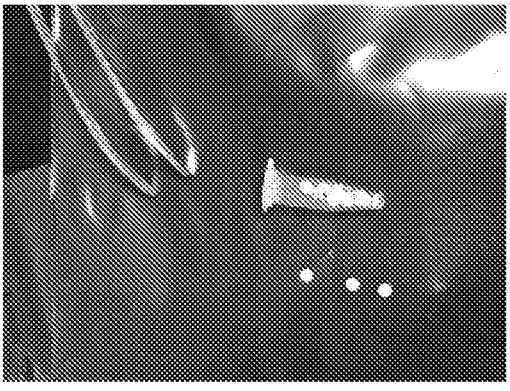


FIG. 9C



FIGS. 9A-9C

FIG. 10A



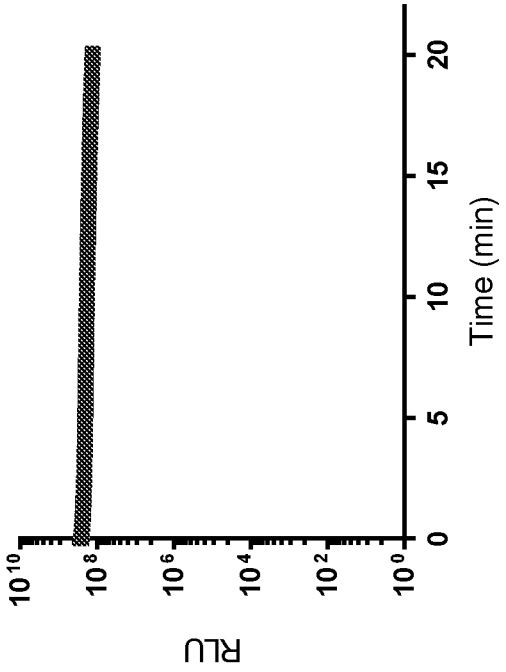
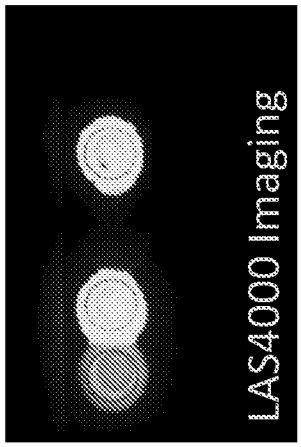


FIG. 10B



FIGS. 10A-10B

FIG. 11A

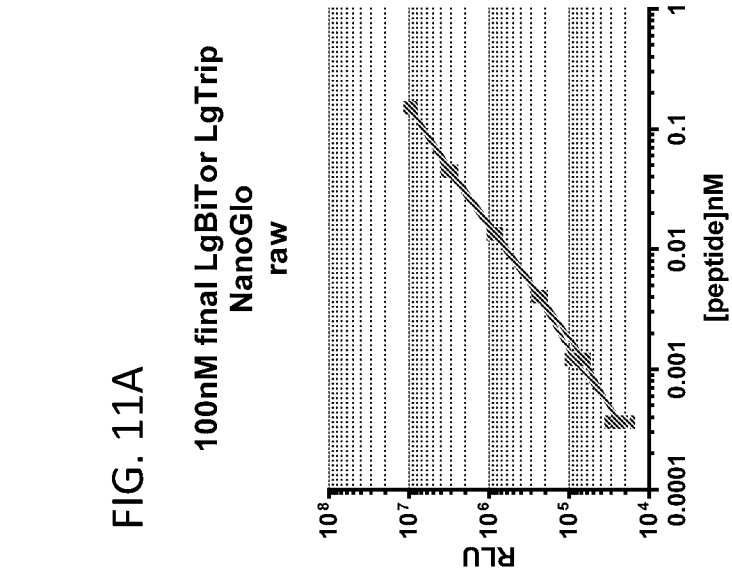
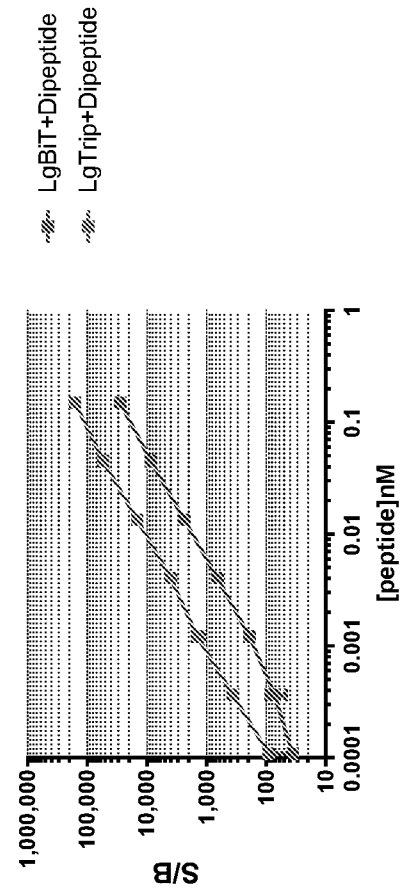


FIG. 11B



FIGS. 11A-11B

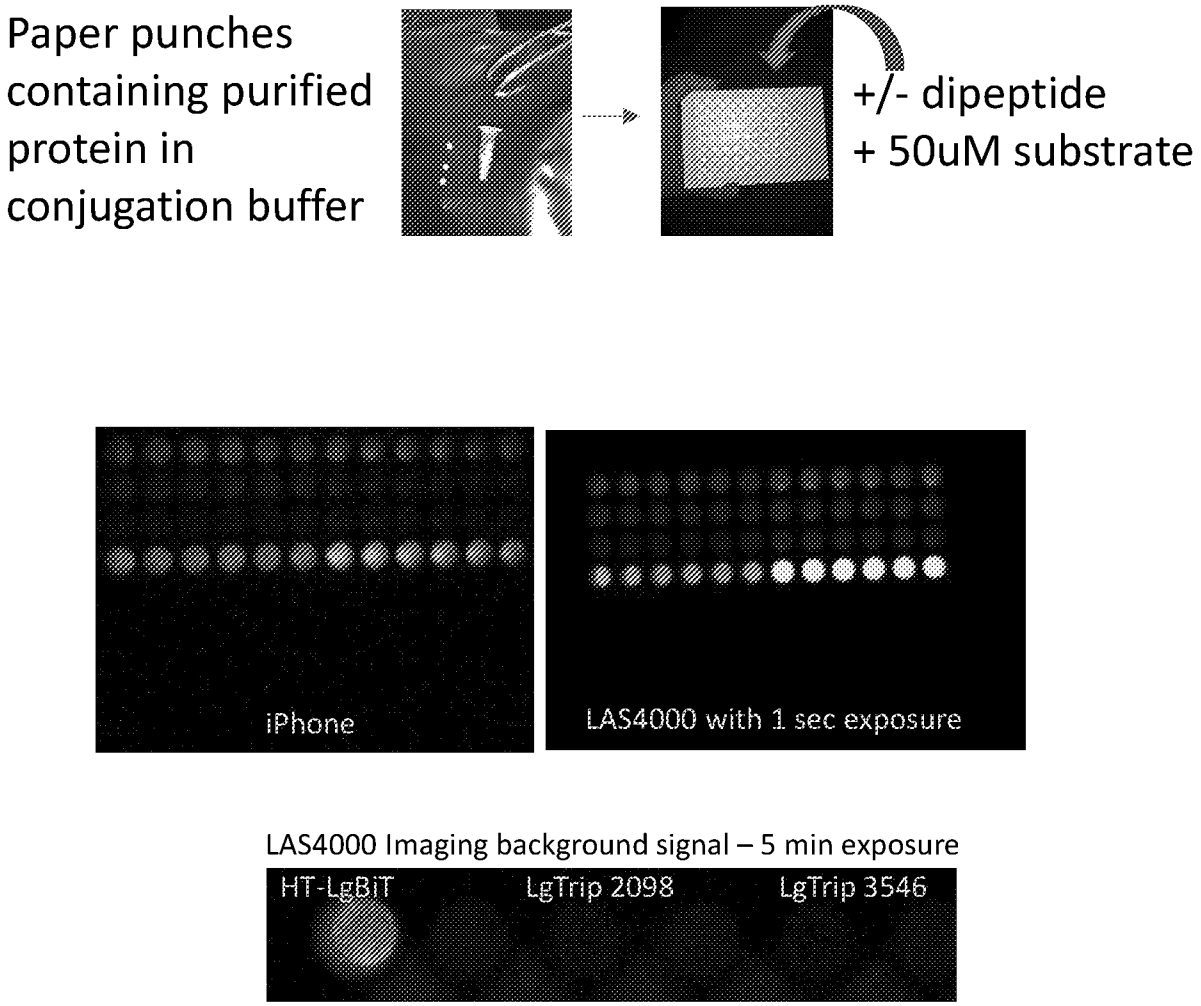

Paper punches containing purified protein in conjugation buffer
+/- dipeptide
+ 50uM substrate
iPhone
LAS4000 with 1 sec exposure
LAS4000 Imaging background signal – 5 min exposure
HT-LgBiT
LgTrip 2098
LgTrip 3546

FIG. 12

FIG. 13A

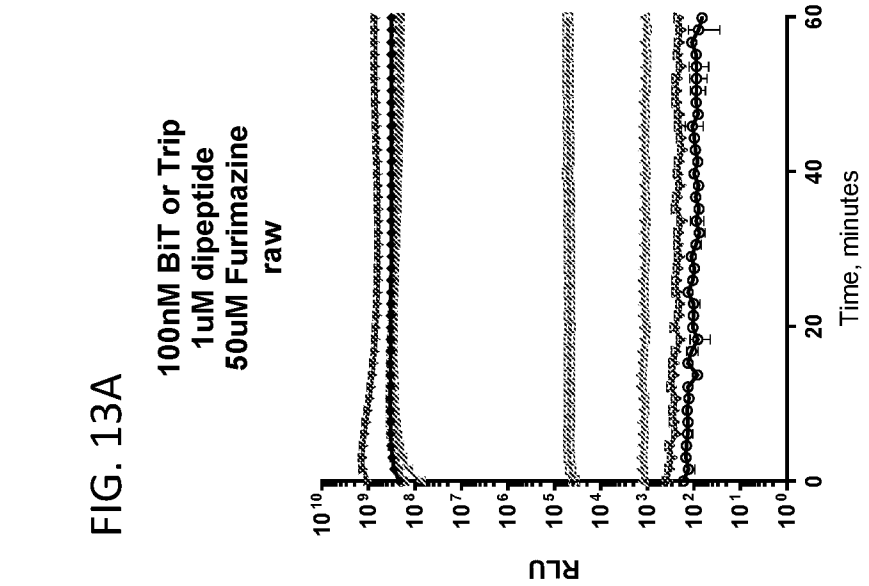
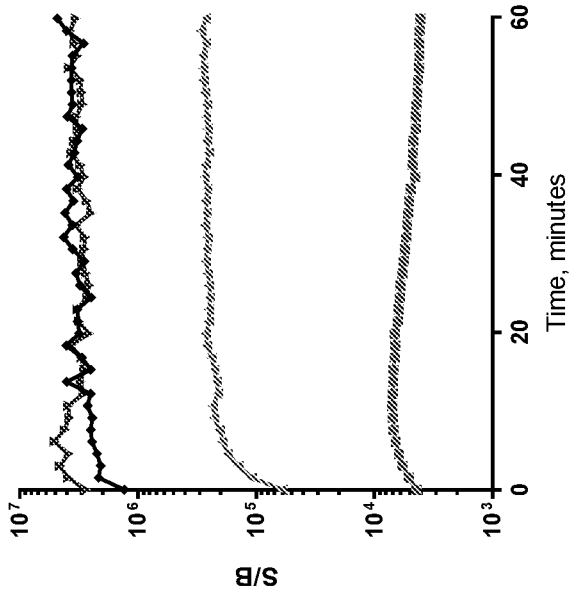


FIG. 13B



Day 3 at 25°C

FIGS. 13A-13B

FIG. 14A

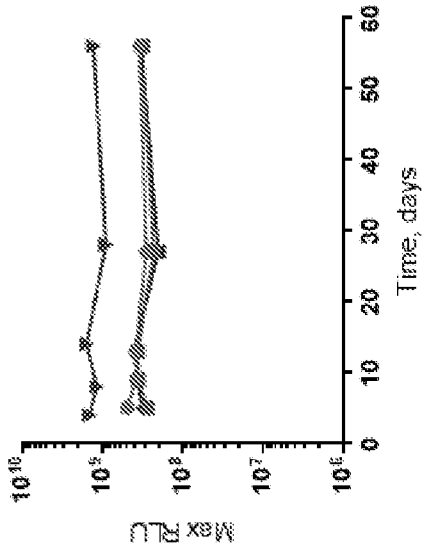


FIG. 14B

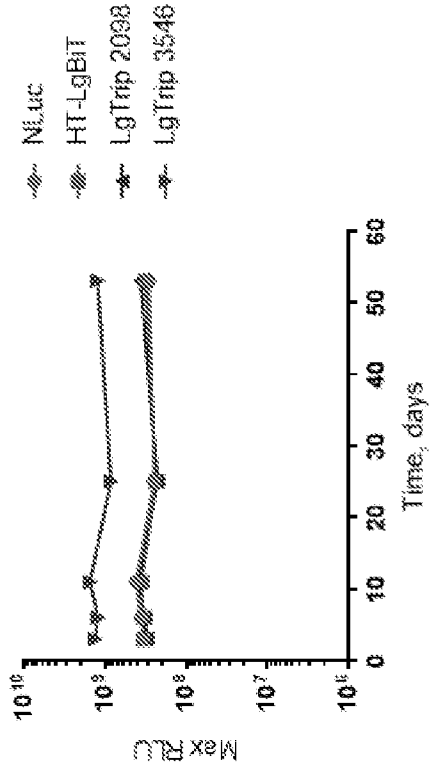
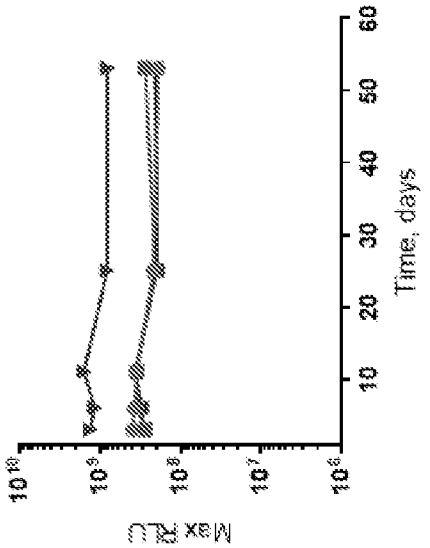


FIG. 14C



FIGS. 14A-14C

FIG. 15A

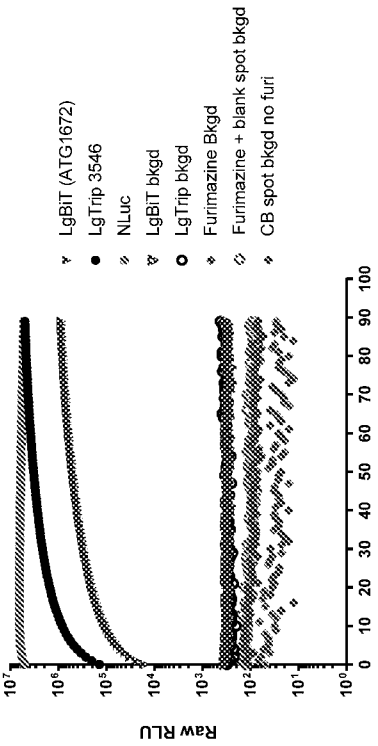


FIG. 15B

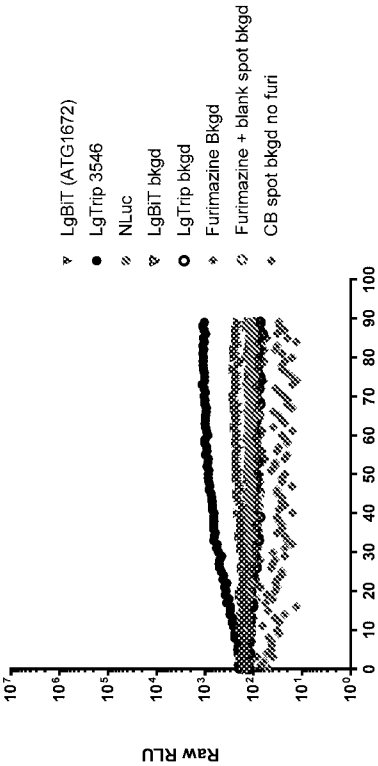


FIG. 15C

FIG. 15D

FIGS. 15A-15D

FIG. 16A

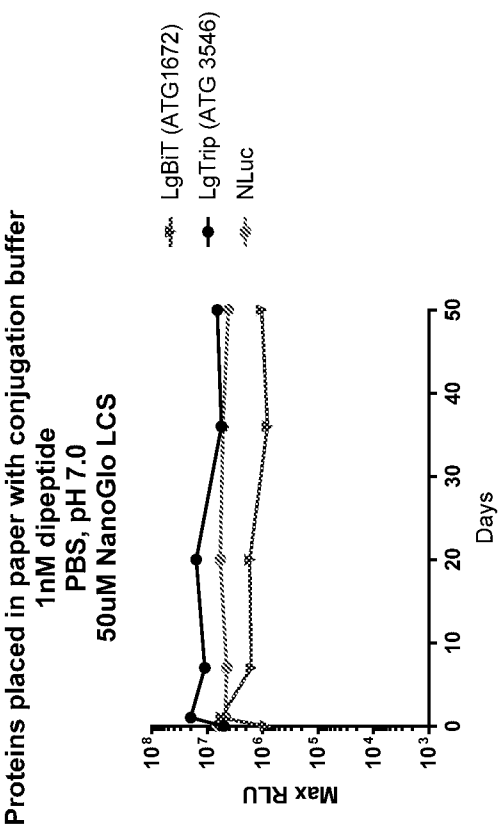
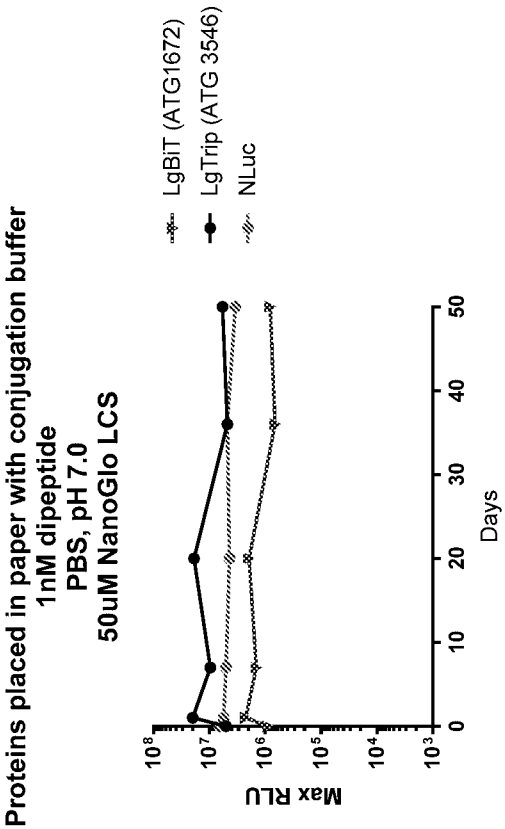


FIG. 16B



FIGS. 16A-16B

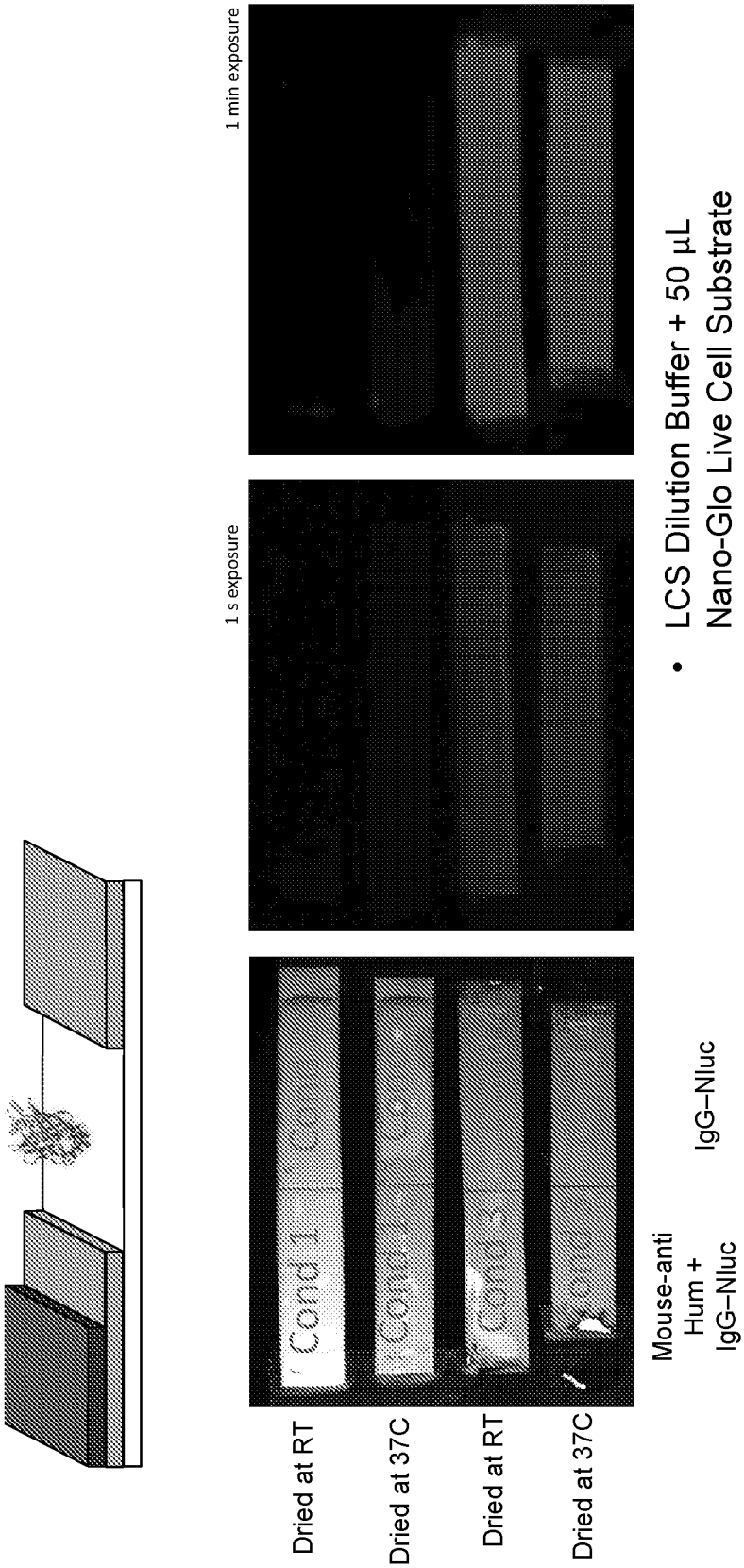

1 min exposure
1 s exposure
• LCS Dilution Buffer + 50 µL
Nano-Glo Live Cell Substrate
Cond 1
Cond 2
Cond 3
Cond 4
Dried at RT
Dried at 37C
Dried at RT
Dried at 37C
Mouse-anti Hum + IgG–Nluc
IgG–Nluc

FIG. 17

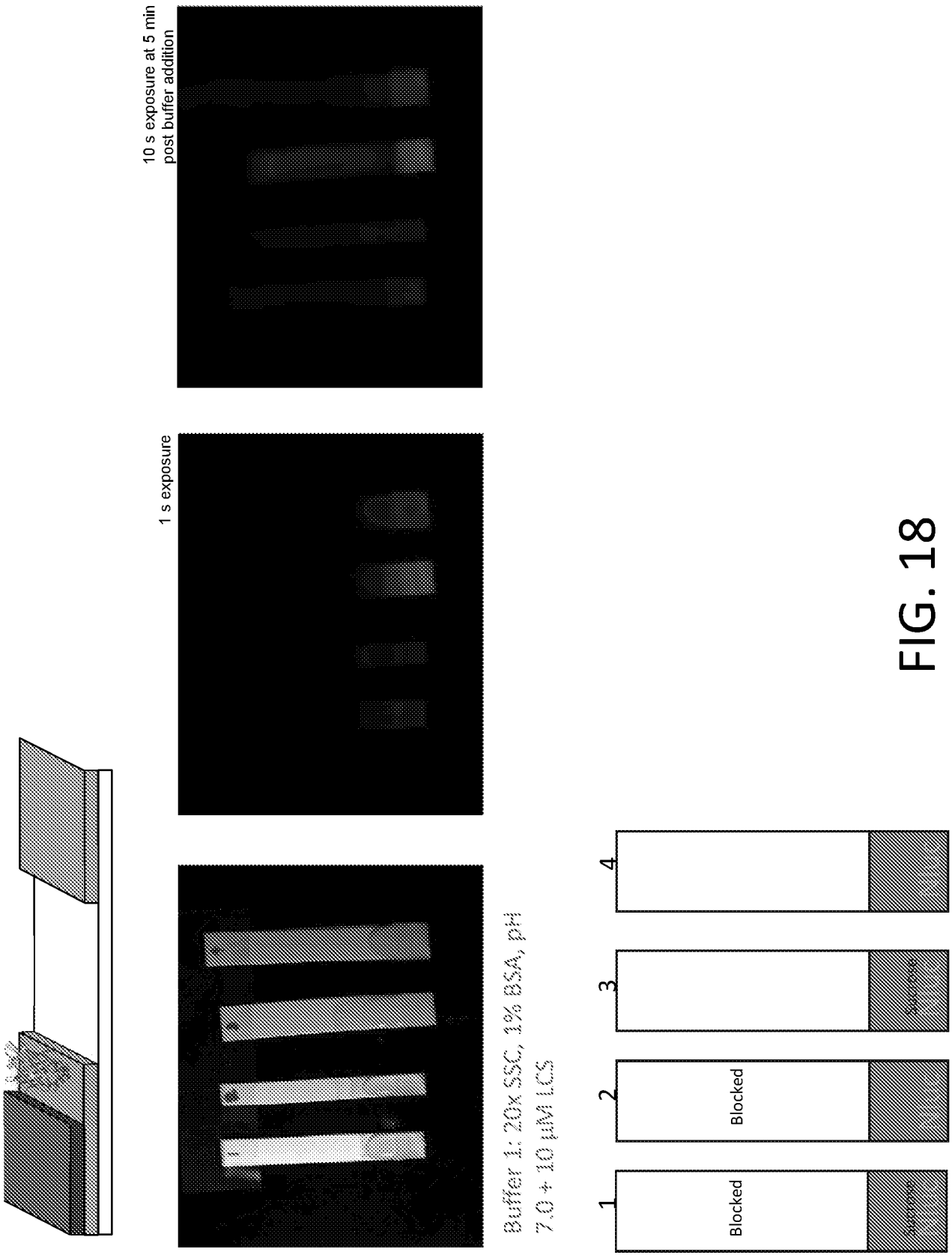

10 s exposure at 5 min post buffer addition
1 s exposure
Buffer 1: 20x SSC, 1% BSA, pH 7.0 + 10 μM LCS
1
2
3
4
Blocked
Blocked
Aptamer
Aptamer
Aptamer

FIG. 18

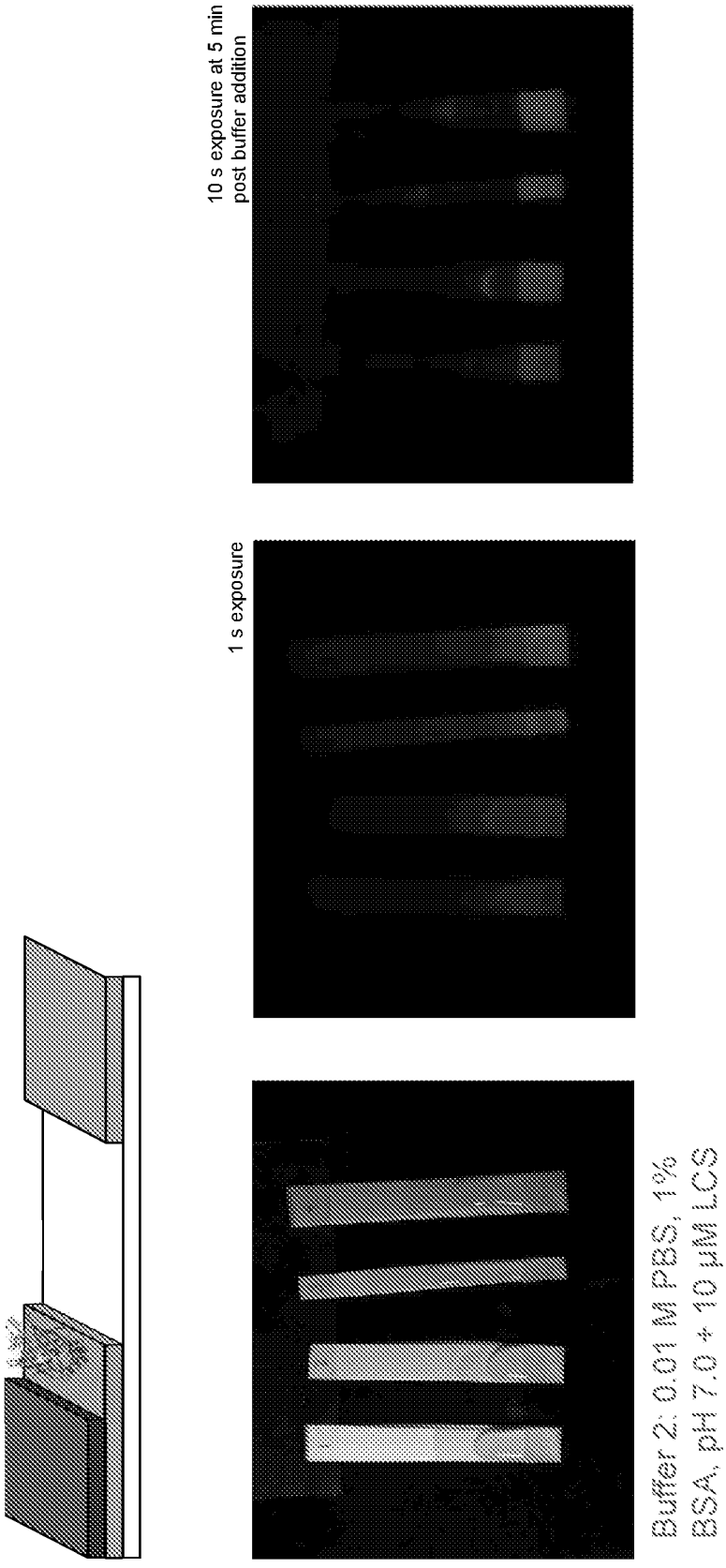

10 s exposure at 5 min post buffer addition
1 s exposure
Buffer 2: 0.01 M PBS, 1% BSA, pH 7.0 + 10 μM LCS

FIG. 19

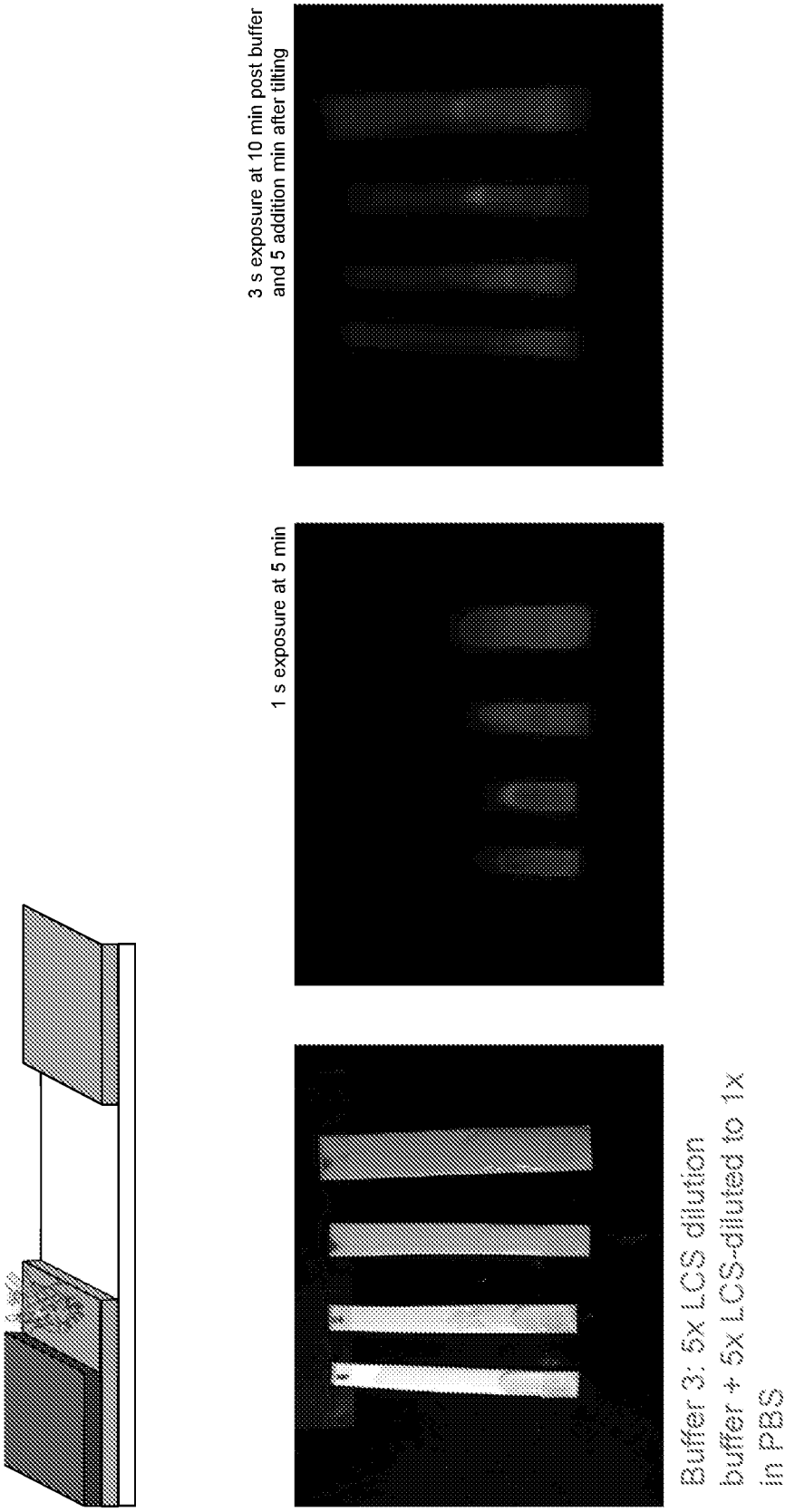
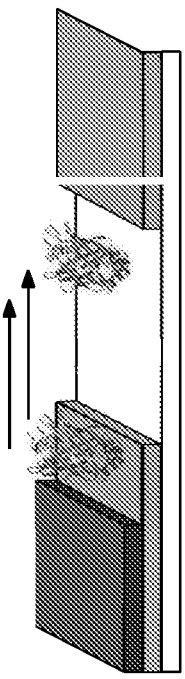

3 s exposure at 10 min post buffer and 5 addition min after tilting
1 s exposure at 5 min
Buffer 3: 5x LCS dilution buffer + 5x LCS-diluted to 1x in PBS

FIG. 20



Membrane type	Expected Rate
FF170HP	1.5 cm/min
HFC18002	1.3 cm/min
HFC13502	1.7 cm/min
HFC09002	2.6 cm/min
HFC12002	2.0 cm/min
HFC07502	3.2 cm/min
FF170HP	neg ctrl

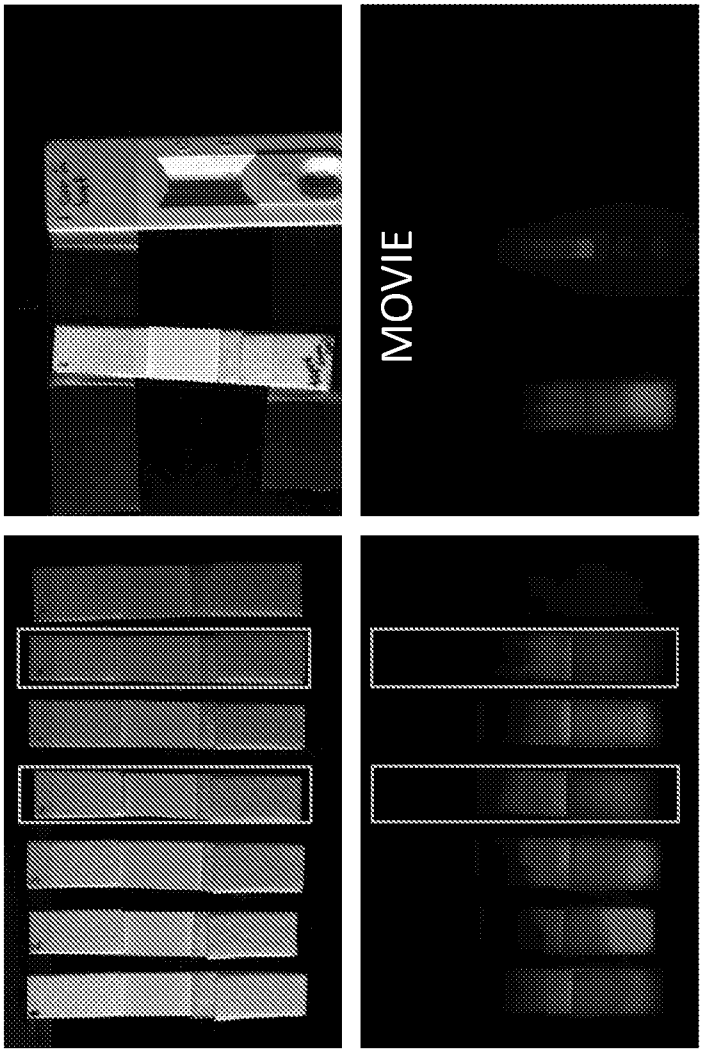


FIG. 21

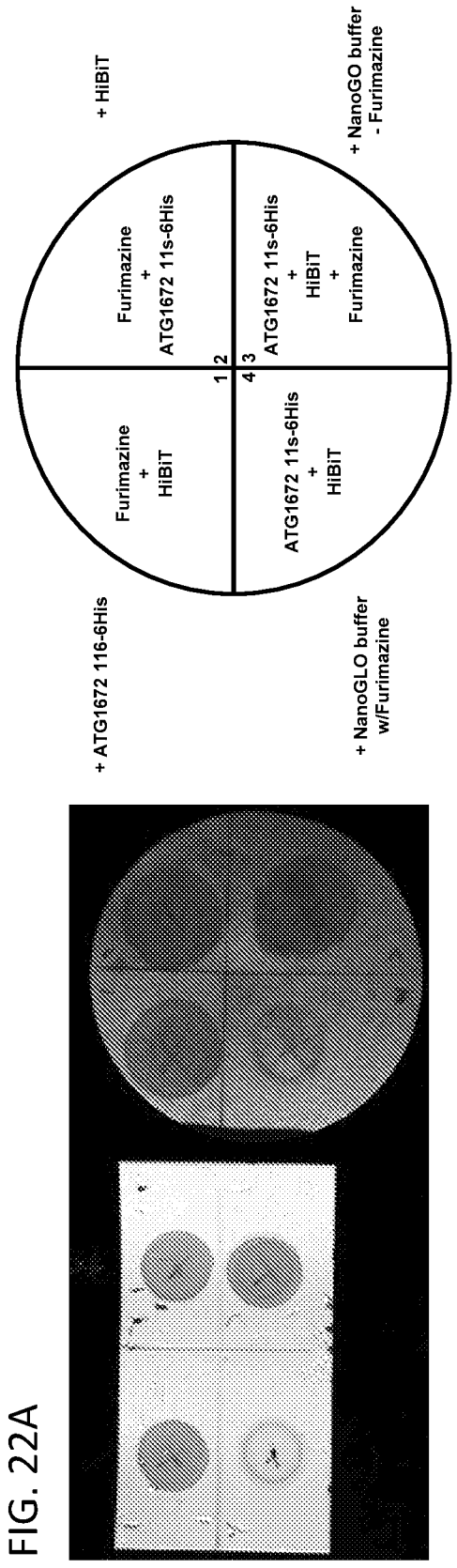


FIG. 22A

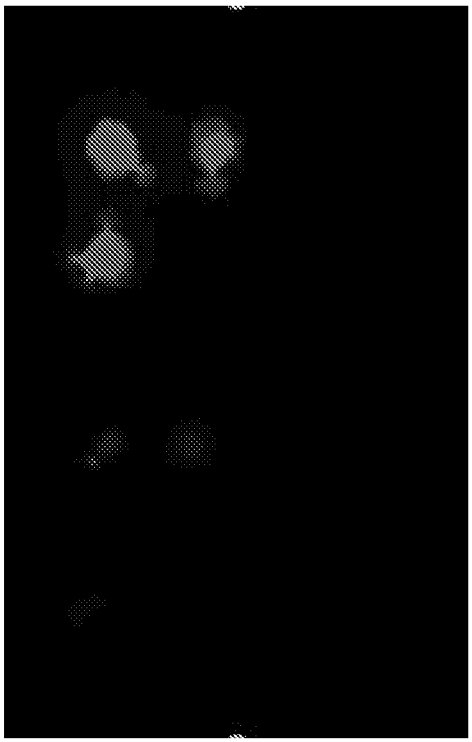


FIG. 22B

FIGS. 22A-22B

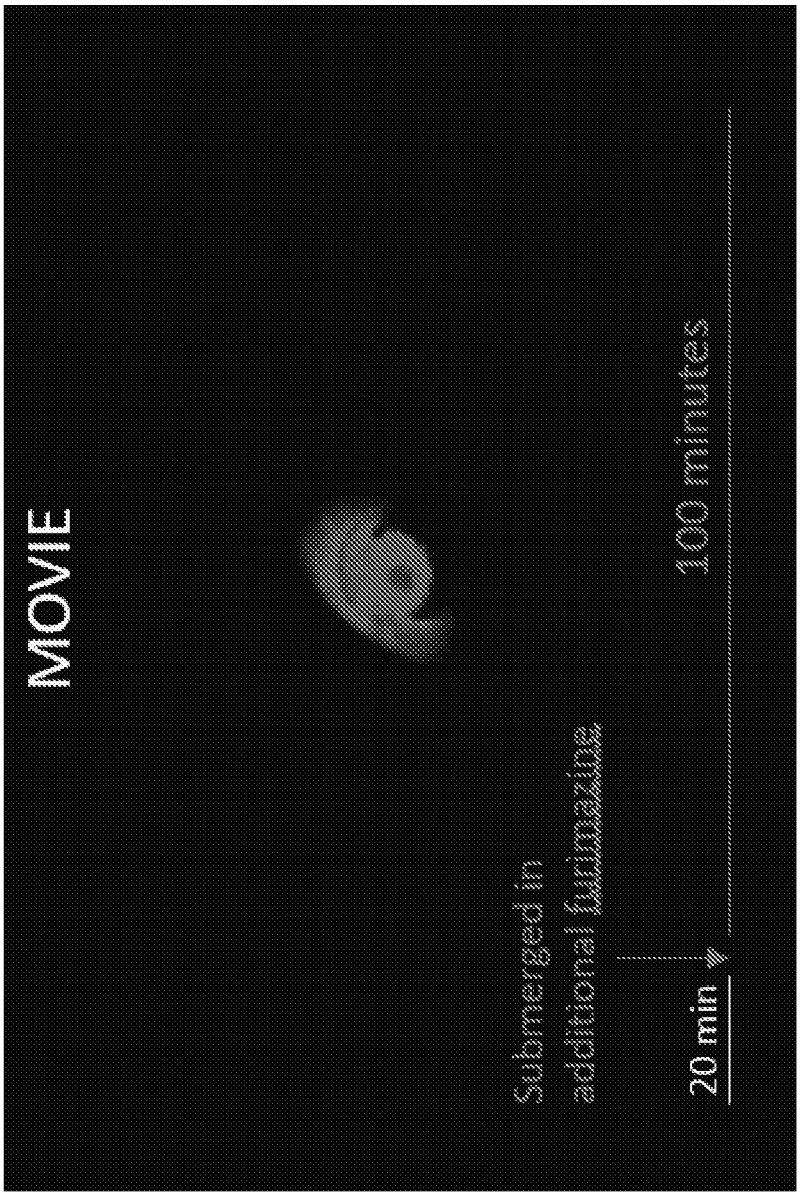

MOVIE
Submerged in
additional luminaire
20 min
100 minutes

FIG. 23

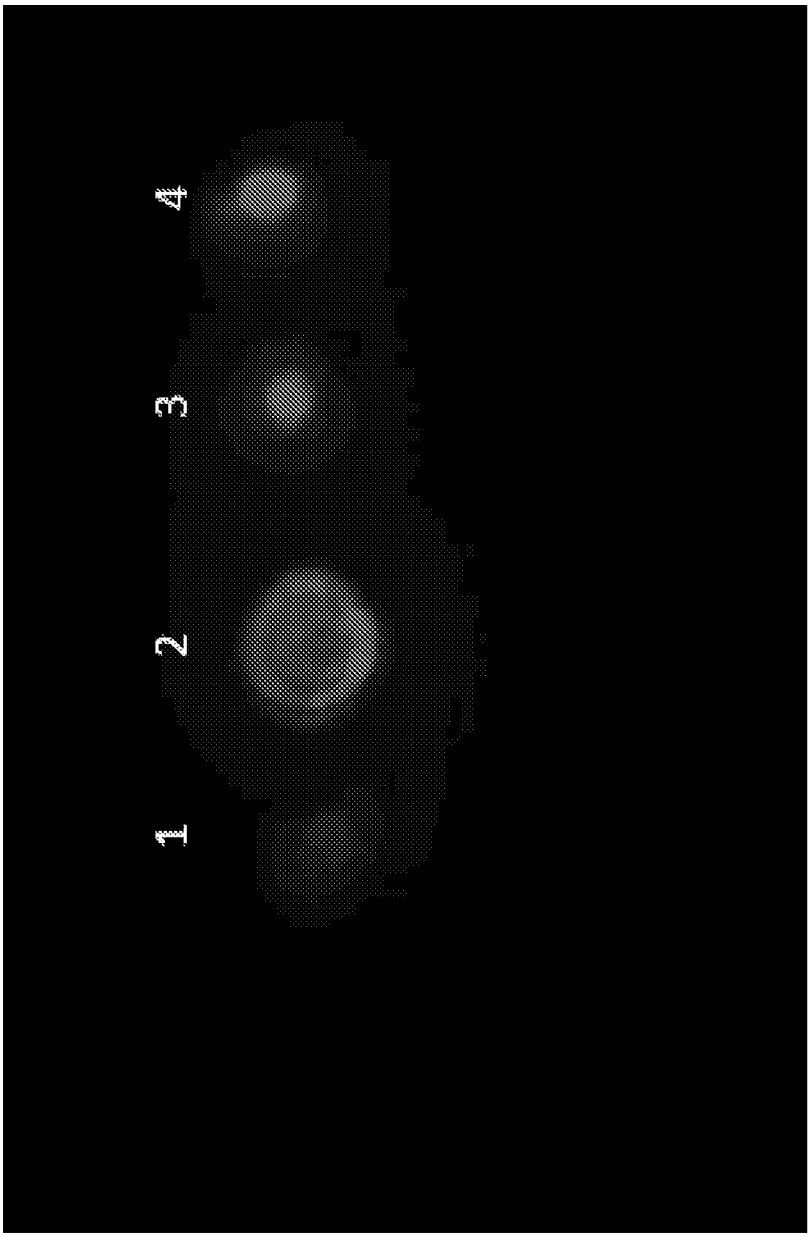


FIG. 24

FIG. 25A

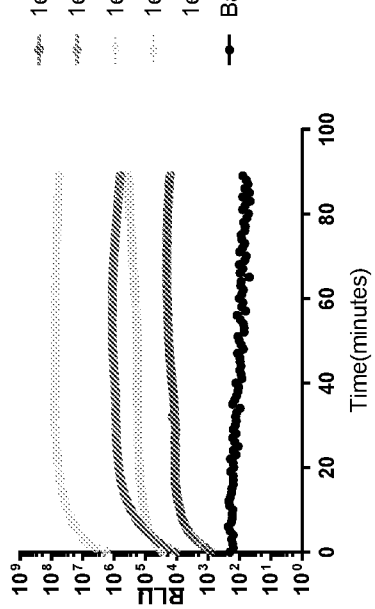


FIG. 25B

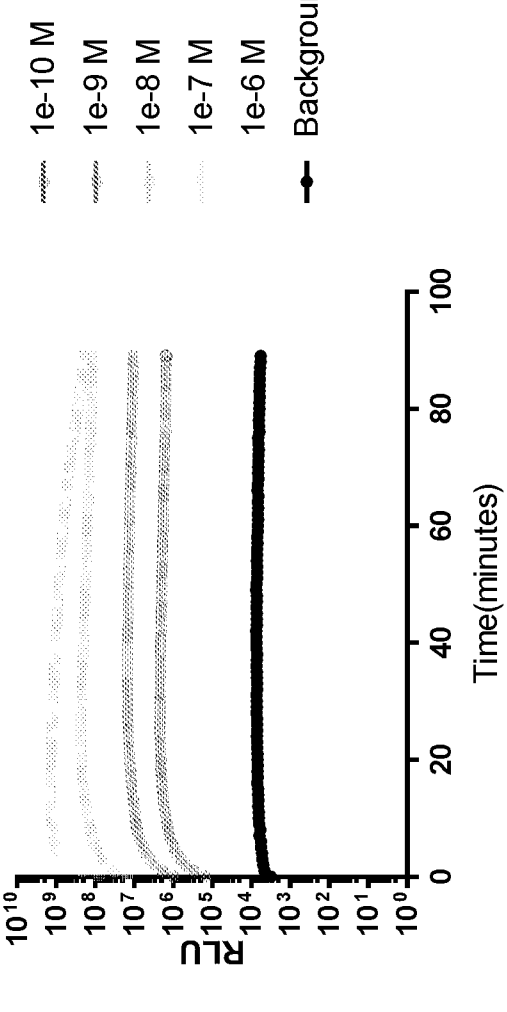
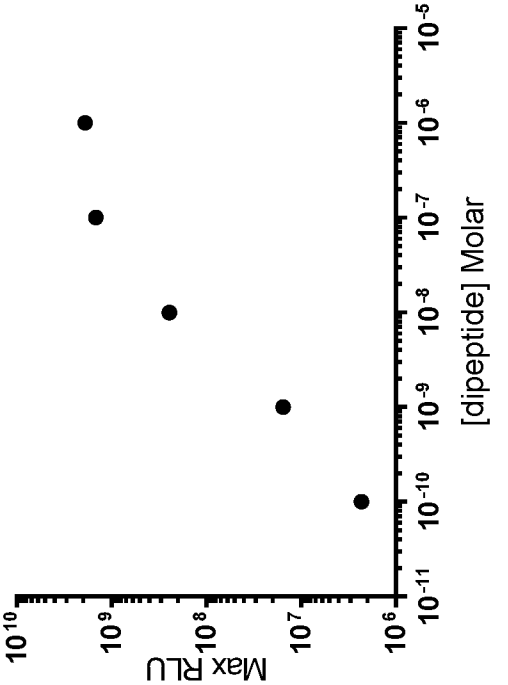


FIG. 25C



FIGS. 25A-25C

FIG. 26A
Reconstitution of LgTrip + substrate lyocake

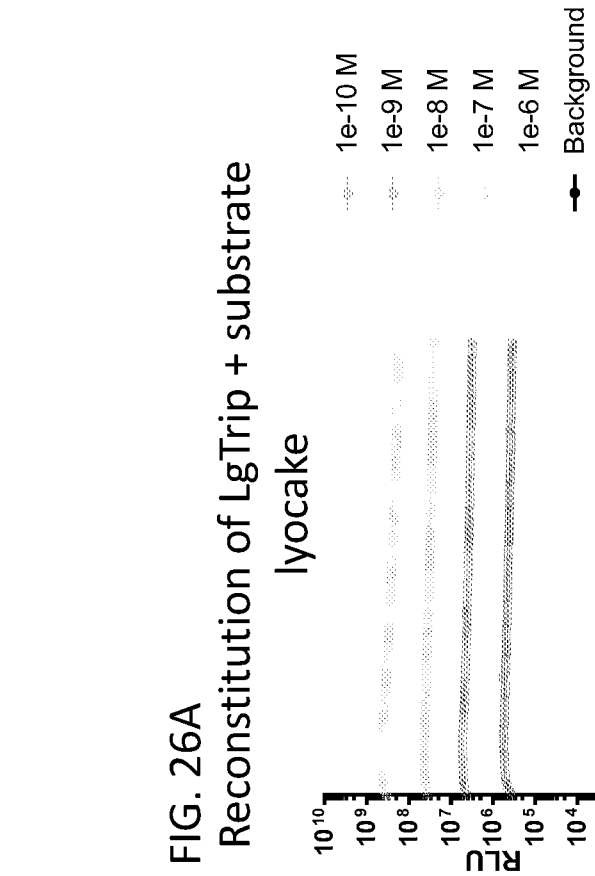
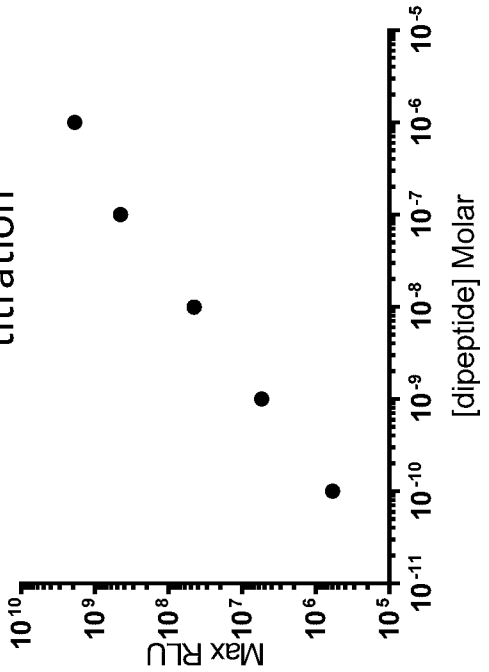


FIG. 26B
Summary of Fig 26A dipeptide titration



FIGS. 26A-26B

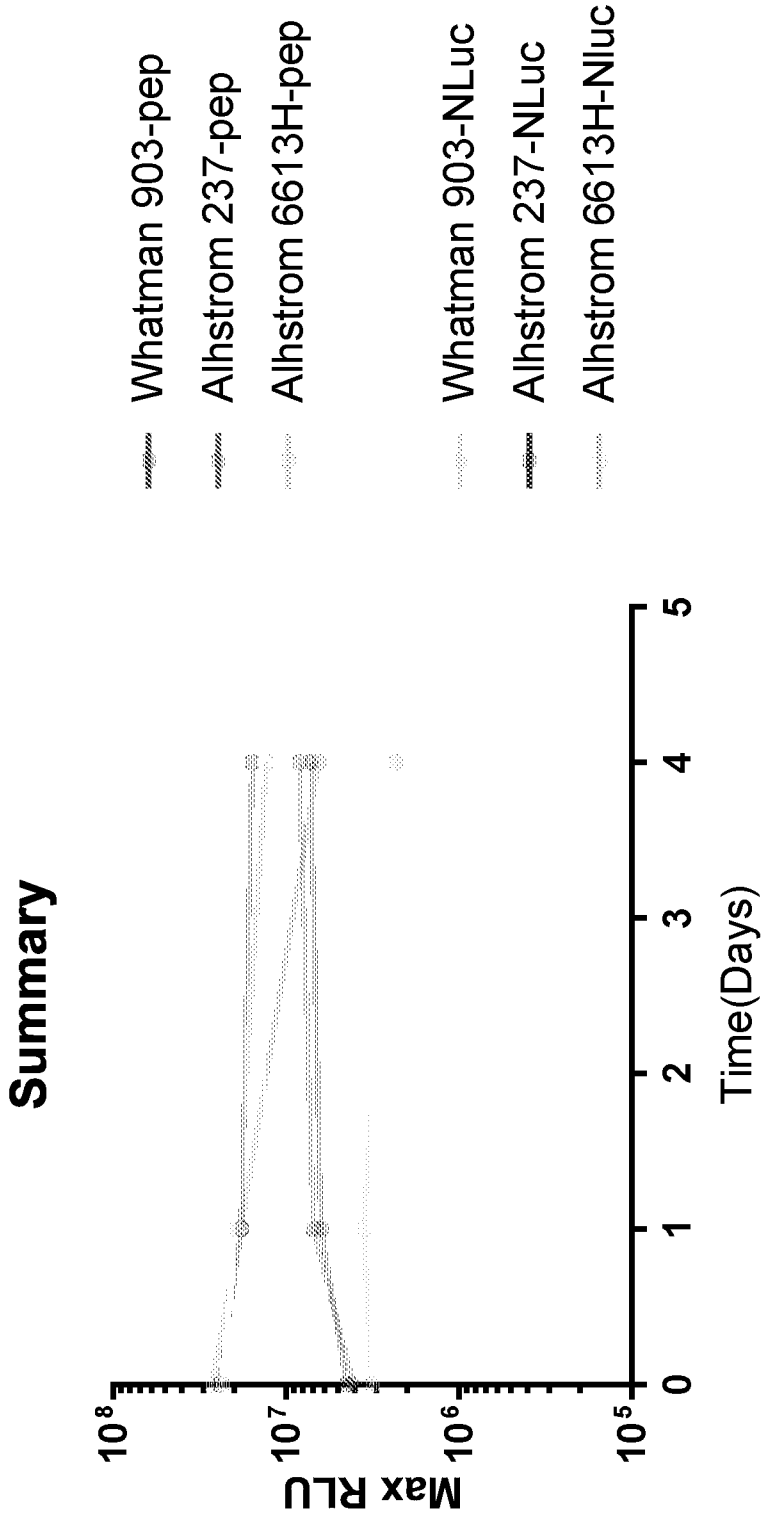

Summary
Whatman 903-pep
Alhstrom 237-pep
Alhstrom 6613H-pep
Whatman 903-NLuc
Alhstrom 237-NLuc
Alhstrom 6613H-Nluc
Max RLU
10^8
10^7
10^6
10^5
0
1
2
3
4
5
Time(Days)

FIG. 27

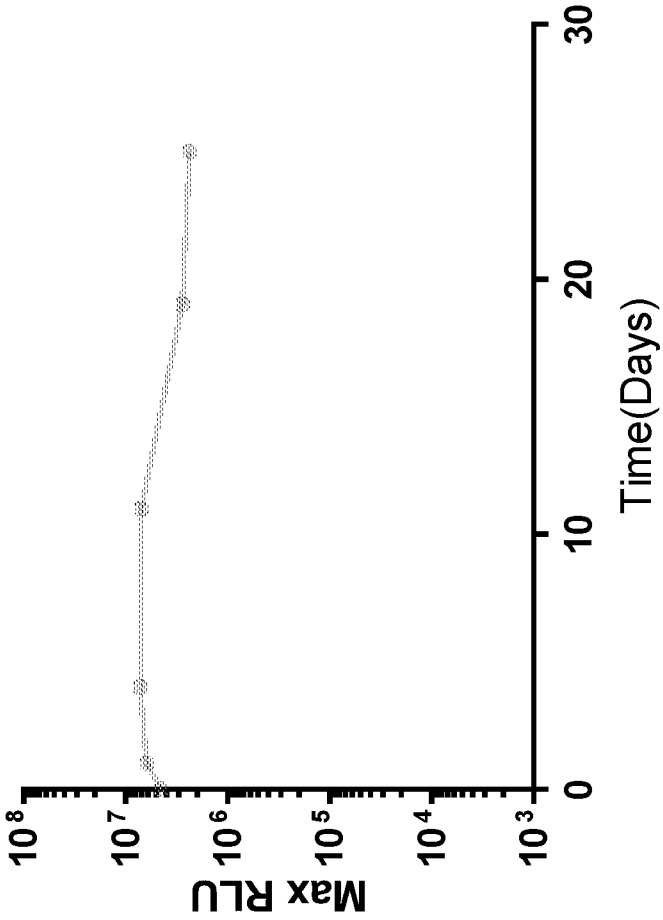


FIG. 28

FIG. 29A

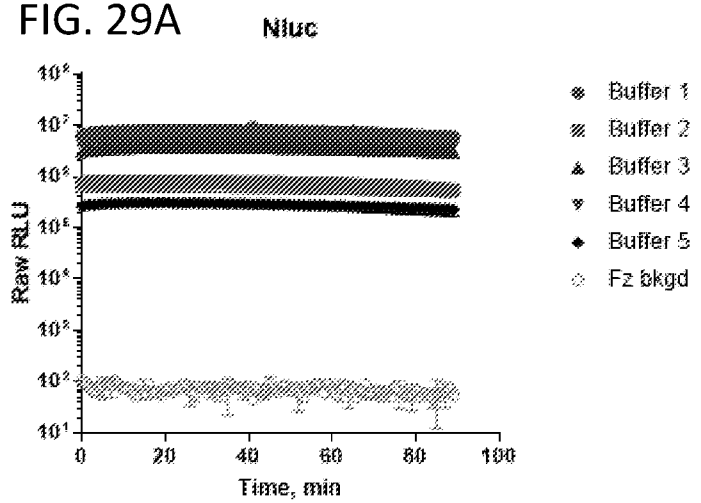


FIG. 29B

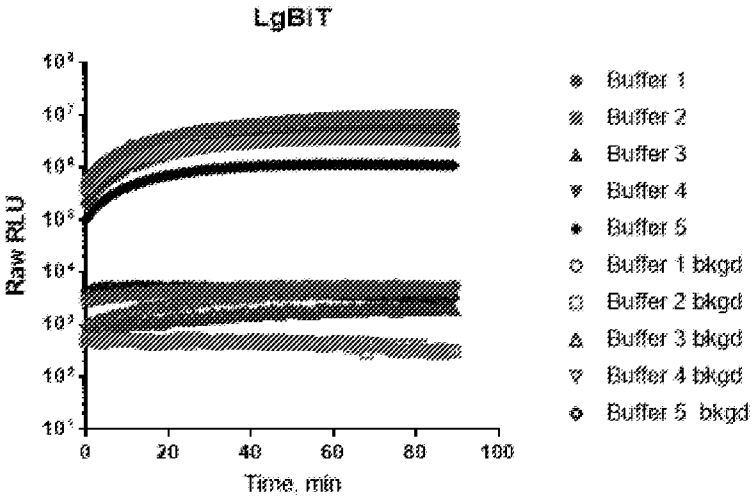
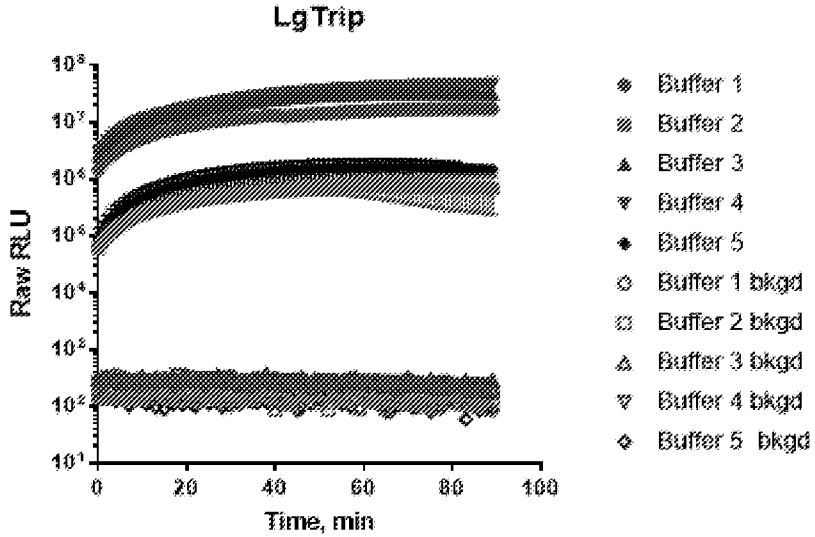


FIG. 29C



FIGS. 29A-29C

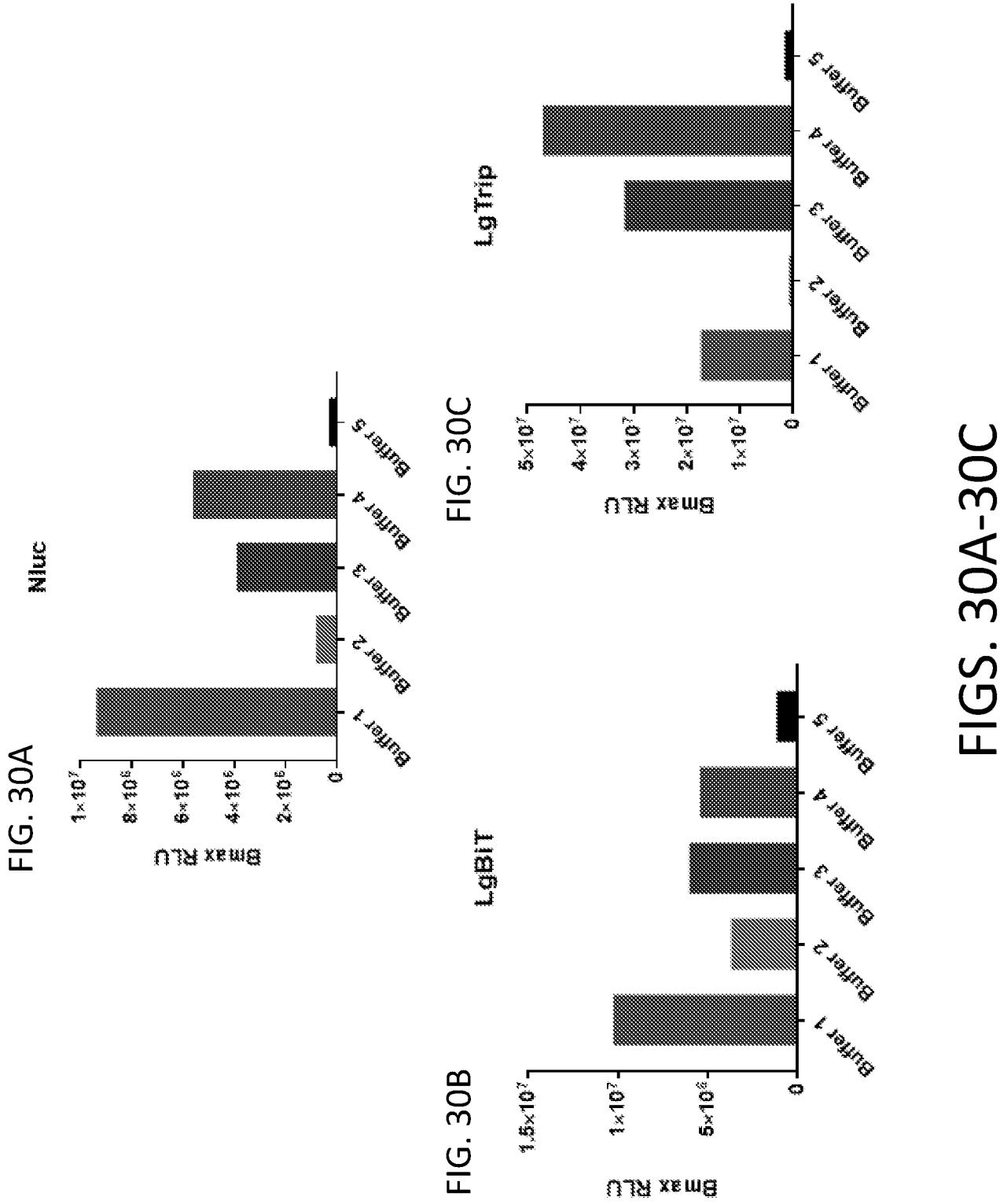


FIG. 30A
Nluc
Bmax RLU
1×10^7
8×10^6
6×10^6
4×10^6
2×10^6
0
Buffer 1
Buffer 2
Buffer 3
Buffer 4
Buffer 5
FIG. 30B
LgBiT
Bmax RLU
1.5×10^7
1×10^7
5×10^6
0
Buffer 1
Buffer 2
Buffer 3
Buffer 4
Buffer 5
FIG. 30C
LgTrip
Bmax RLU
5×10^7
4×10^7
3×10^7
2×10^7
1×10^7
0
Buffer 1
Buffer 2
Buffer 3
Buffer 4
Buffer 5

FIGS. 30A-30C

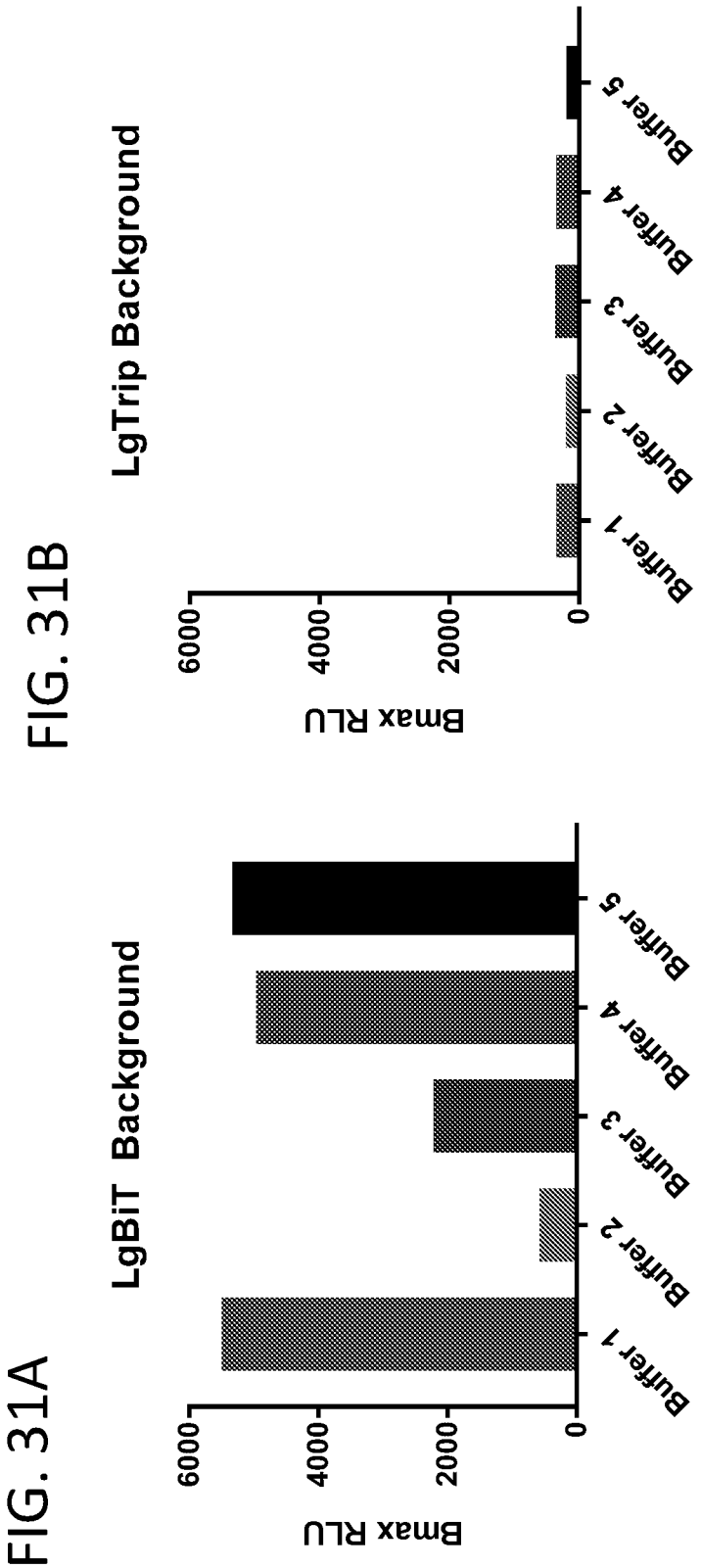


FIG. 31A

FIG. 31B

FIGS. 31A-31B

FIG. 32A

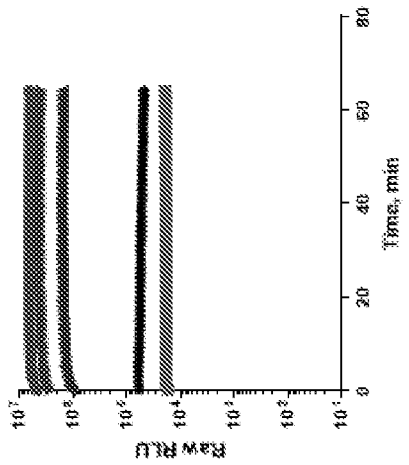


FIG. 32B

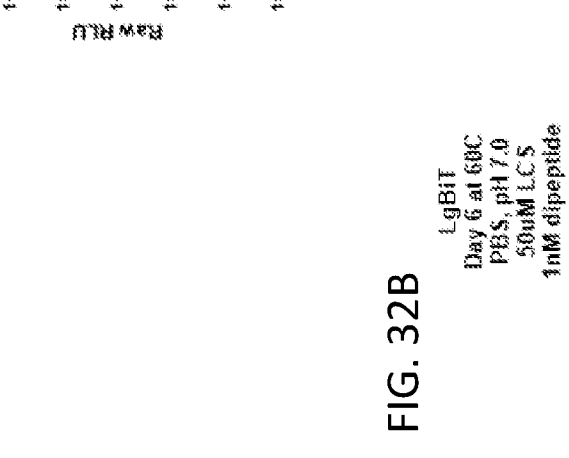
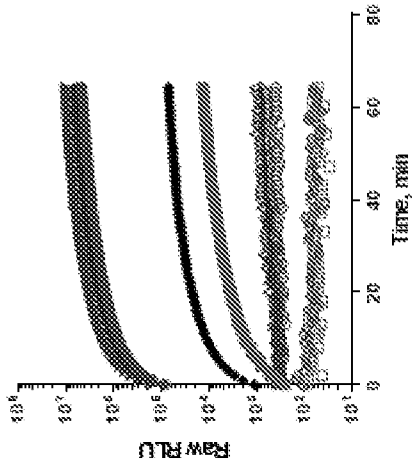


FIG. 32C



FIGS. 32A-32F

FIG. 32D

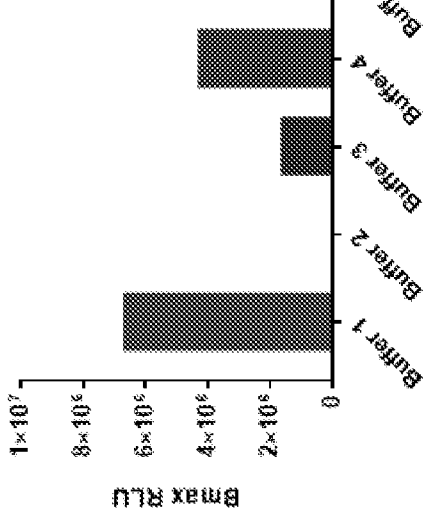


FIG. 32E

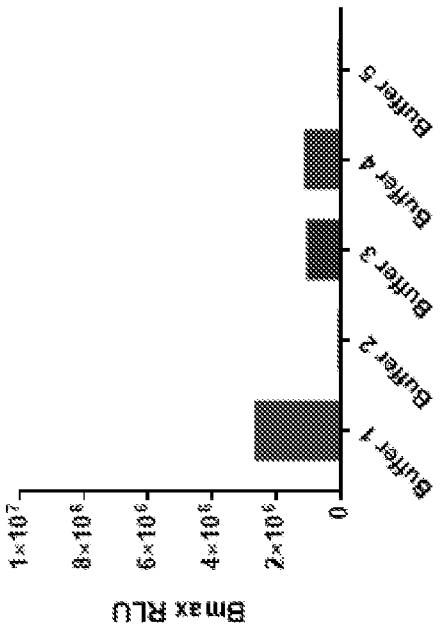
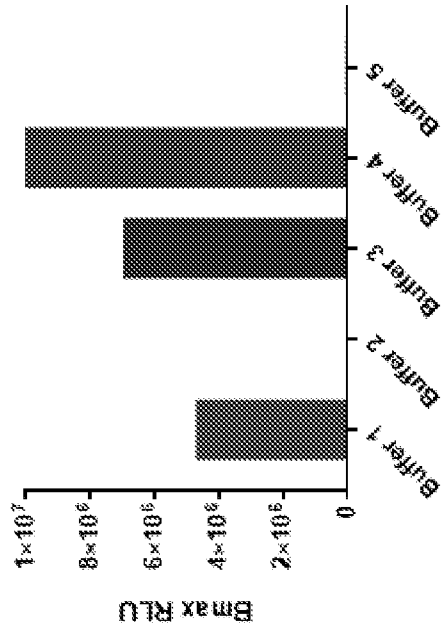


FIG. 32F



FIGS. 32A-32F

Tablet or lyocake embodiments containing all assay components that can be used in cuvettes, test tubes, bottles, snap test type formats, etc.

FIG. 33

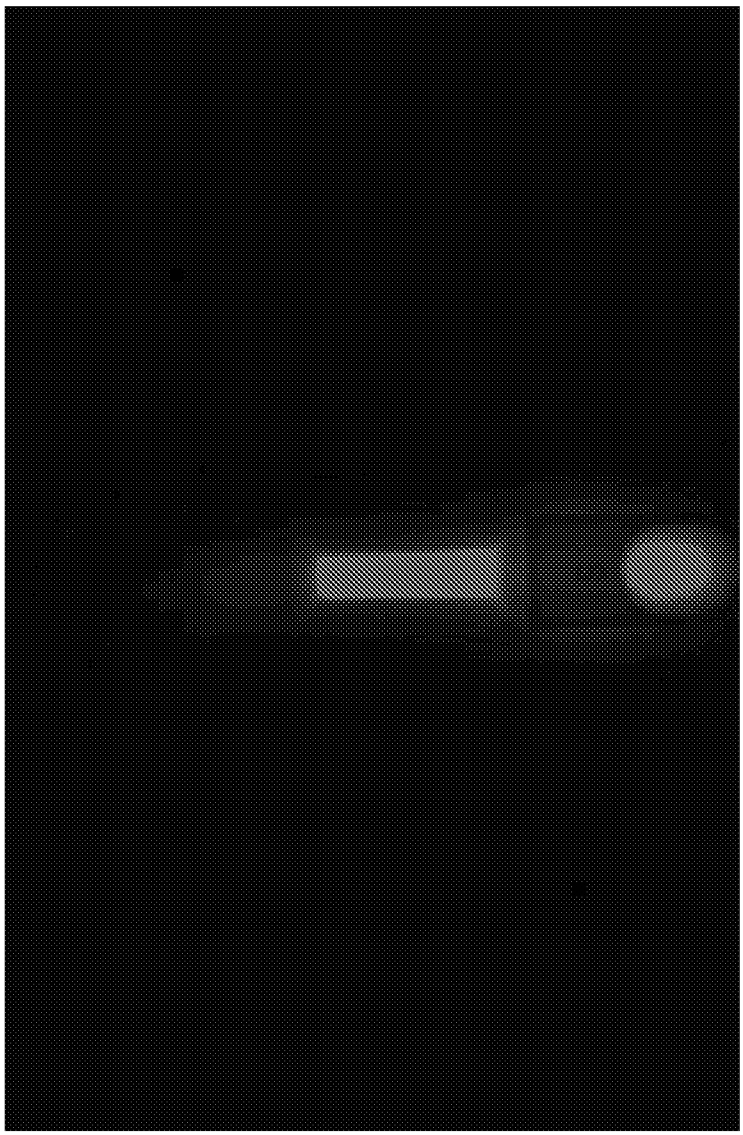


FIG. 34

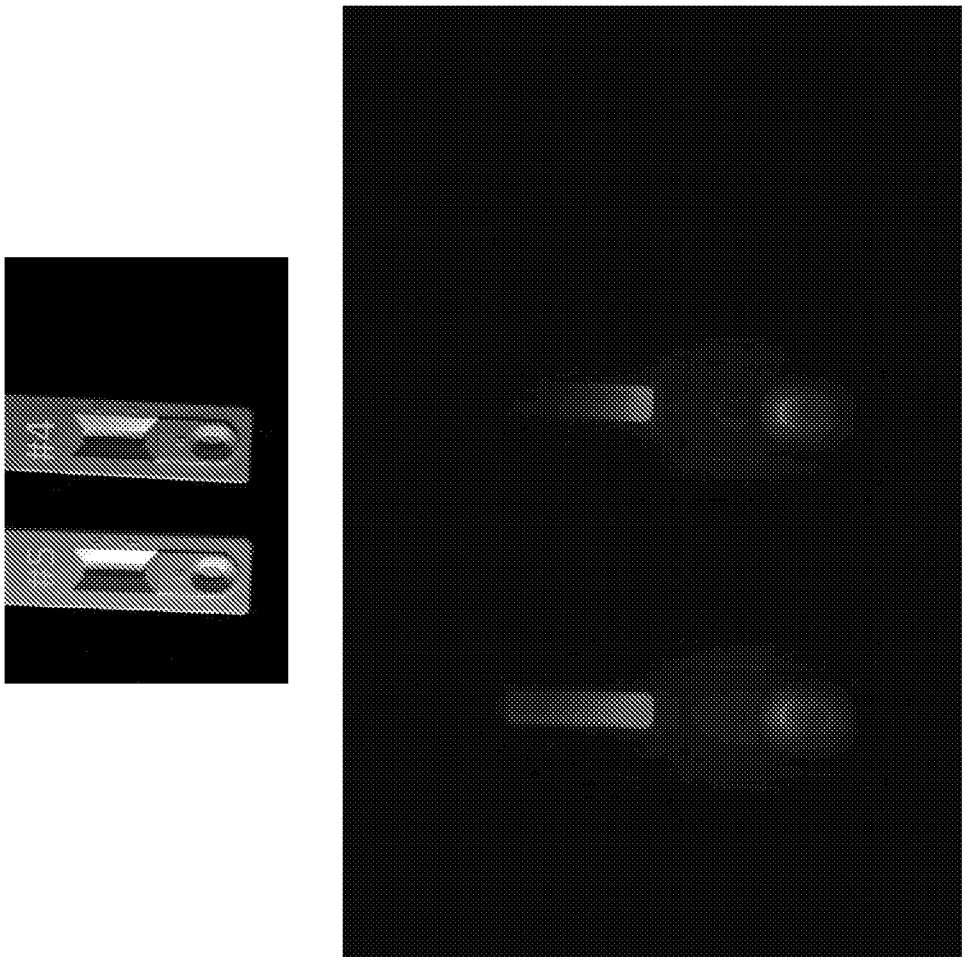


FIG. 35

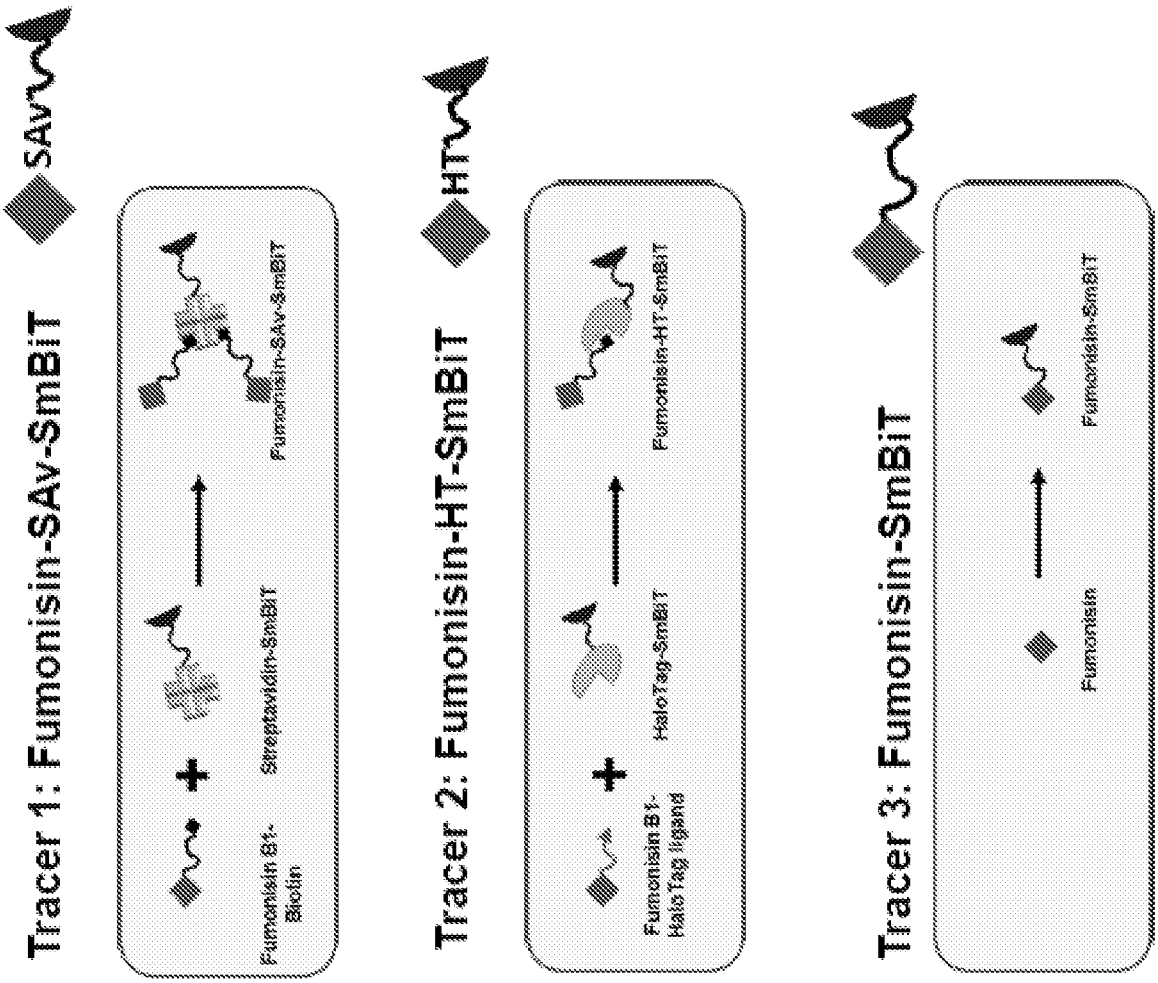

Tracer 1: Fumonisin-SAv-SmBiT
SAv
Fumonisin B1-Biotin
Streptavidin-SmBiT
Fumonisin-SAv-SmBiT
Tracer 2: Fumonisin-HT-SmBiT
HT
Fumonisin B1-HaloTag ligand
HaloTag-SmBiT
Fumonisin-HT-SmBiT
Tracer 3: Fumonisin-SmBiT
Fumonisin
Fumonisin-SmBiT

FIG. 36

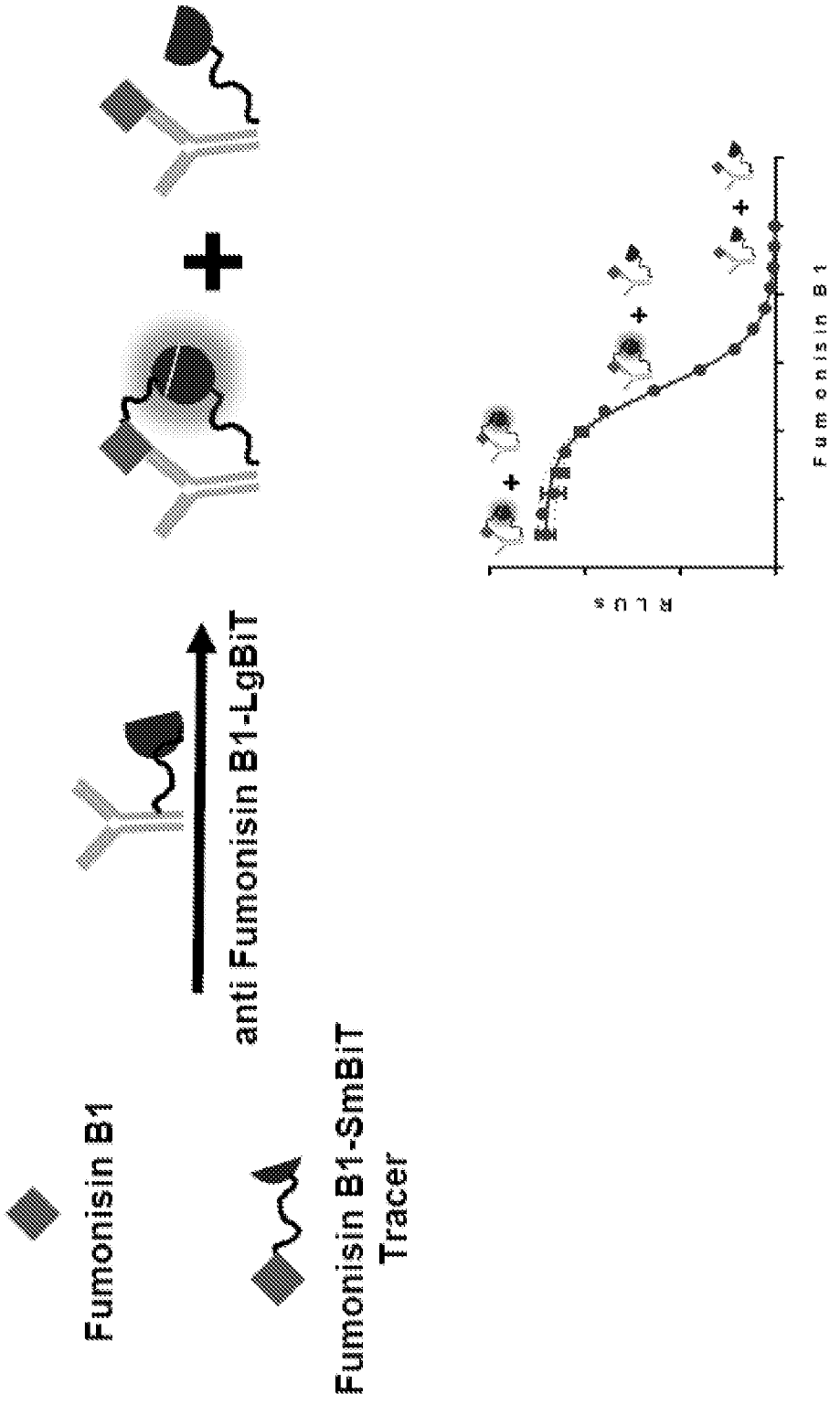

Fumonisin B1
anti Fumonisin B1-LgBiT
Fumonisin B1-SmBiT
Tracer
RLUs
Fumonisin B1

FIG. 37

FIG. 38A

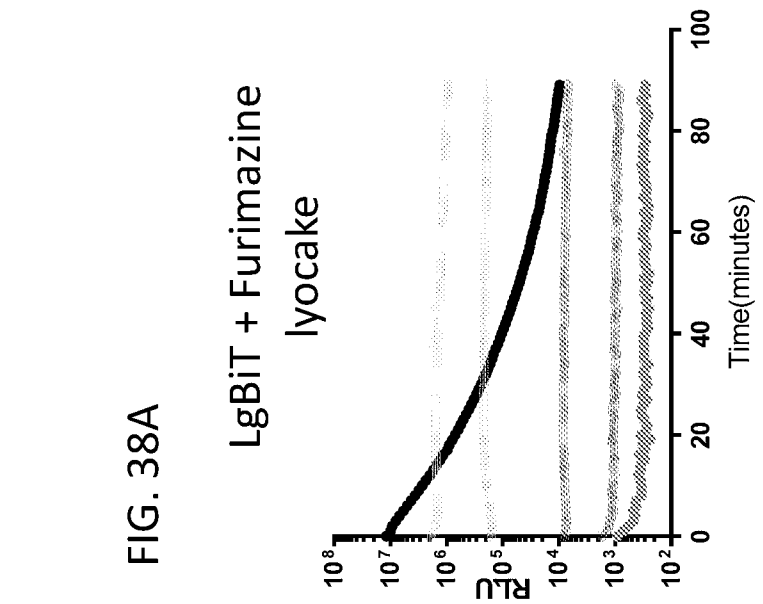
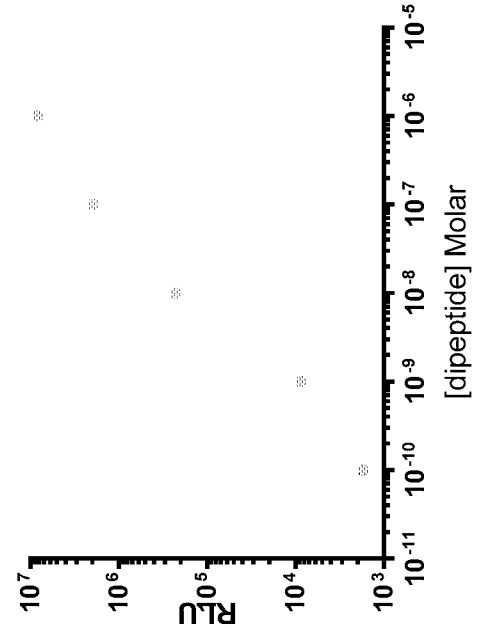


FIG. 38B



FIGS. 38A-38B

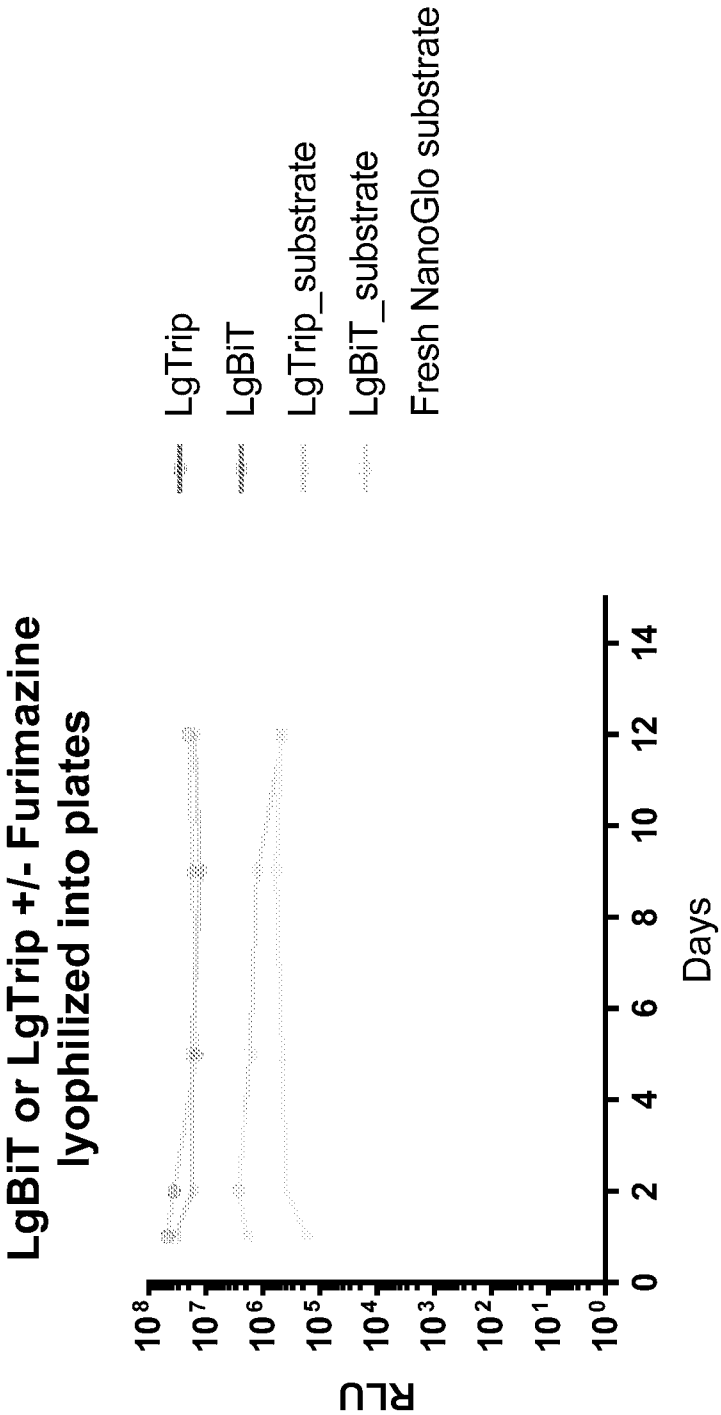

LgBiT or LgTrip +/- Furimazine
lyophilized into plates
LgTrip
LgBiT
LgTrip_substrate
LgBiT_substrate
Fresh NanoGlo substrate
RLU
Days
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
0
2
4
6
8
10
12
14

FIG. 39

FIG. 40A

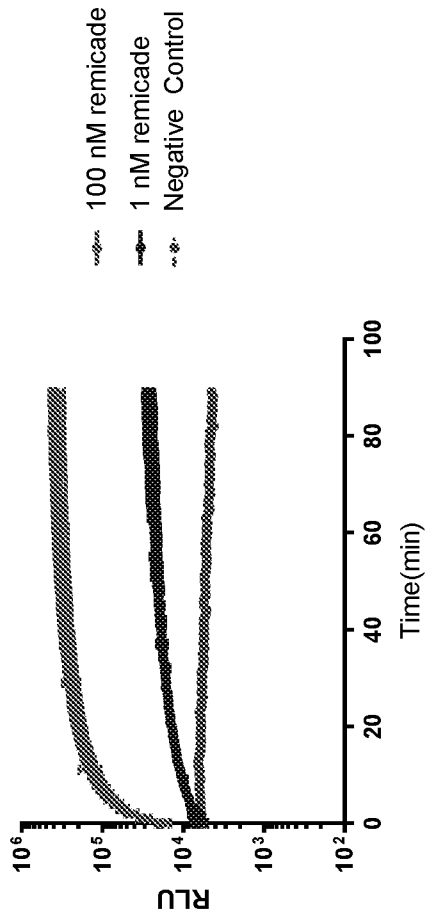
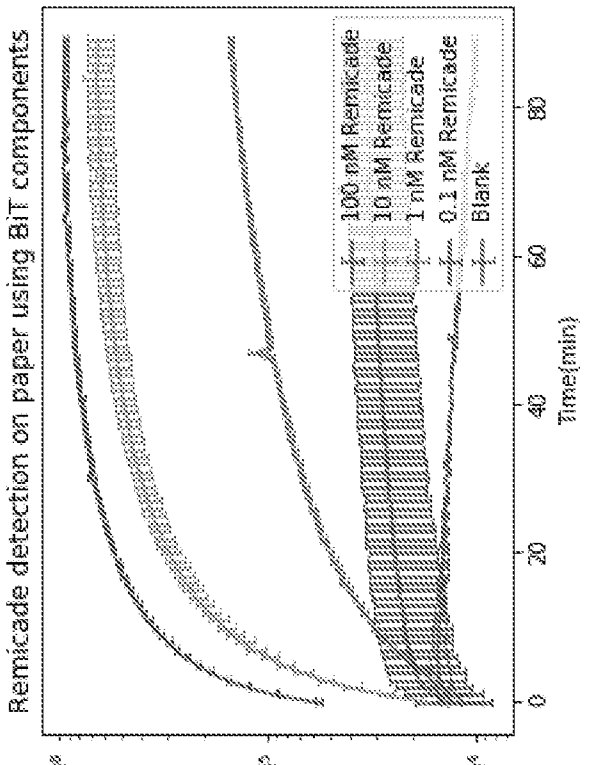
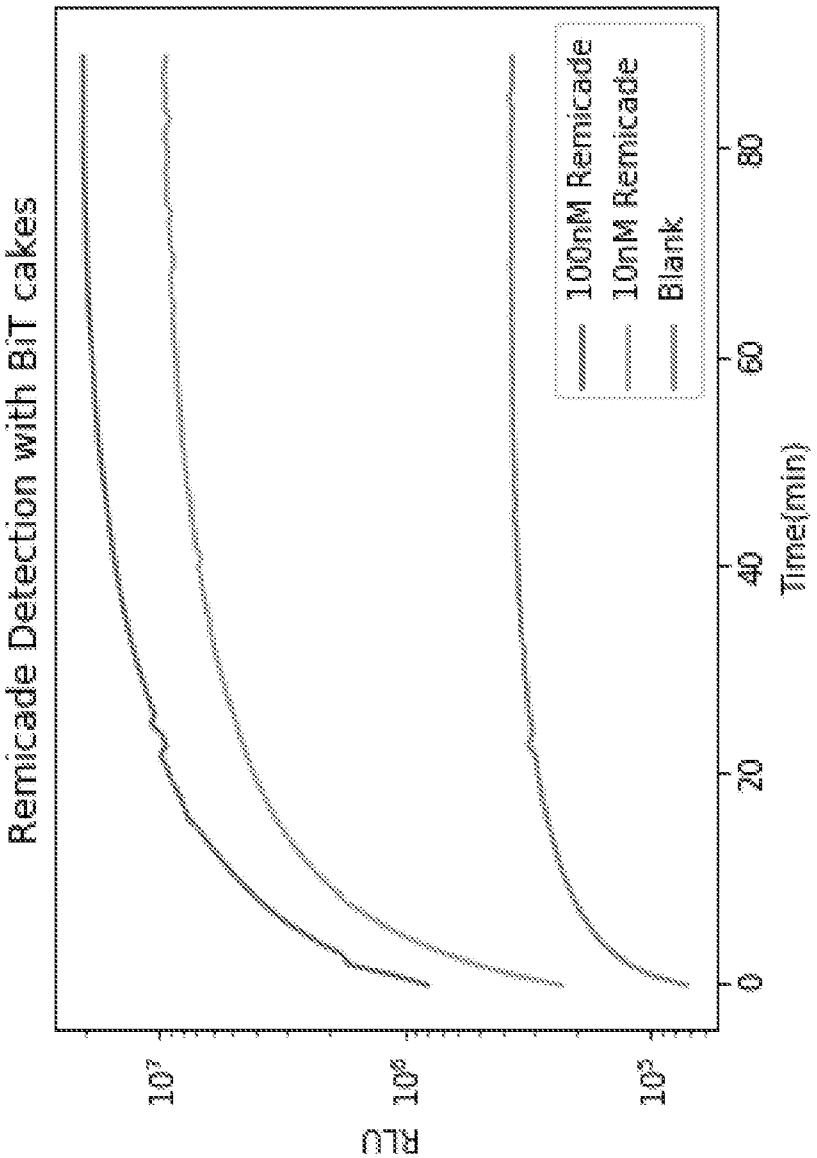


FIG. 40B



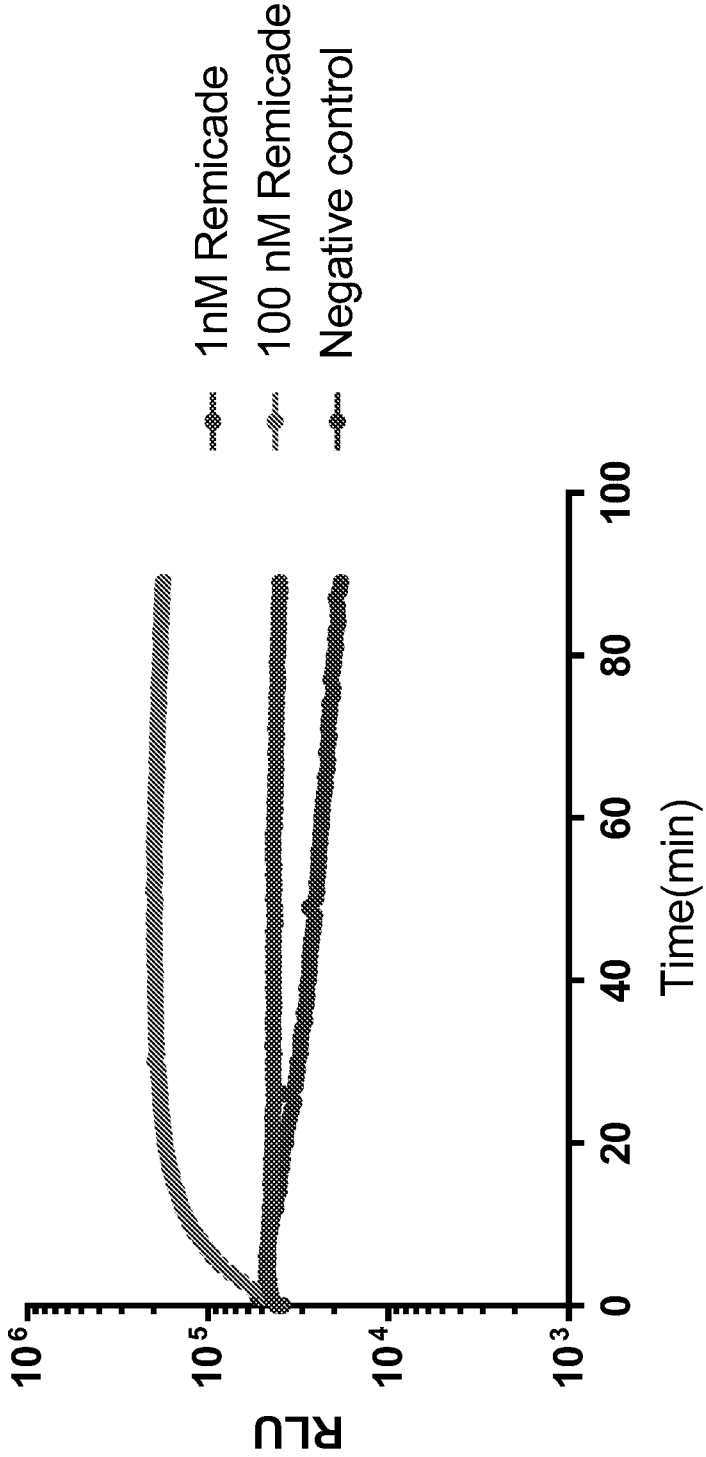
FIGS. 40A-40C

FIG. 40C



FIGS. 40A-40C

FIG. 41A



FIGS. 41A-41C

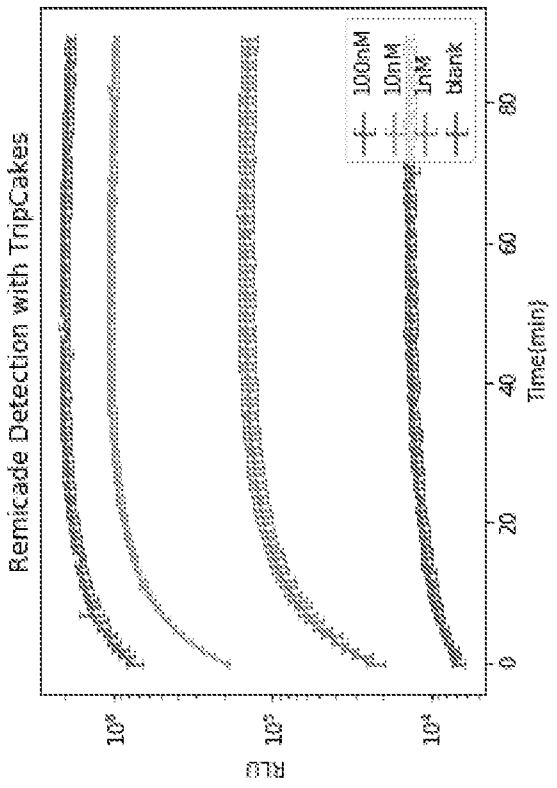


FIG. 41B

FIG. 41C

FIGS. 41A-41C

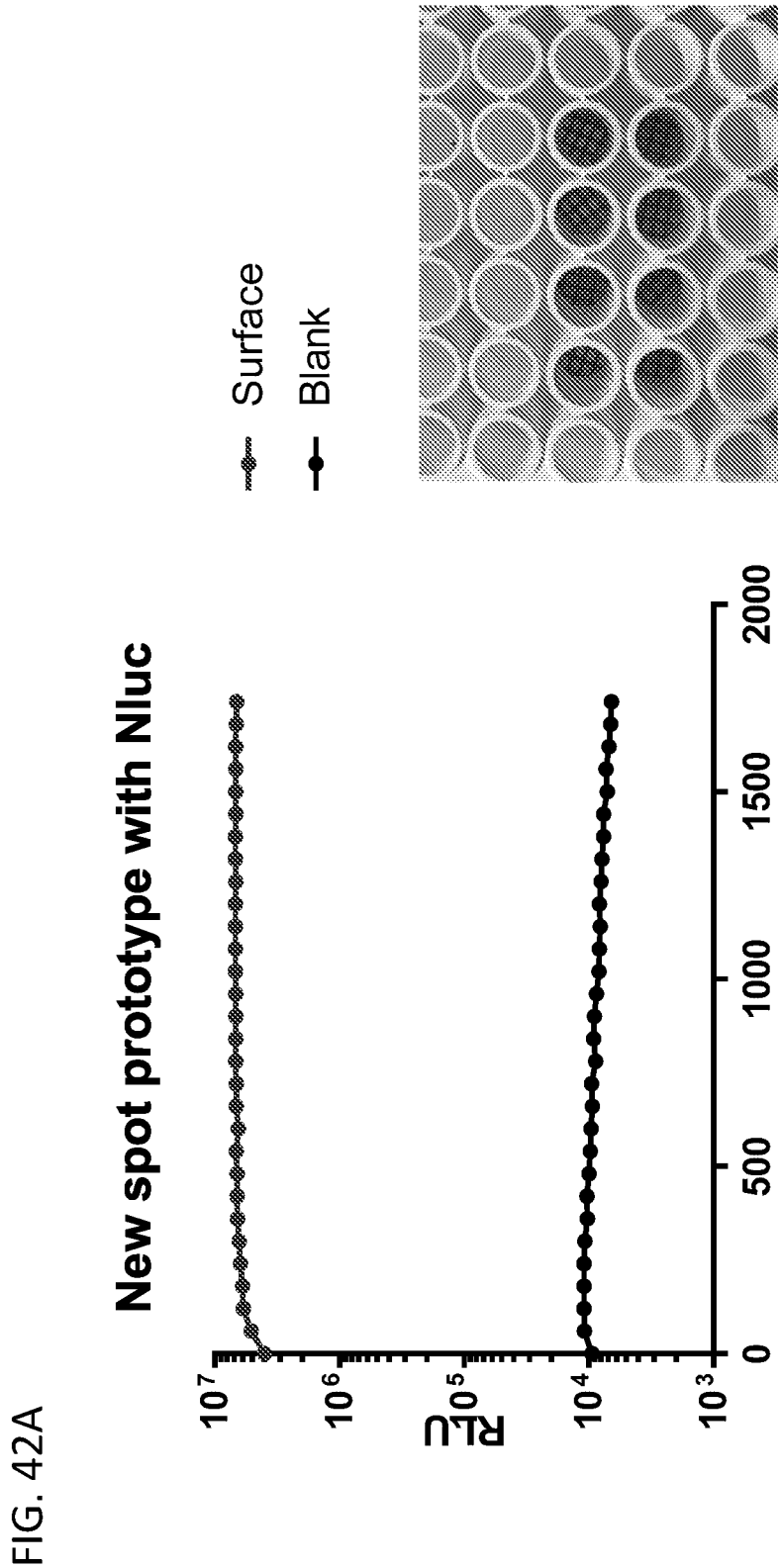


FIG. 42A

FIGS. 42A-42E

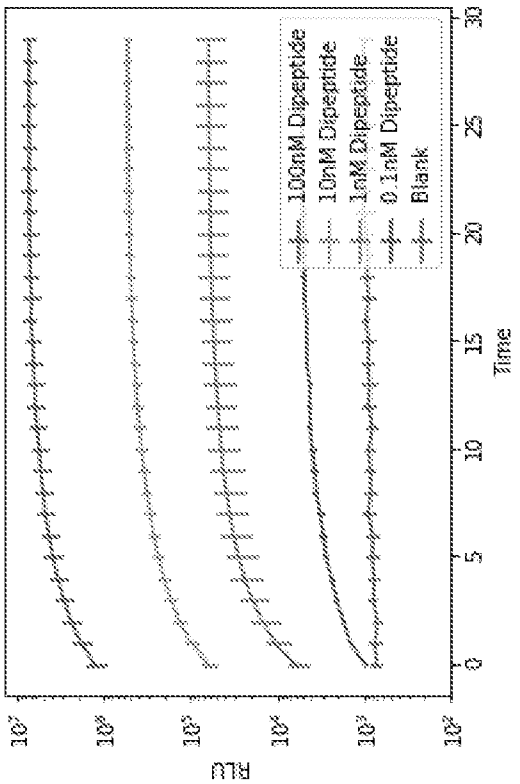


FIG. 42B

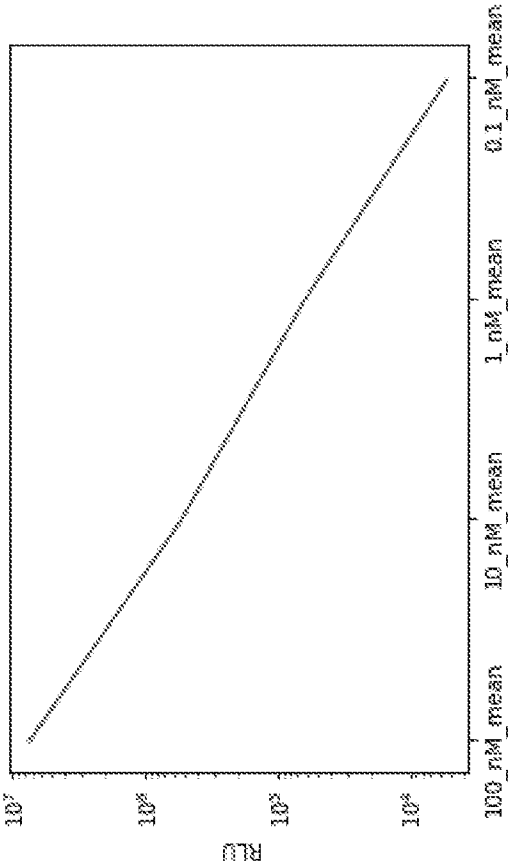


FIG. 42C

FIGS. 42A-42E

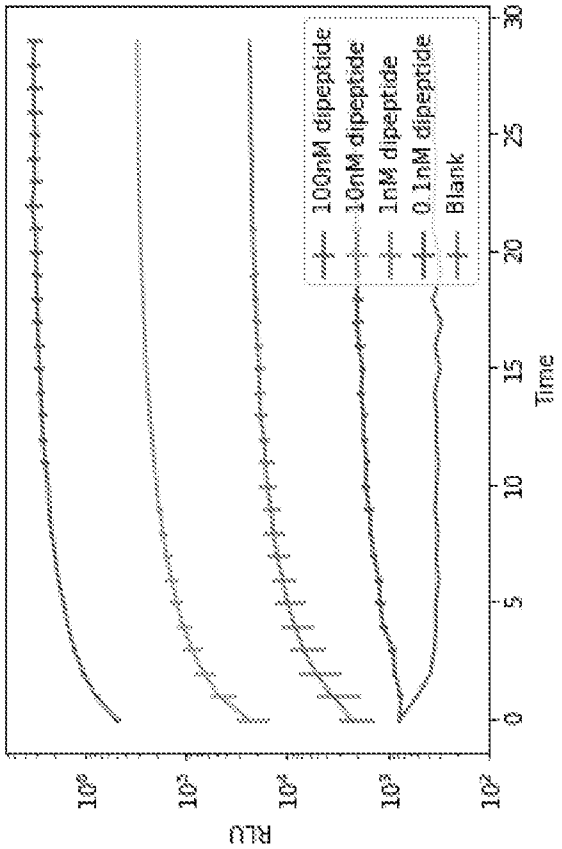


FIG. 42D

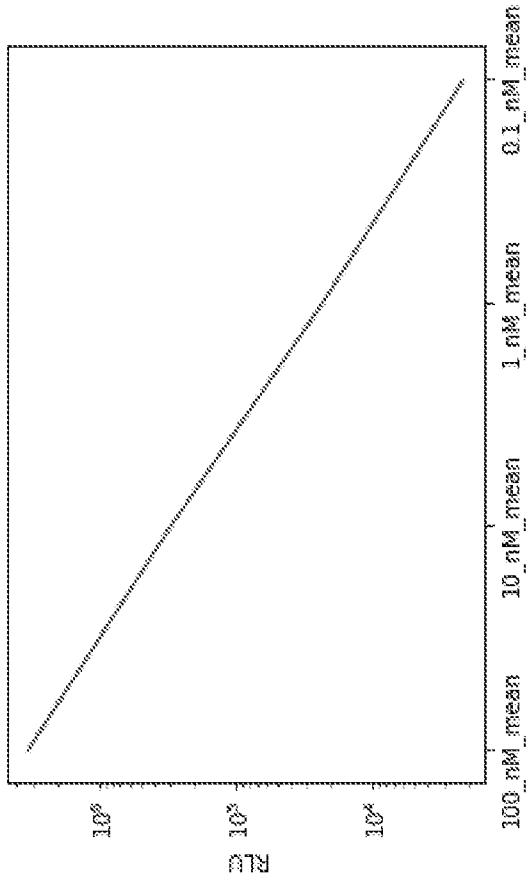


FIG. 42E

FIGS. 42A-42E

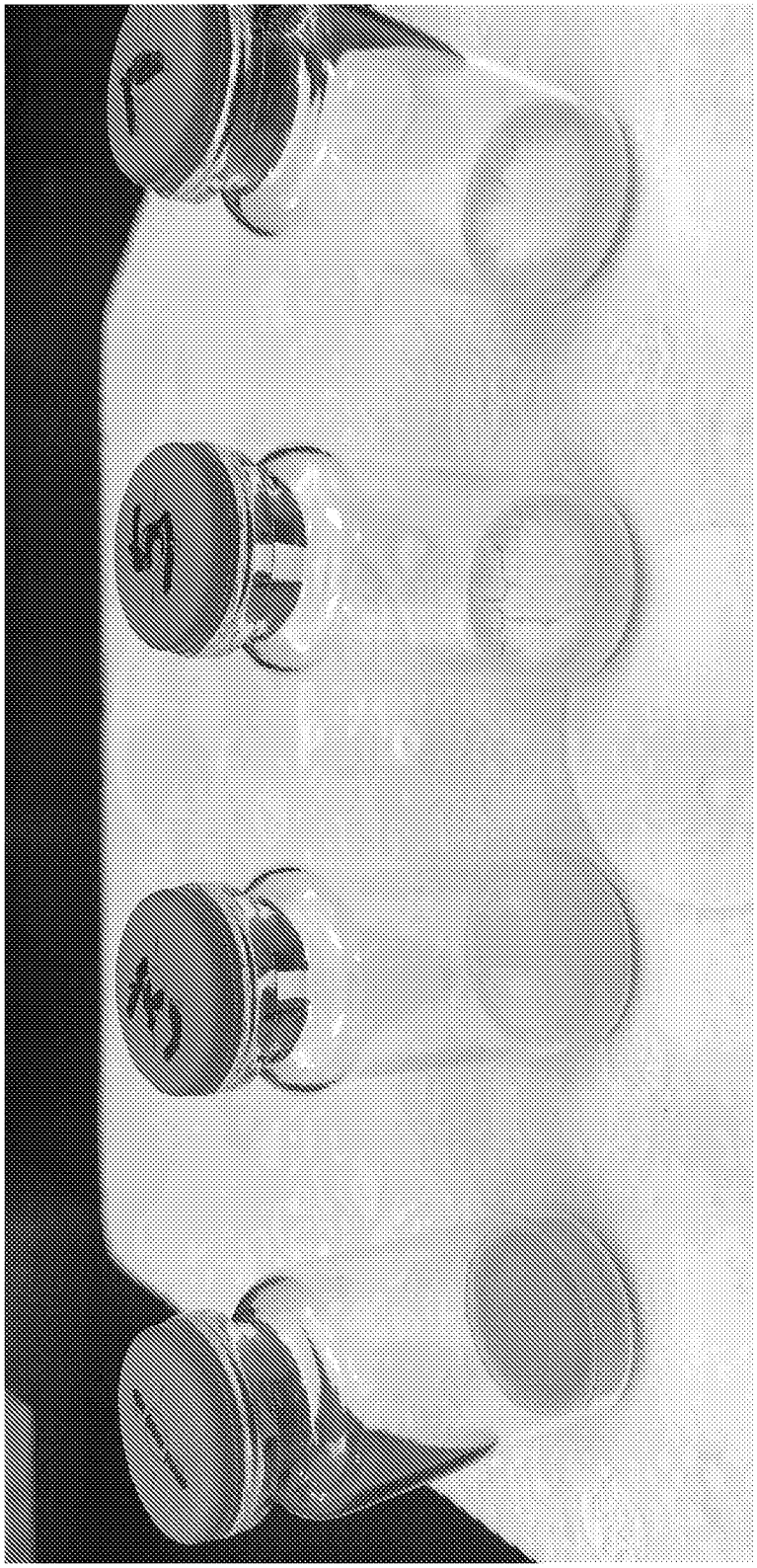


FIG. 43

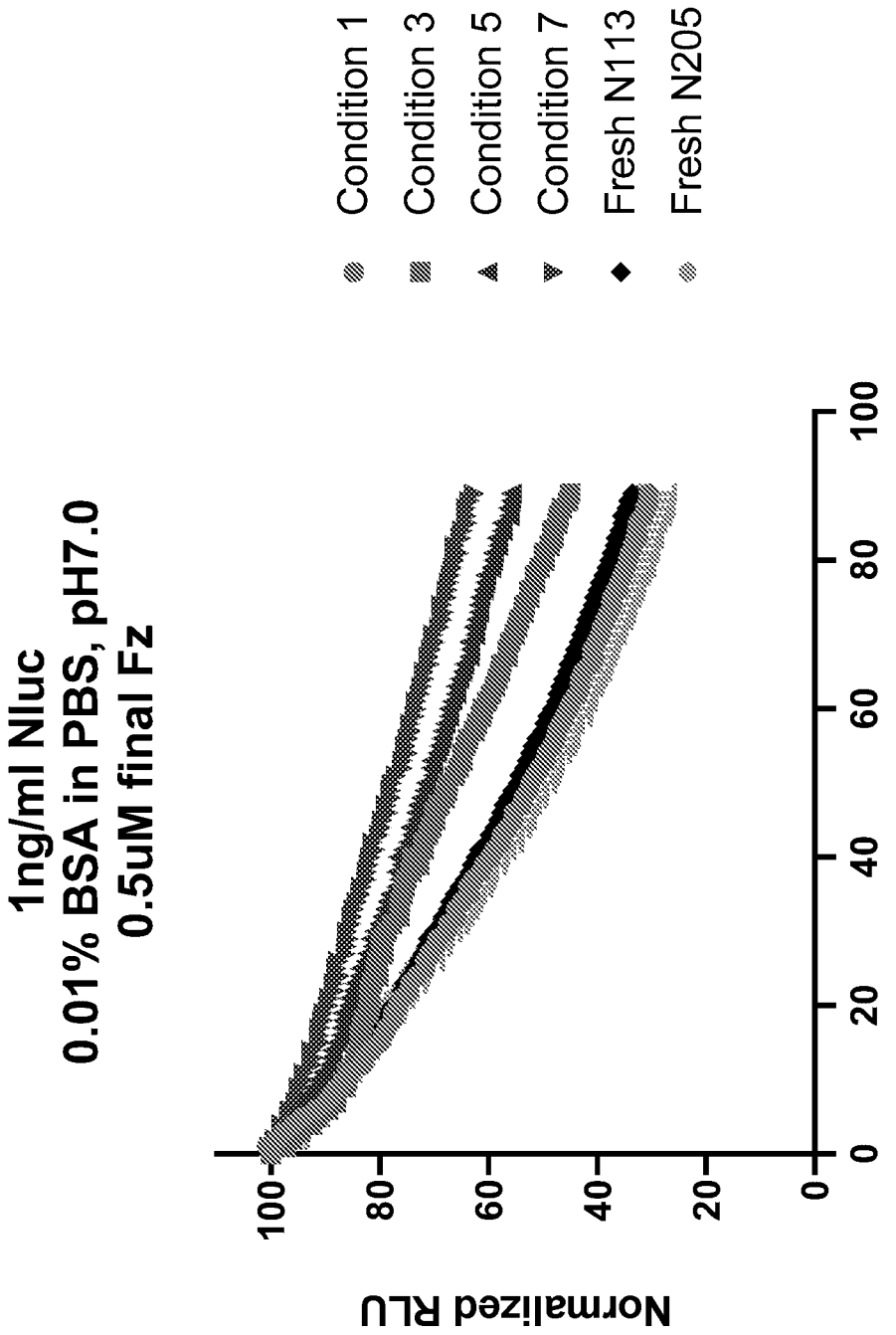

1ng/ml Nluc
0.01% BSA in PBS, pH7.0
0.5uM final Fz
Condition 1
Condition 3
Condition 5
Condition 7
Fresh N113
Fresh N205
Normalized RLU
Time, minutes
0
20
40
60
80
100

FIG. 44

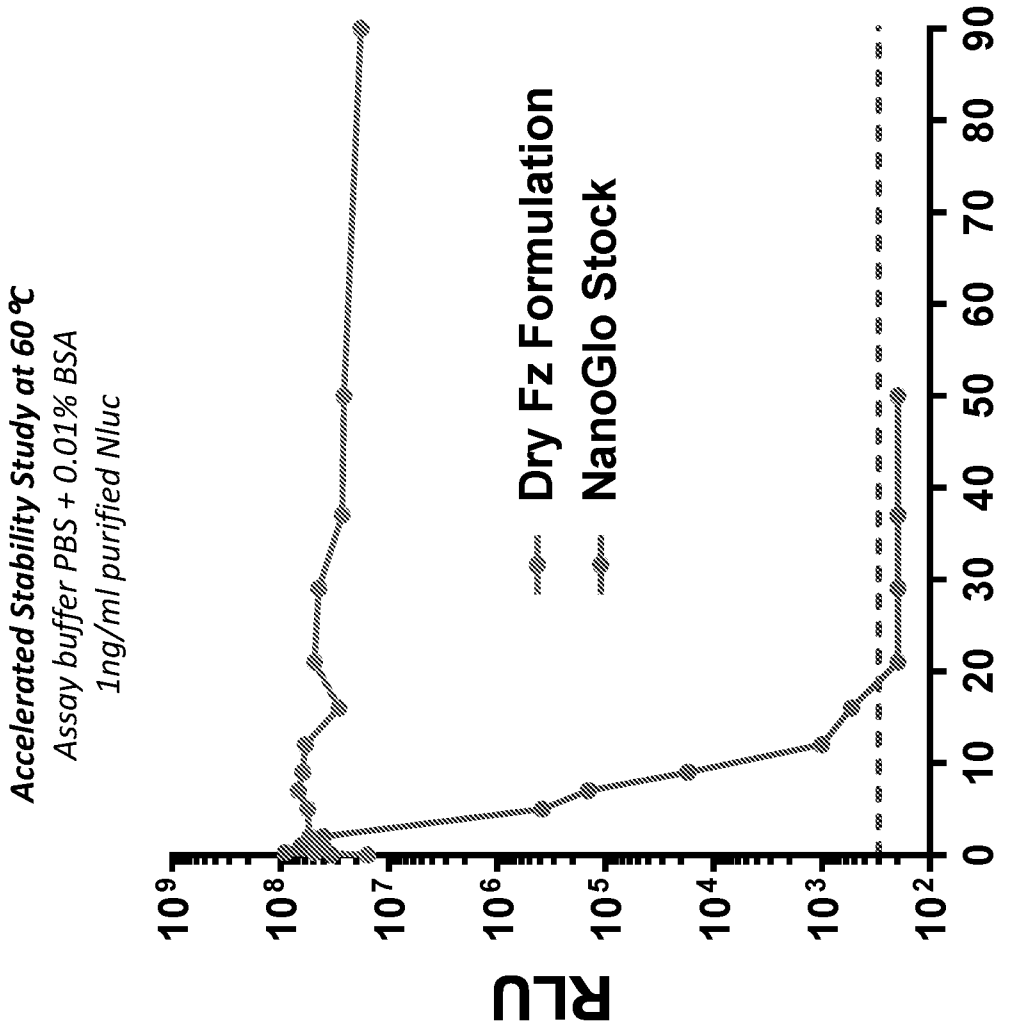

Accelerated Stability Study at 60°C
Assay buffer PBS + 0.01% BSA
1ng/ml purified Nluc
Dry Fz Formulation
NanoGlo Stock
RLU
time, days
10^9
10^8
10^7
10^6
10^5
10^4
10^3
10^2
0
10
20
30
40
50
60
70
80
90

FIG. 45

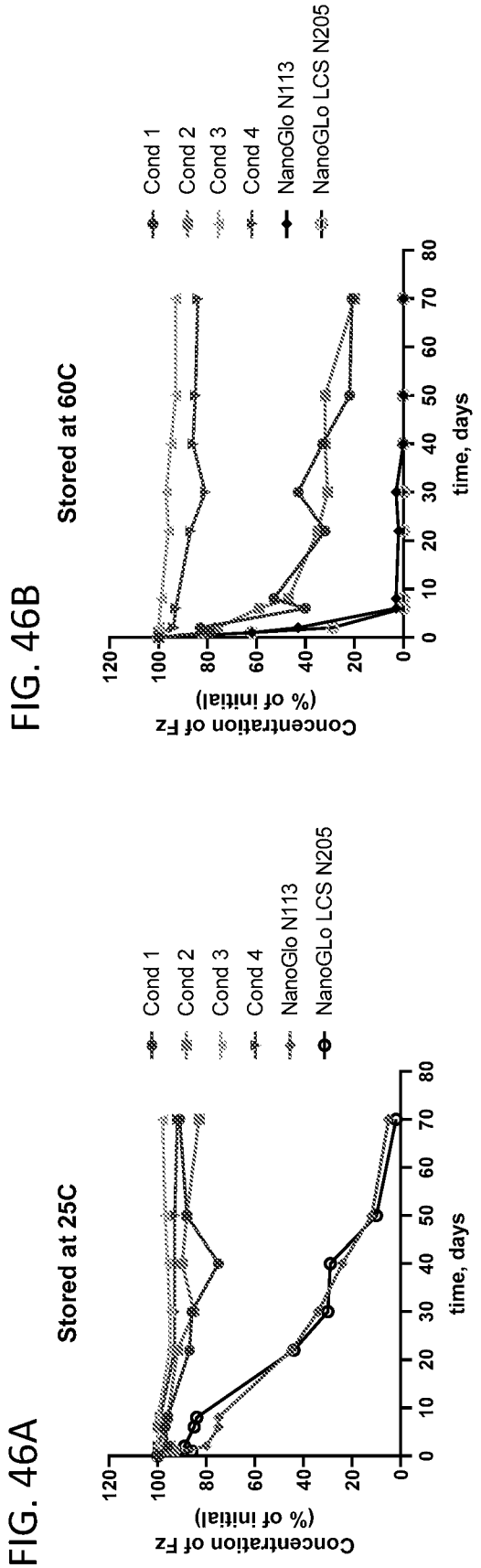


FIG. 46A

FIG. 46B

FIGS. 46A-46B

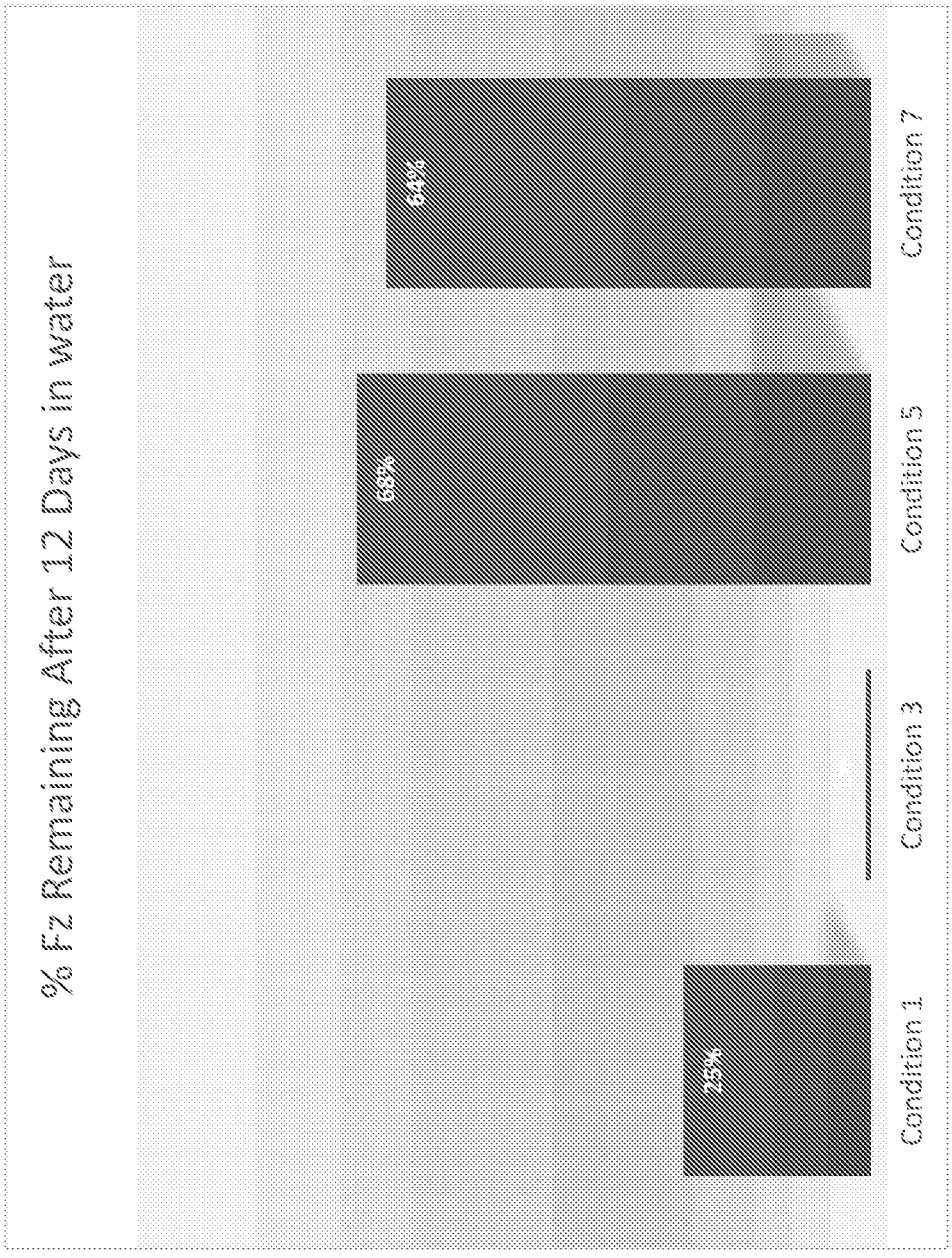

% Fz Remaining After 12 Days in water
15%
68%
84%
Condition 1
Condition 3
Condition 5
Condition 7

FIG. 47

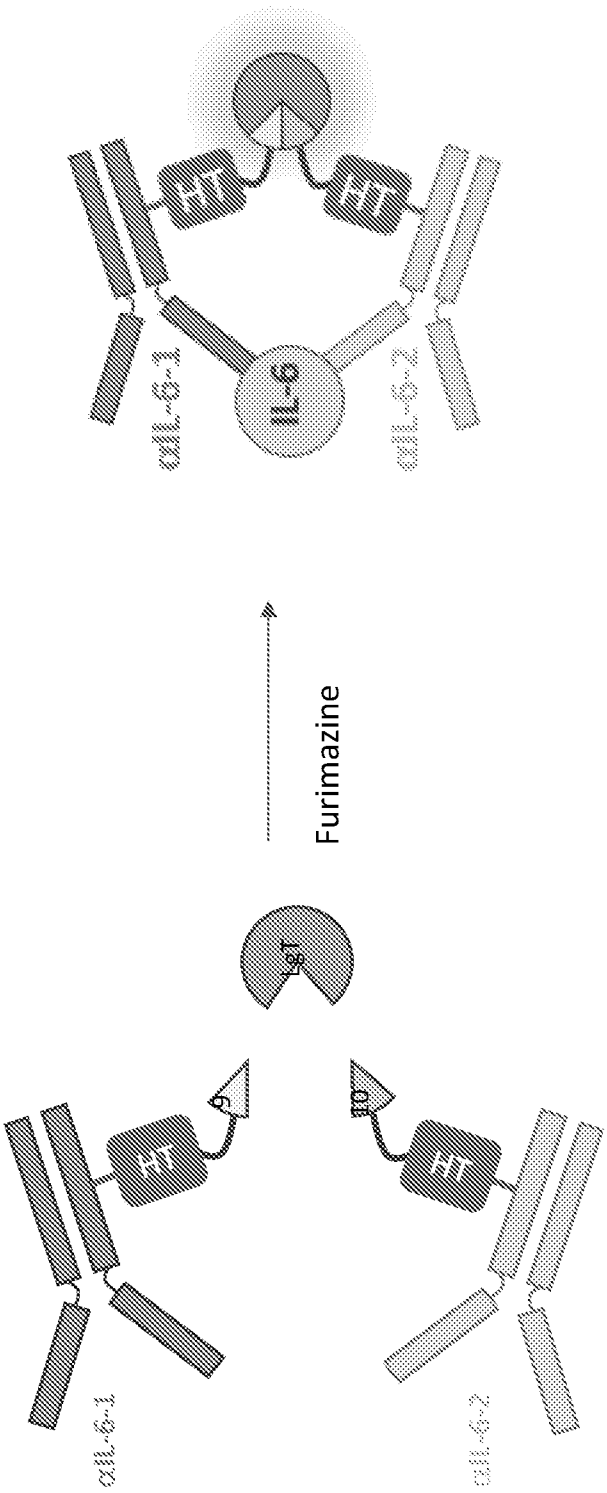
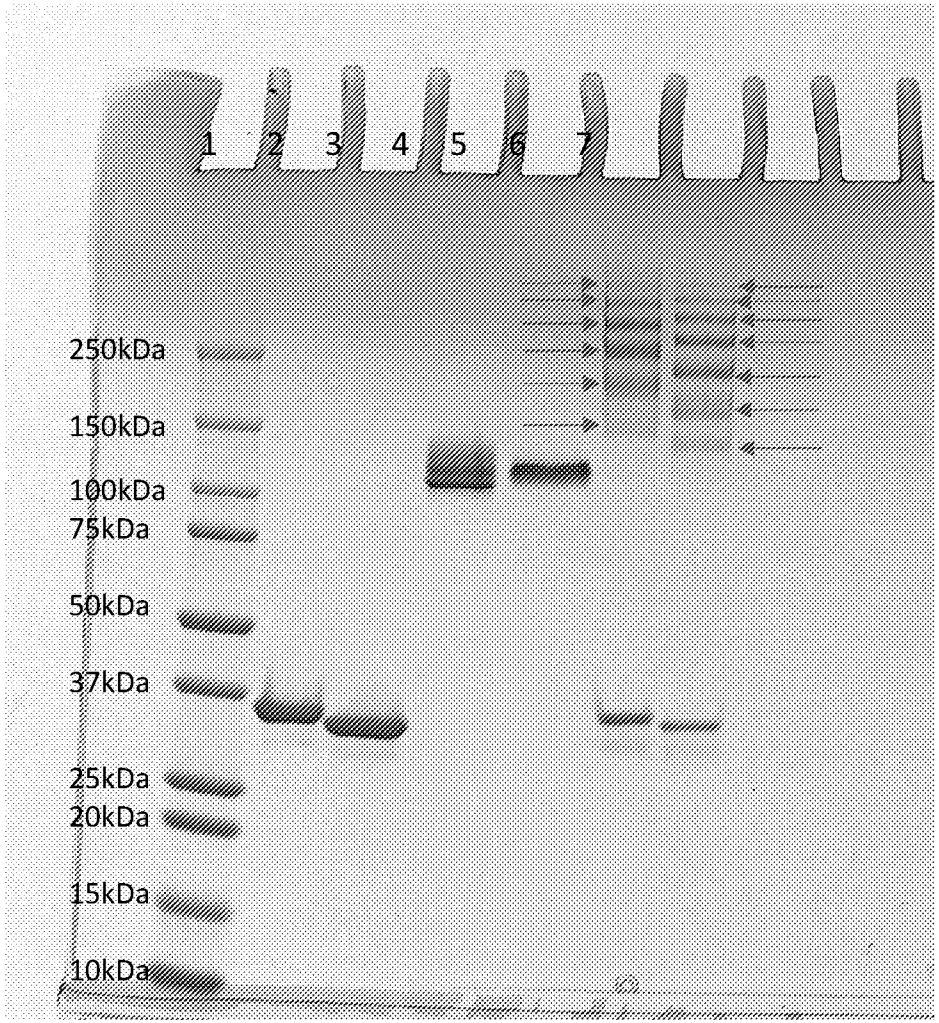


FIG. 48



1) molecular weight standard
2) 6xHis-SmTrip9-24GS-HaloTag fusion purified protein
3) 6xHis-SmTrip10-8GS-HaloTag purified protein
4) unlabeled anti-IL-6 antibody clone 5IL6
5) unlabeled anti-IL-6 antibody clone 505E 9A12 A3 clone
6) clone 5IL6 antibody labeled with SmTrip9-HaloTag fusion protein
7) clone 505E 9A12 A3 clone labeled with SmTrip10-HaloTag fusion protein

FIG. 49

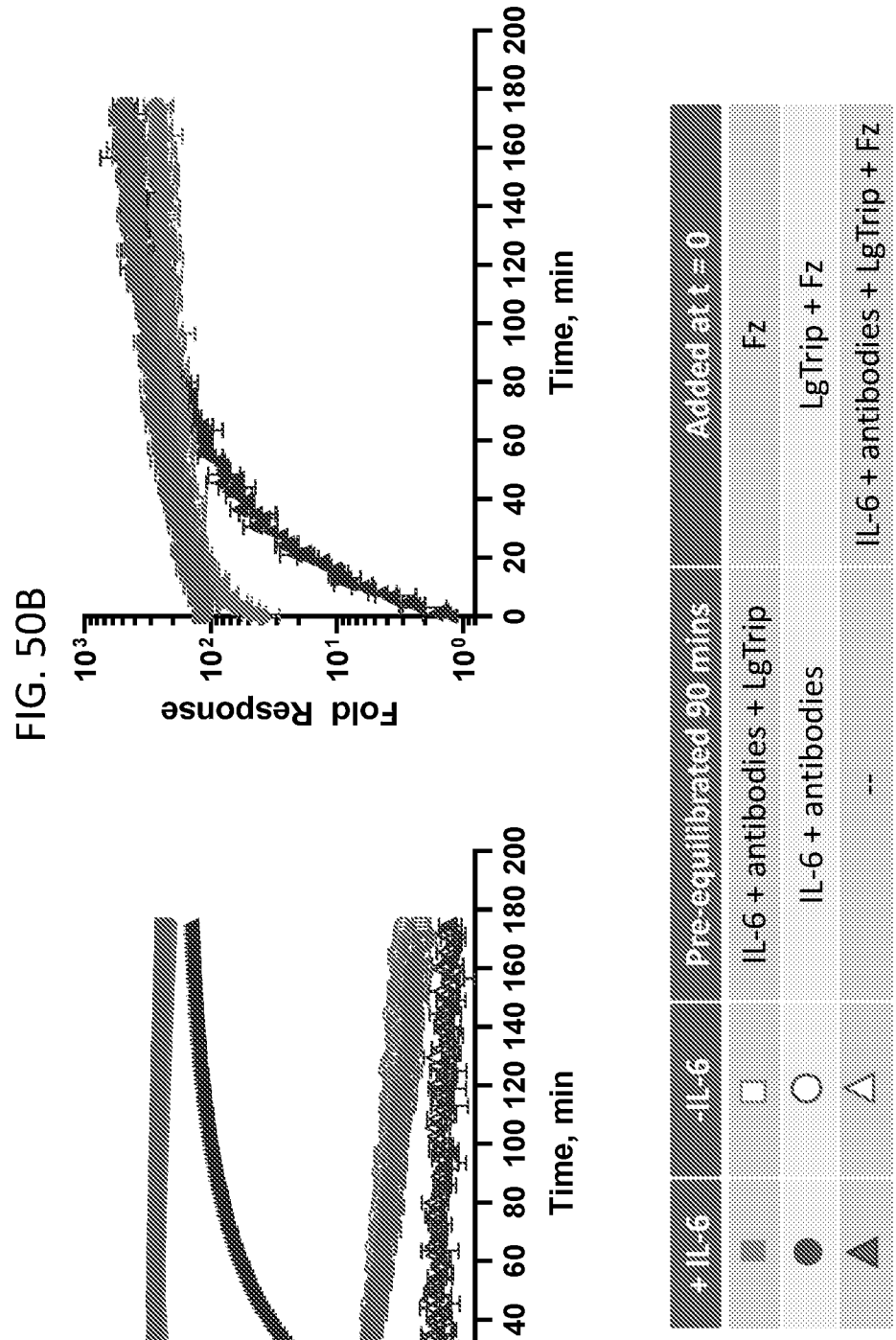


FIG. 50A
Raw RLU
Time, min
FIG. 50B
Fold Response
Time, min
+ IL-6
- IL-6
Pre-equilibrated 90 mins
Added at t = 0
IL-6 + antibodies + LgTrip
Fz
IL-6 + antibodies
LgTrip + Fz
--
IL-6 + antibodies + LgTrip + Fz

FIGS. 50A-50B

FIG. 51A

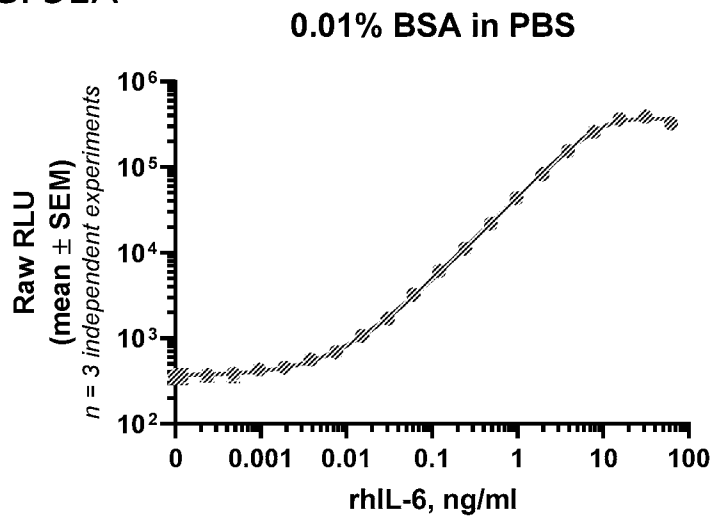
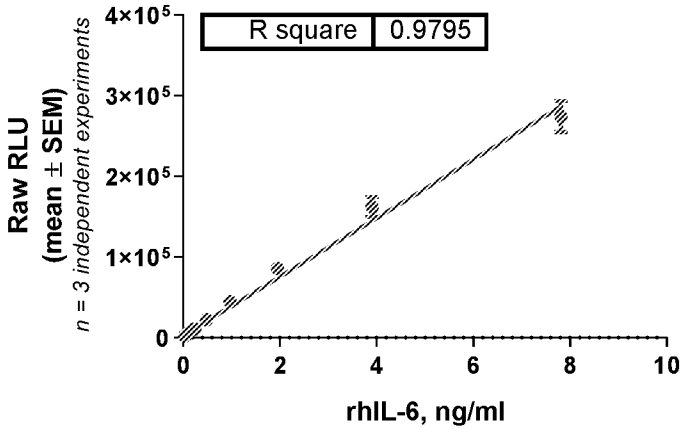


FIG. 51B



	Trip
LOD	2.1 pg/ml
LLOQ	4.3 pg/ml
ULOQ	16,200 pg/ml

LOD = blank + 3*SD_{blank}
LLOQ = blank + 10*SD_{blank}
ULOQ = [high] + 10*$SD_{[hi]}$
→Values then interpolated on the standard curve and < 10% CV

FIGS. 51A-51B

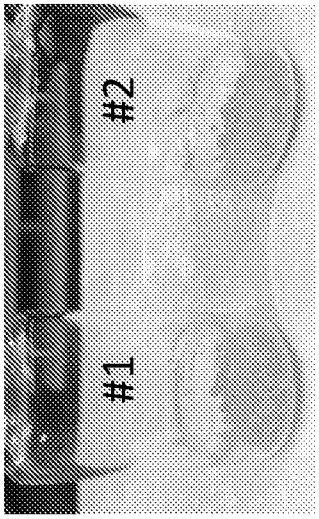


FIG. 52A

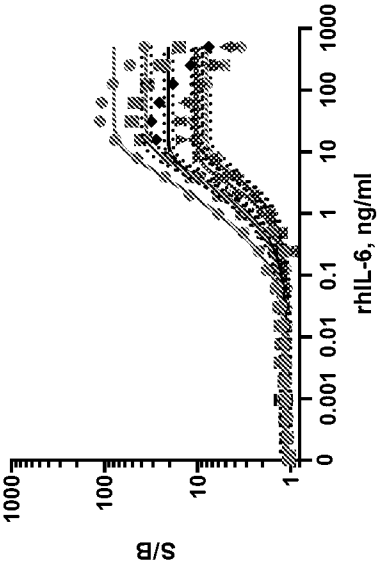


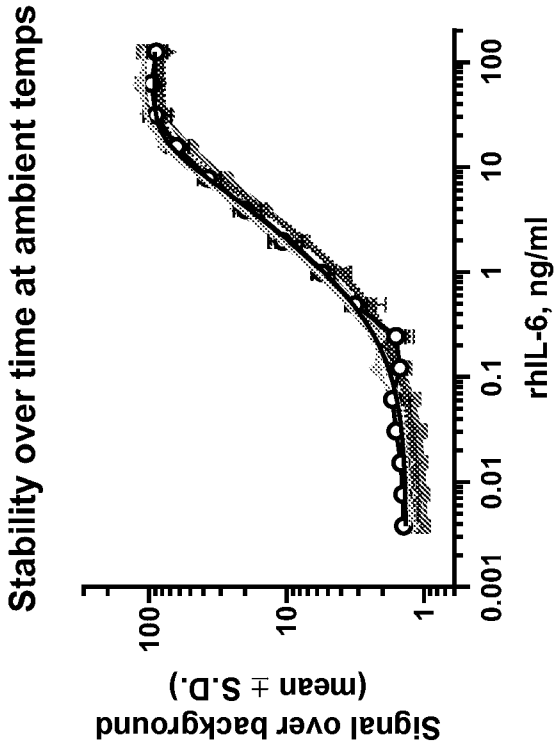
FIG. 52B

FIG. 52C

FIGS. 52A-52C

FIG. 53A

FIG. 53B



FIGS. 53A-53B

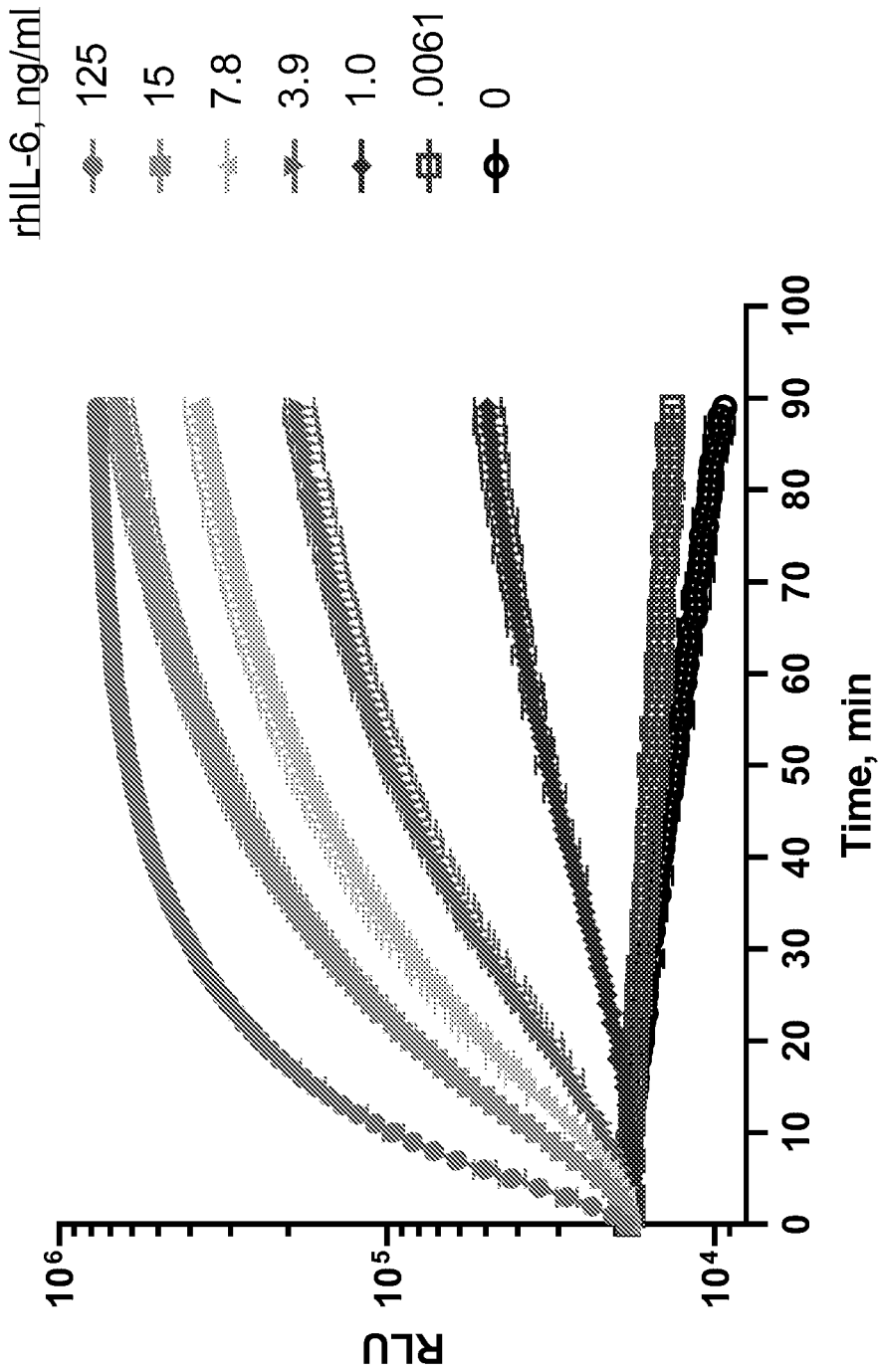

rhIL-6, ng/ml
125
15
7.8
3.9
1.0
.0061
0
RLU
10^6
10^5
10^4
0
10
20
30
40
50
60
70
80
90
100
Time, min

FIG. 54

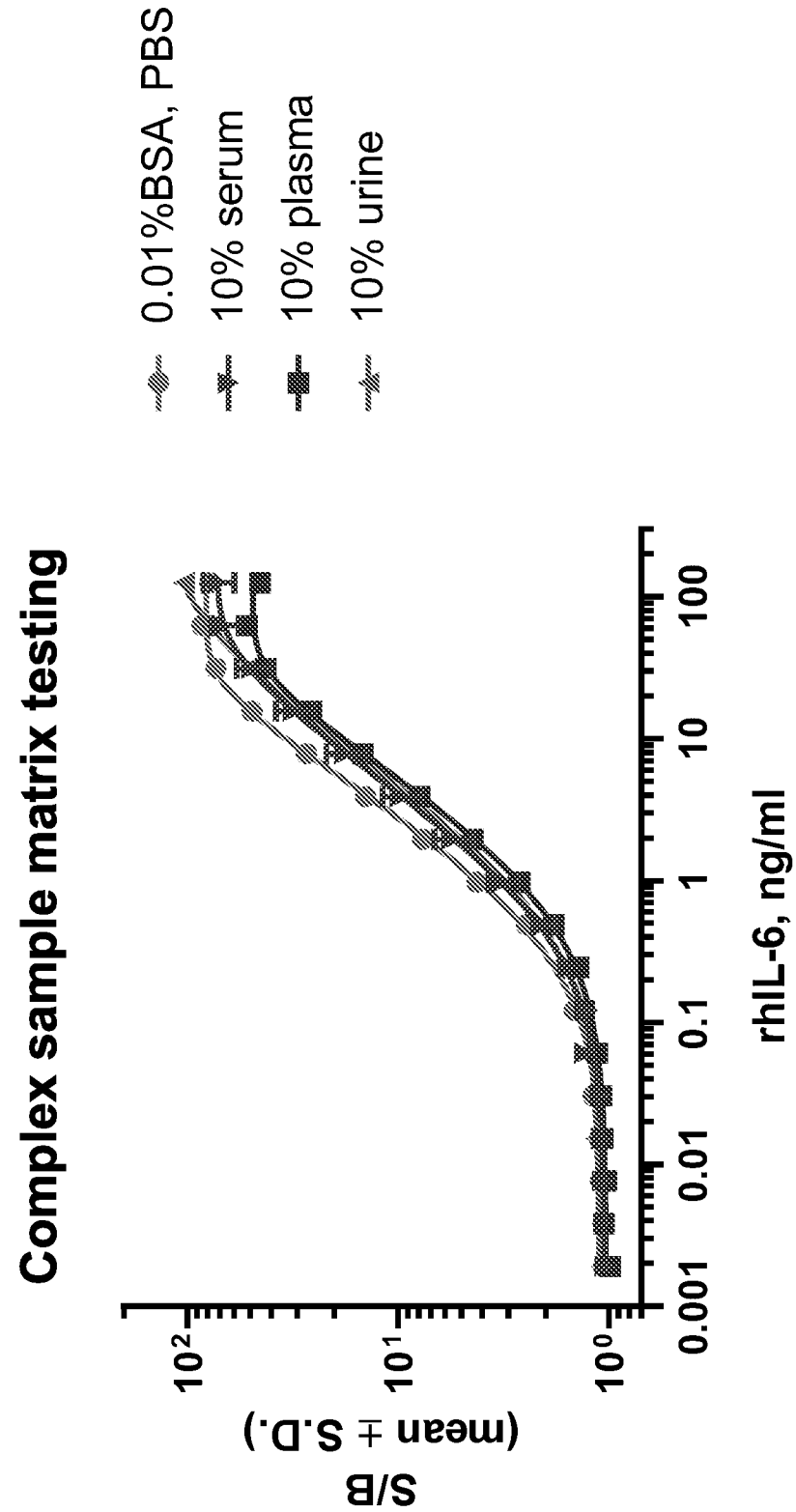

Complex sample matrix testing
0.01%BSA, PBS
10% serum
10% plasma
10% urine
S/B
(mean ± S.D.)
10^2
10^1
10^0
0.001
0.01
0.1
1
10
100
rhIL-6, ng/ml

FIG. 55

FIG. 56A

Add sample and read

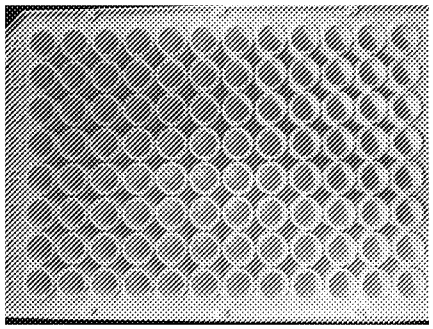
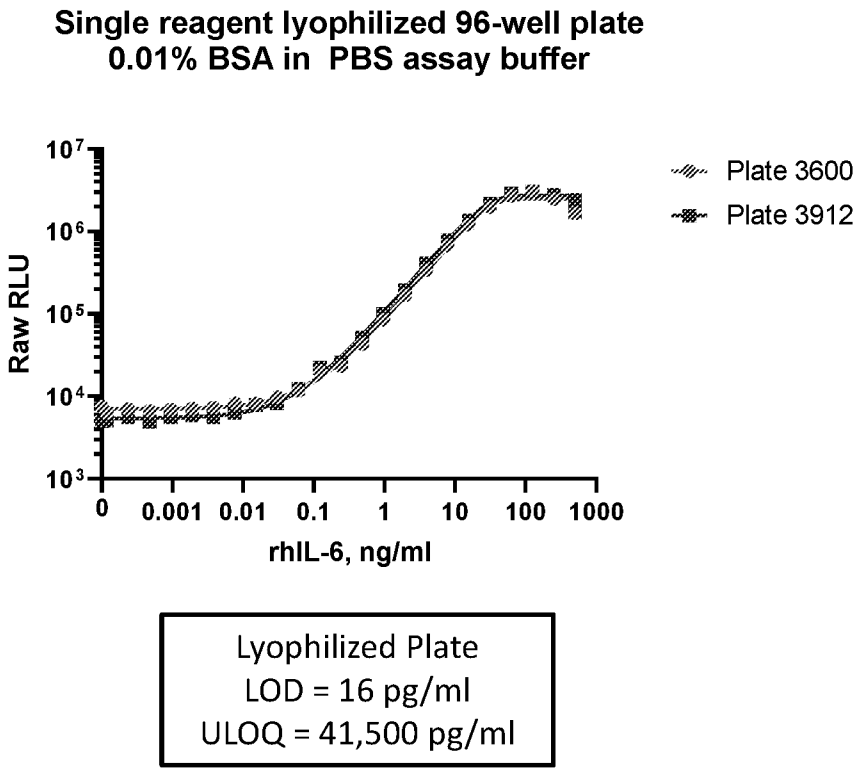


FIG. 56B



Lyophilized Plate
LOD = 16 pg/ml
ULOQ = 41,500 pg/ml

FIGS. 56A-56B

FIG. 57A

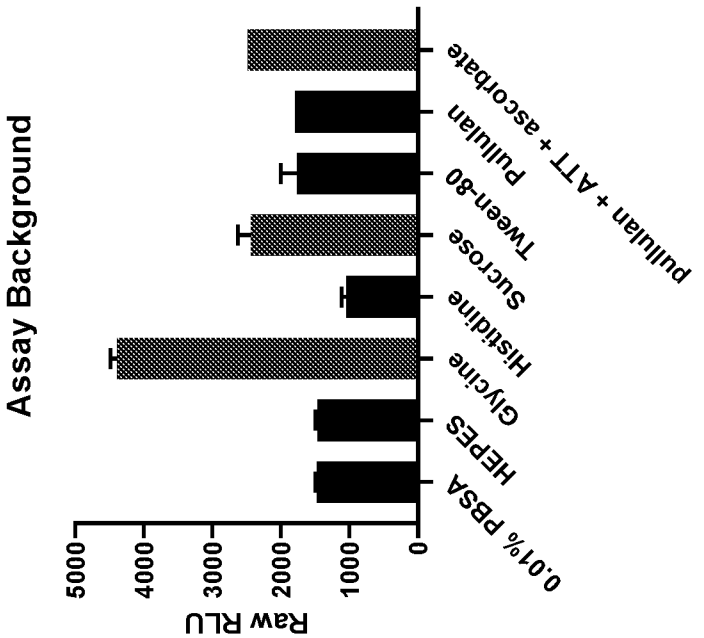
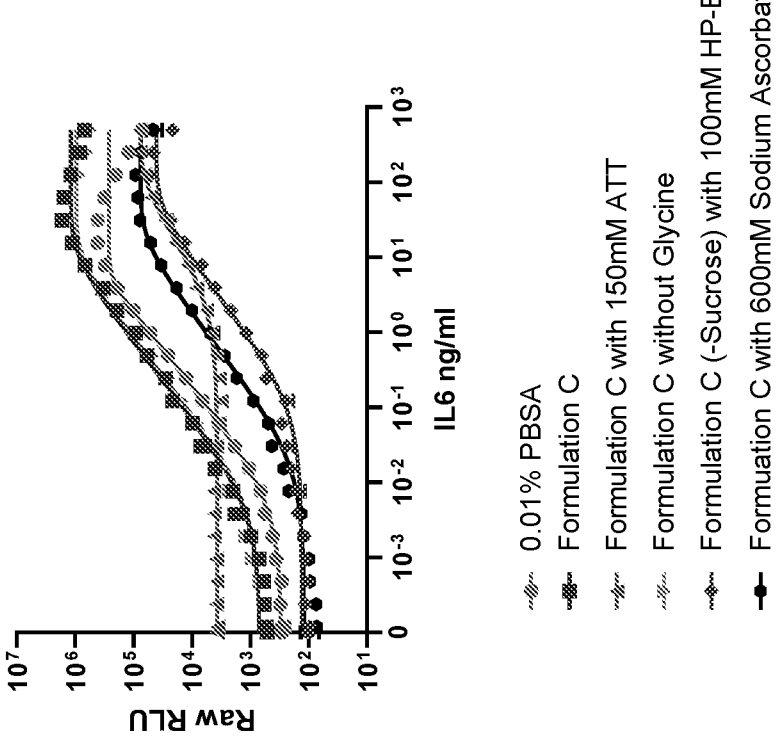


FIG. 57B



FIGS. 57A-57B

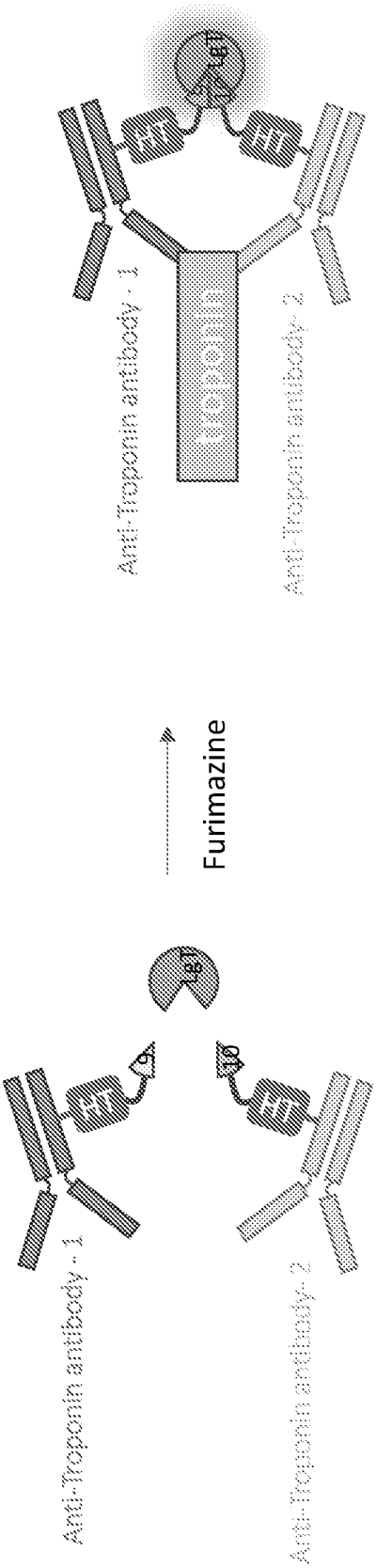

LET
Troponin
Anti-Troponin antibody - 1
Anti-Troponin antibody- 2
Furimazine
LET
Anti-Troponin antibody - 1
Anti-Troponin antibody- 2

FIG. 58

FIG. 59A

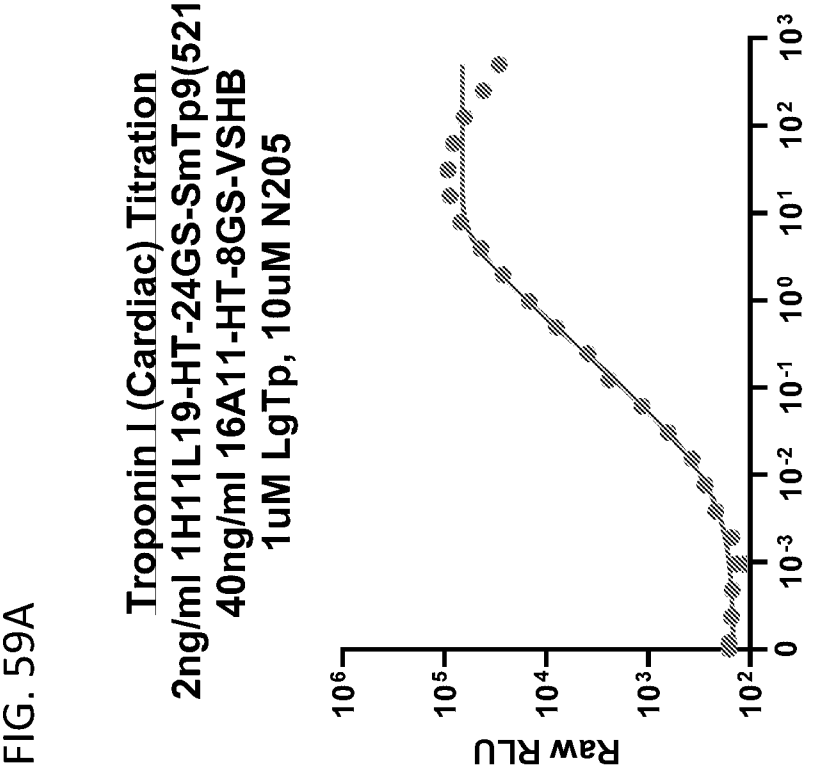
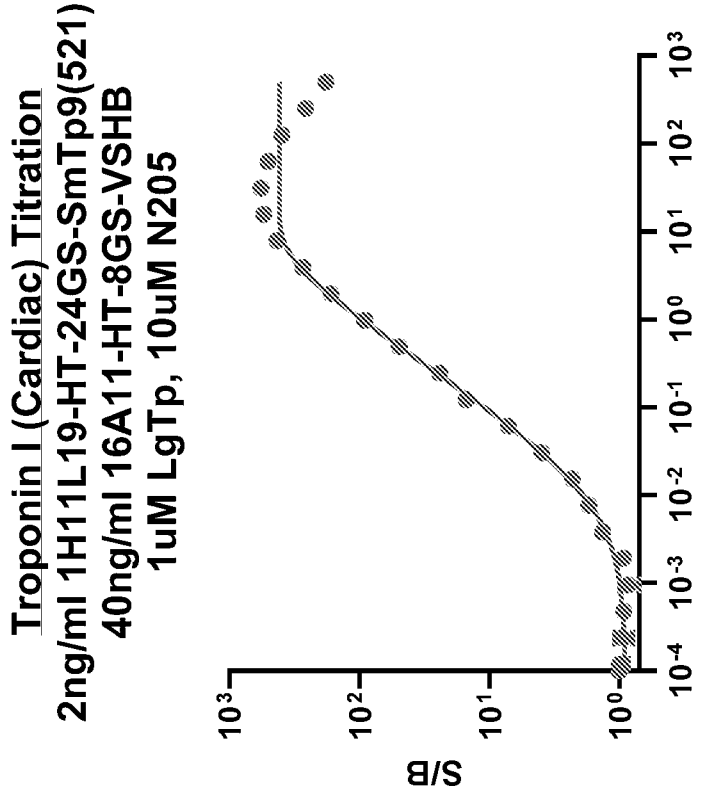


FIG. 59B



FIGS. 59A-59B

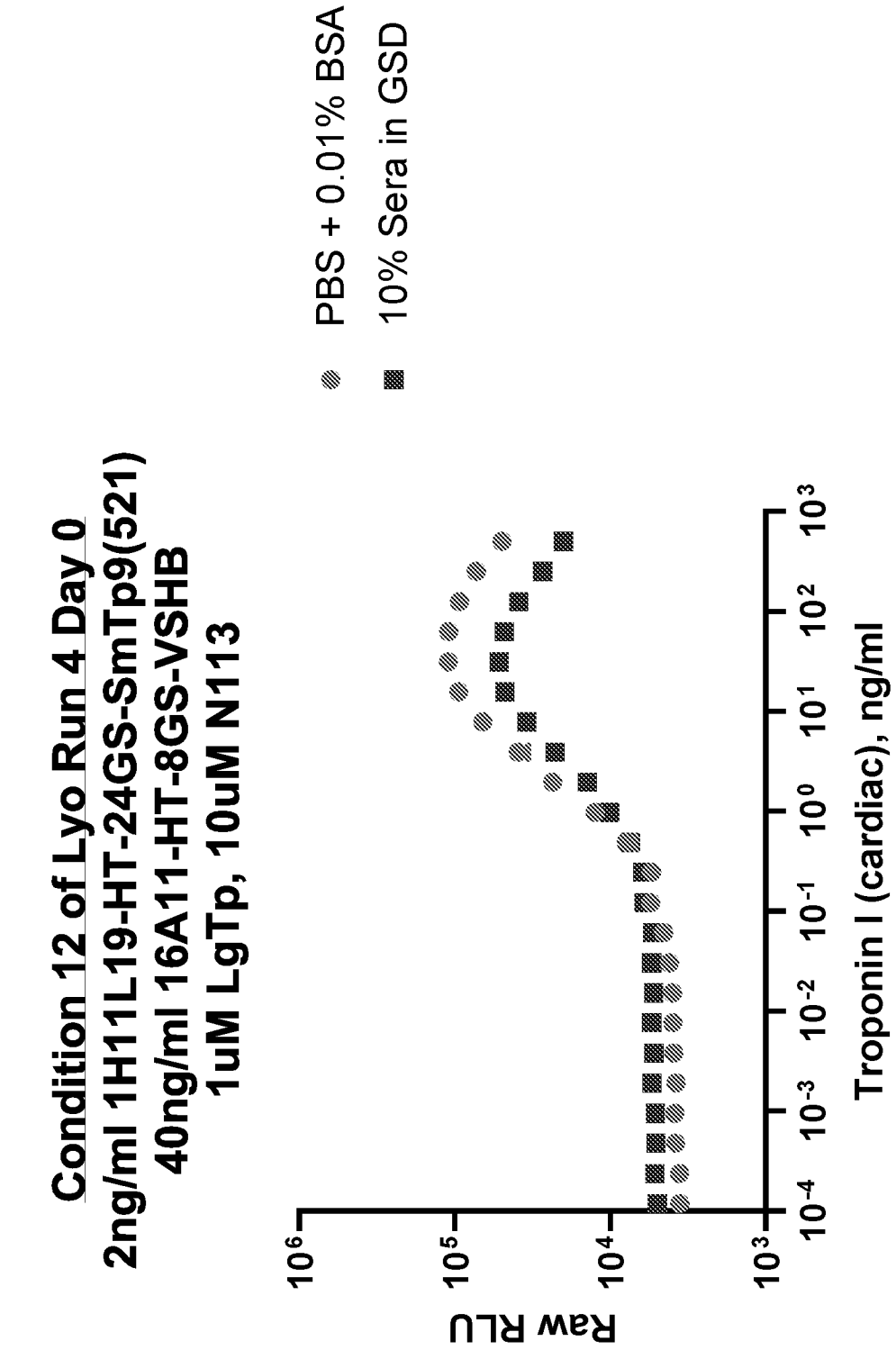

Condition 12 of Lyo Run 4 Day 0
2ng/ml 1H11L19-HT-24GS-SmTp9(521)
40ng/ml 16A11-HT-8GS-VSHB
1uM LgTp, 10uM N113
PBS + 0.01% BSA
10% Sera in GSD
Raw RLU
10^6
10^5
10^4
10^3
10^{-4} 10^{-3} 10^{-2} 10^{-1} 10^0 10^1 10^2 10^3
Troponin I (cardiac), ng/ml

FIG. 60

FIG. 61A

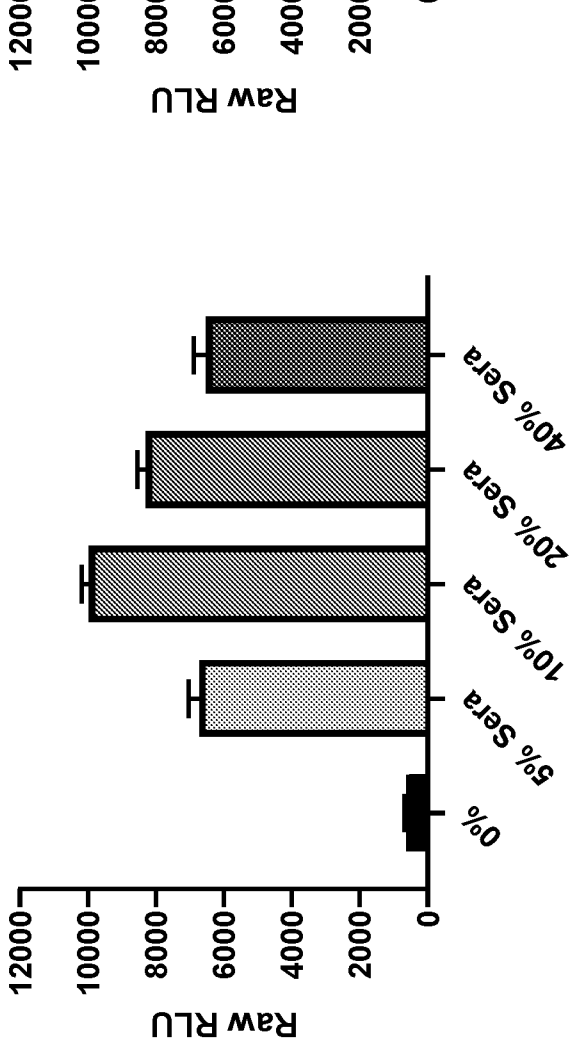
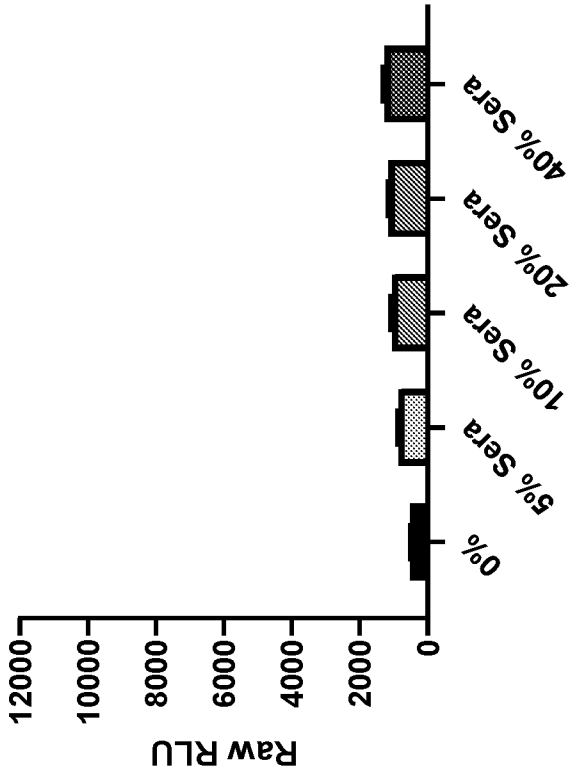


FIG. 61B



Diluting samples with general serum diluent (GSD) preferred over PBSA. GSD mitigates non-specific IgG effects Using N205 as substrate

FIGS. 61A-61B

FIG. 62A

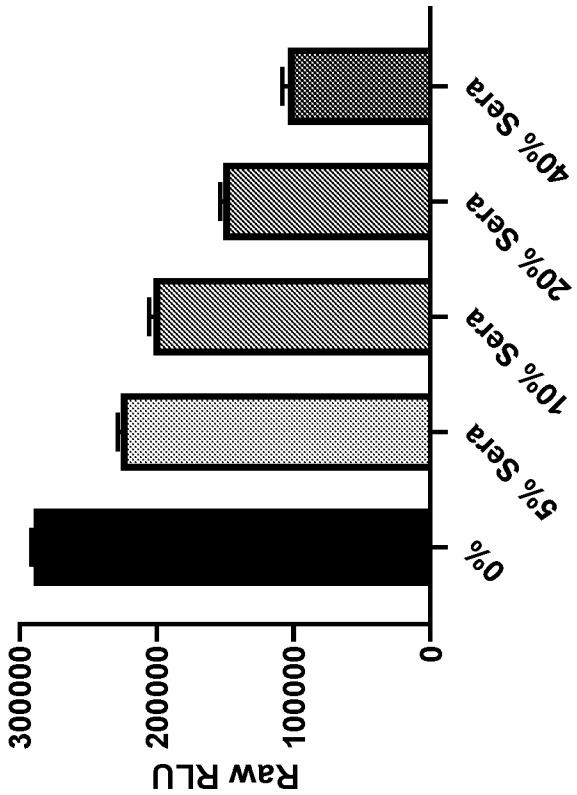
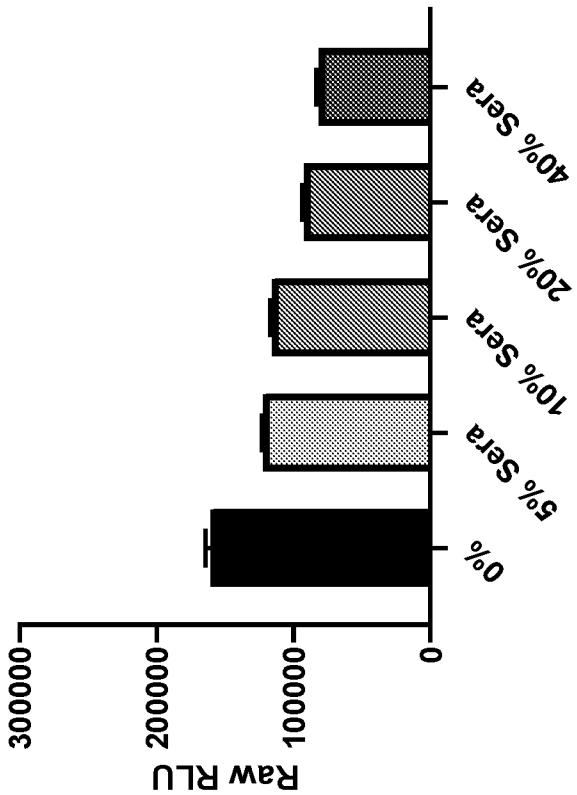


FIG. 62B



FIGS. 62A-62B

FIG. 63A

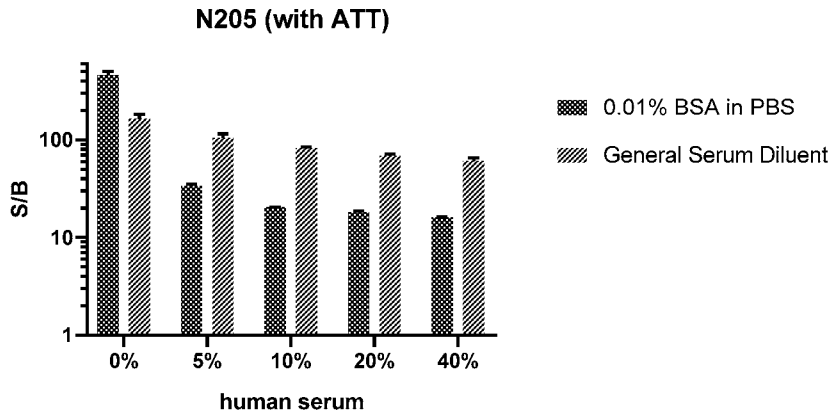


FIG. 63B

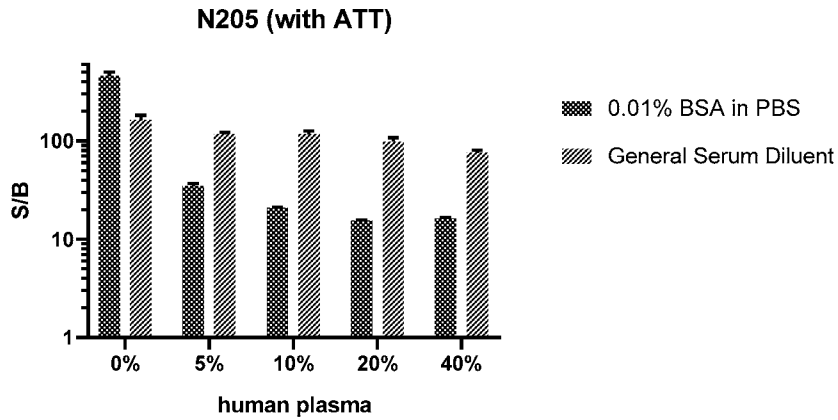


FIG. 63C

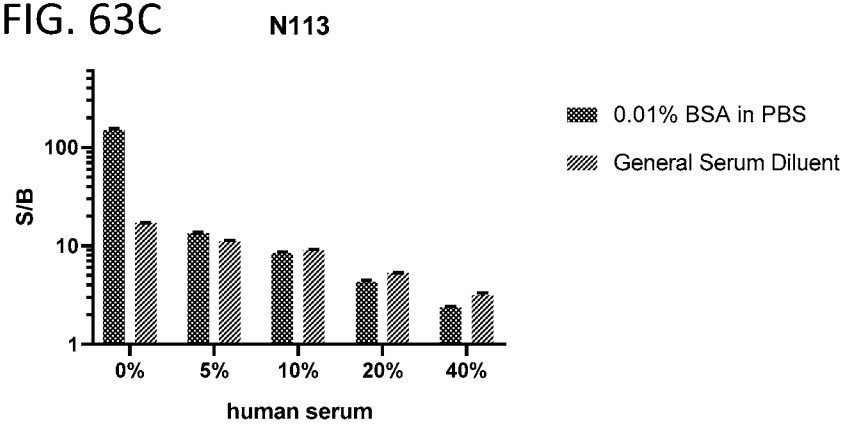
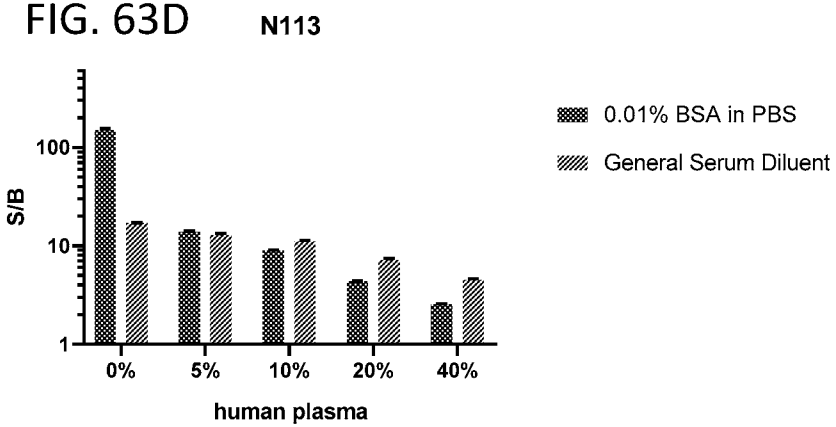


FIG. 63D



FIGS. 63A-63D

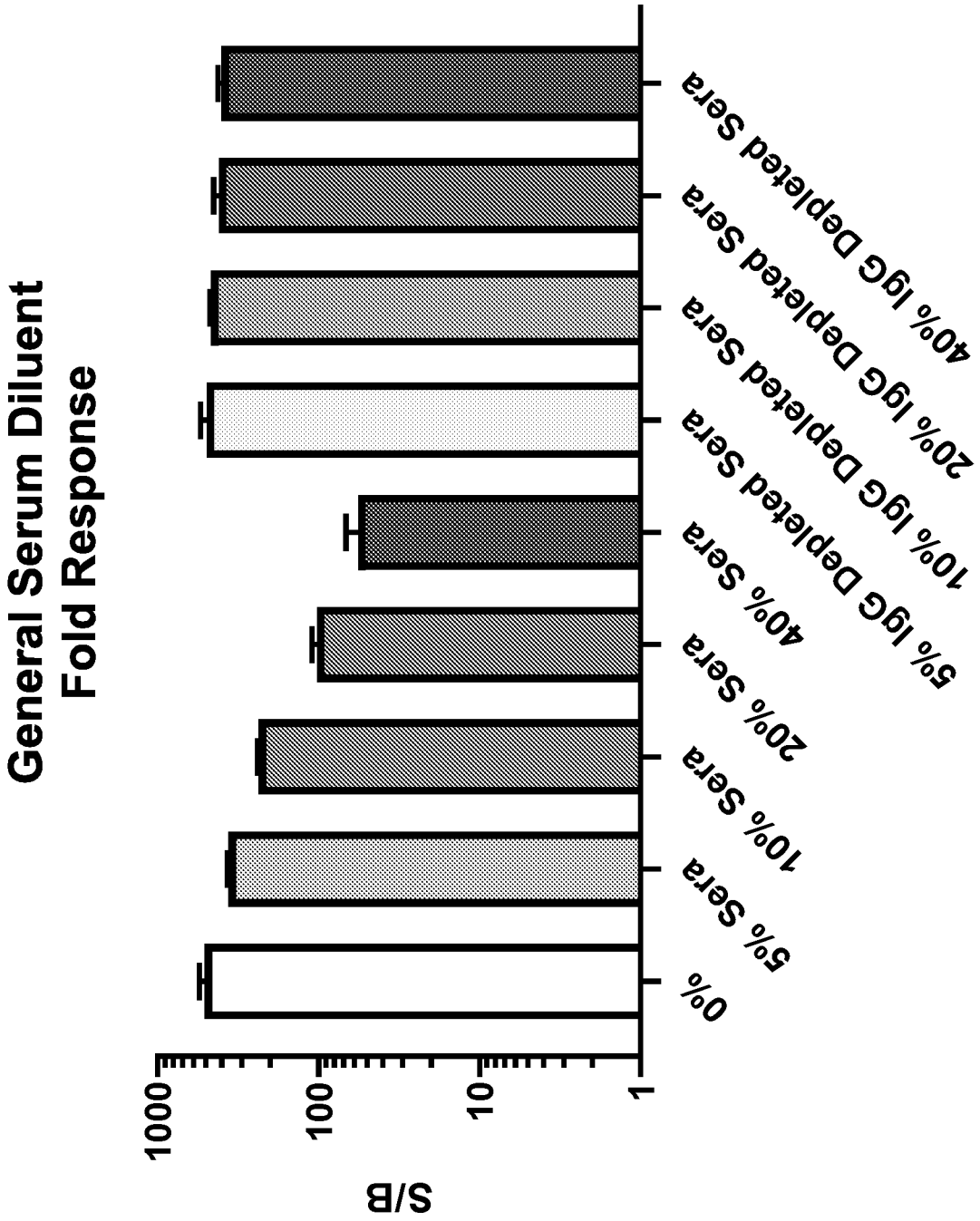

General Serum Diluent
Fold Response
S/B
1000
100
10
1
0%
5% Sera
10% Sera
20% Sera
40% Sera
5% IgG Depleted Sera
10% IgG Depleted Sera
20% IgG Depleted Sera
40% IgG Depleted Sera

FIG. 64

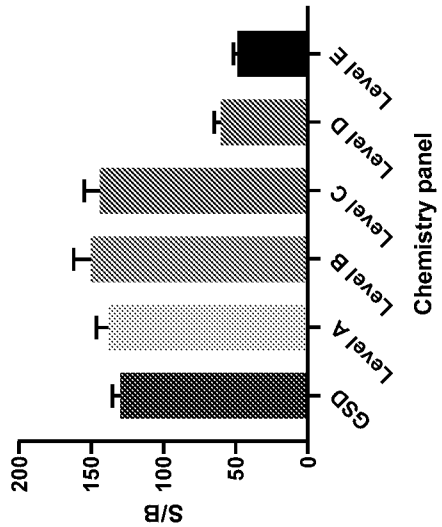


FIG. 65C

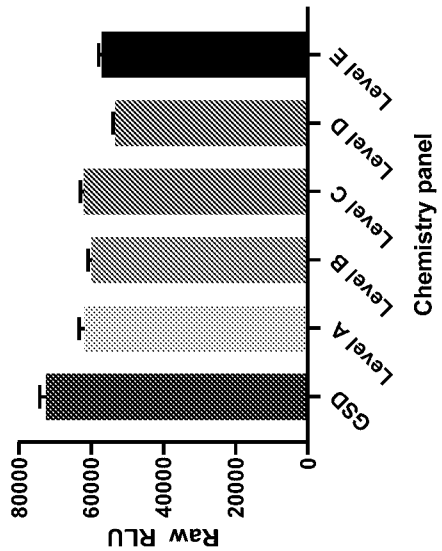


FIG. 65B

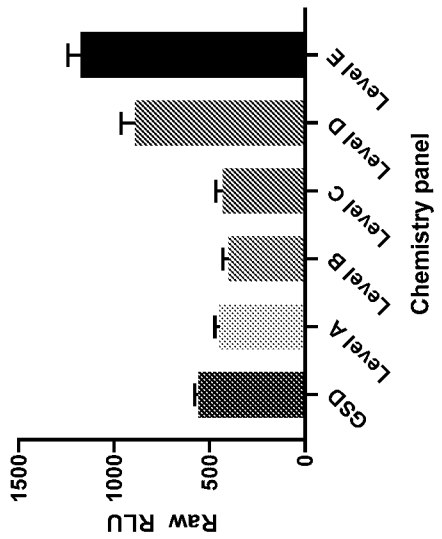


FIG. 65A

FIGS. 65A-65C

FIG. 66A

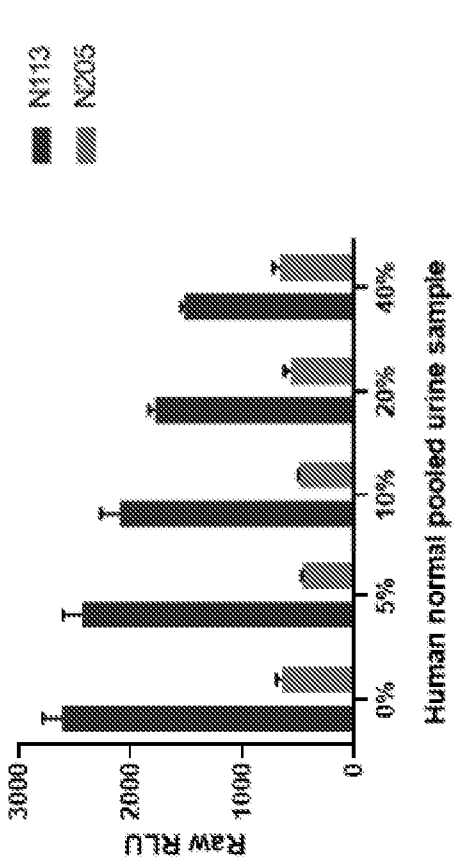


FIG. 66B

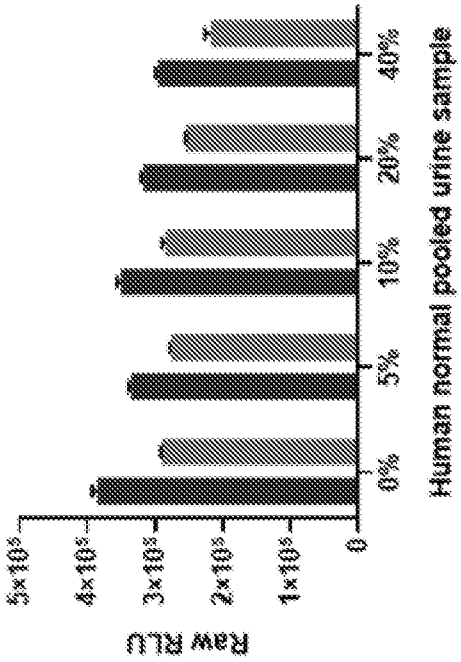


FIG. 66C

FIGS. 66A-66C

FIG. 67A

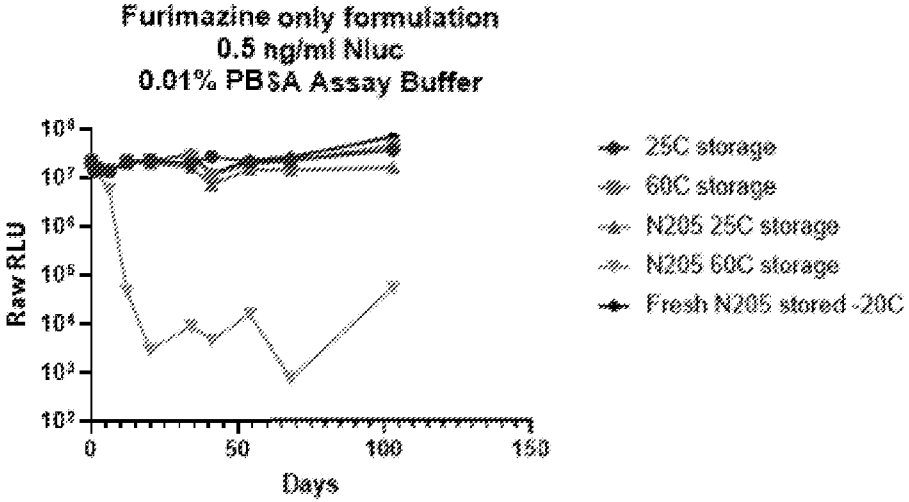


FIG. 67B

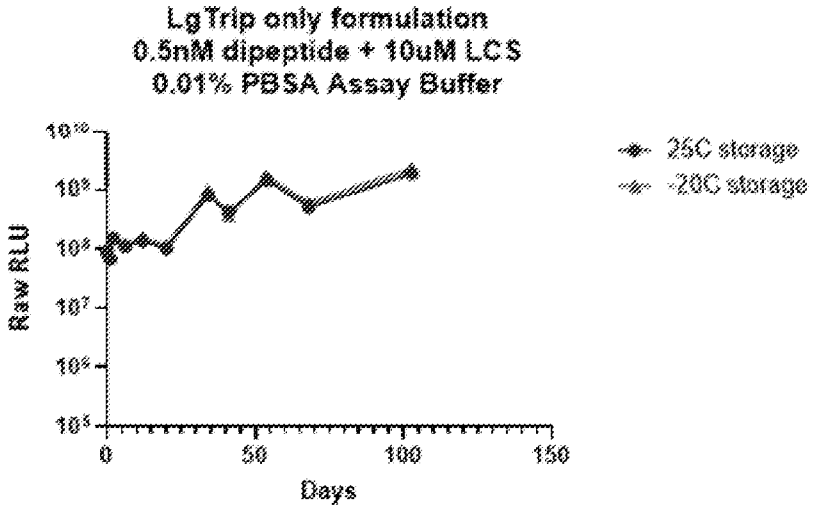
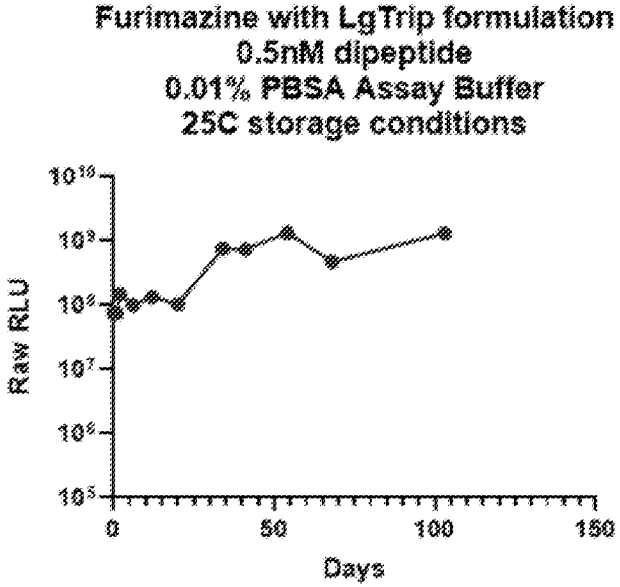


FIG. 67C



FIGS. 67A-76C

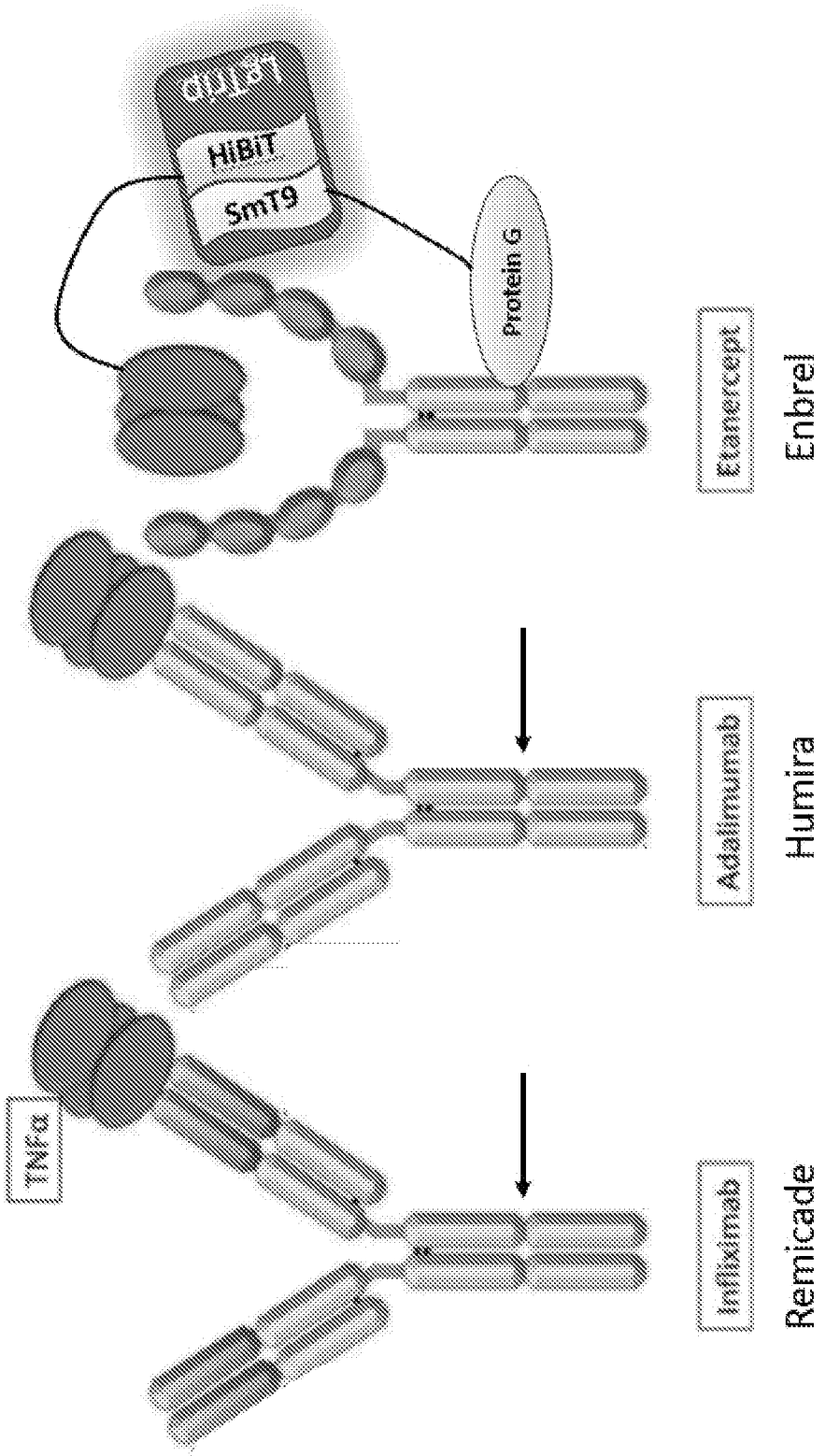

αLL/β1
HiBiT
SmT9
Protein G
Etanercept
Enbrel
Adalimumab
Humira
Infliximab
Remicade
TNFα

FIG. 68

FIG. 69A

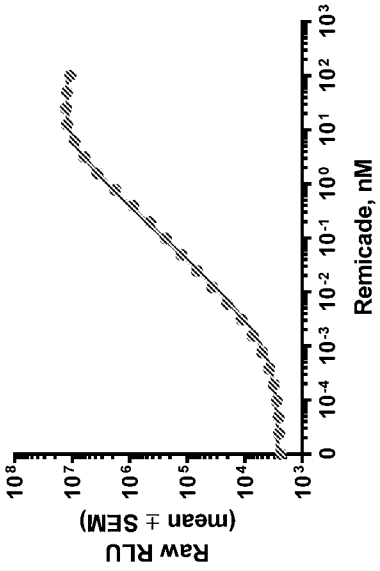


FIG. 69B

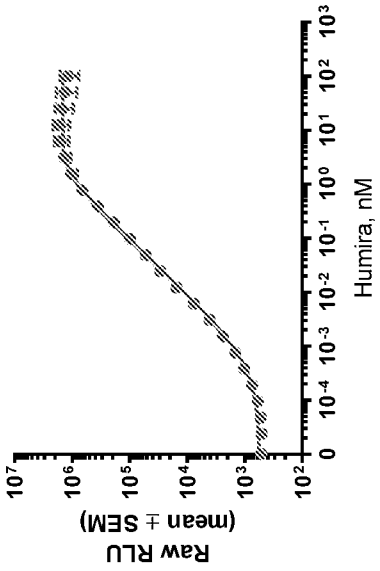
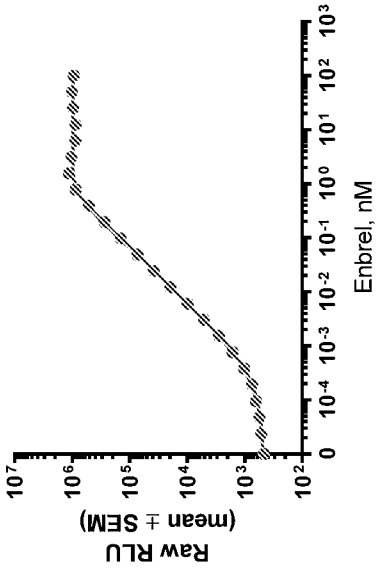


FIG. 69C



FIGS. 69A-69C

FIG. 70A

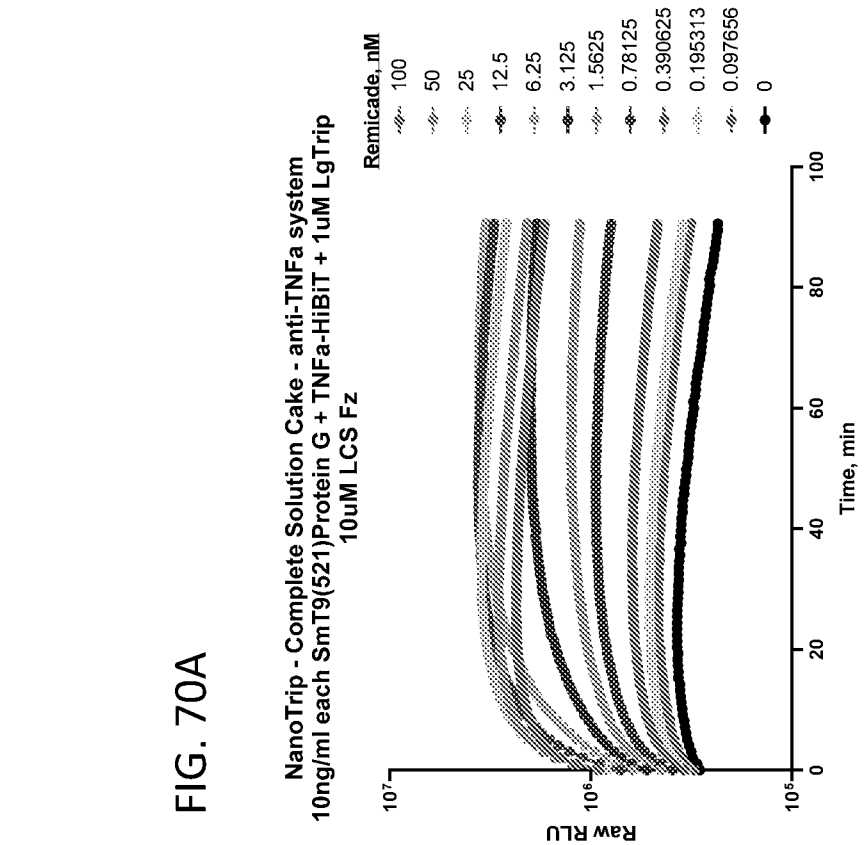
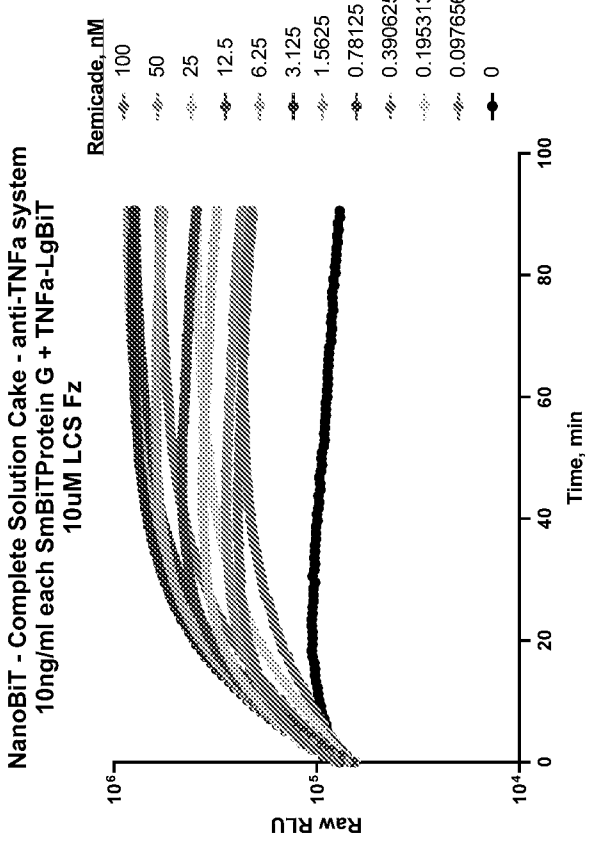


FIG. 70B



FIGS. 70A-70B

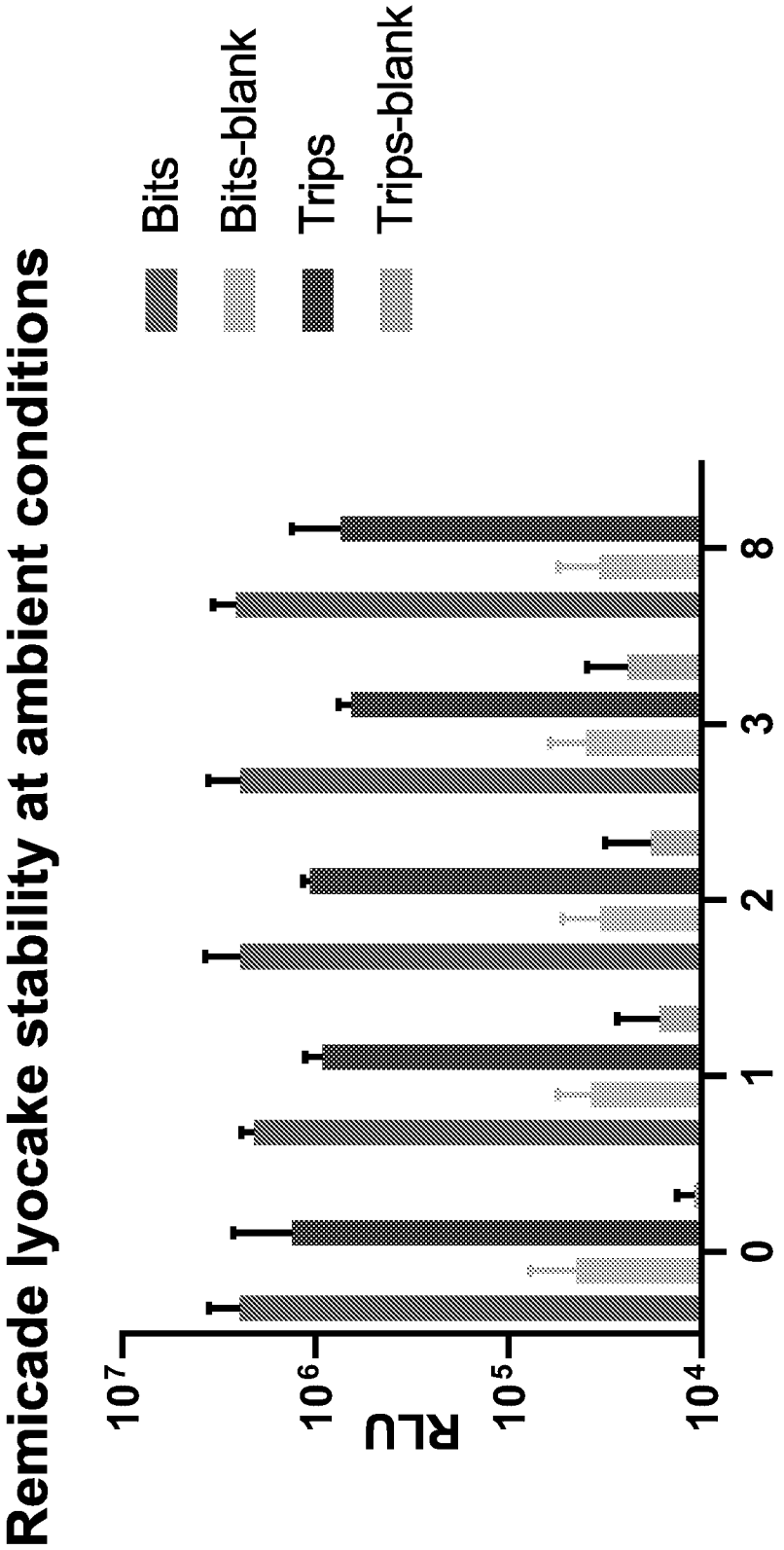

Remicade lyocake stability at ambient conditions
Bits
Bits-blank
Trips
Trips-blank
RLU
10^7
10^6
10^5
10^4
0
1
2
3
8
Days at 25 C

FIG. 71

FIG. 72A

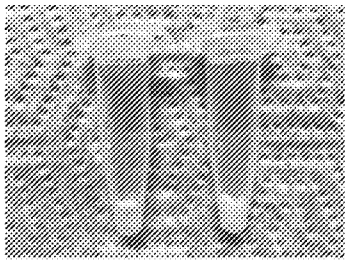


FIG. 72B

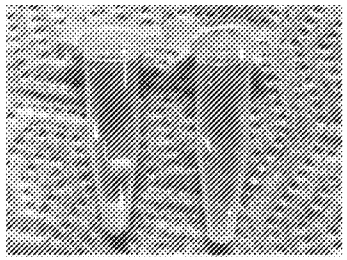


FIG. 72C

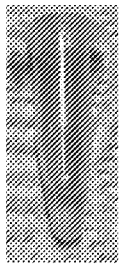


FIG. 72D

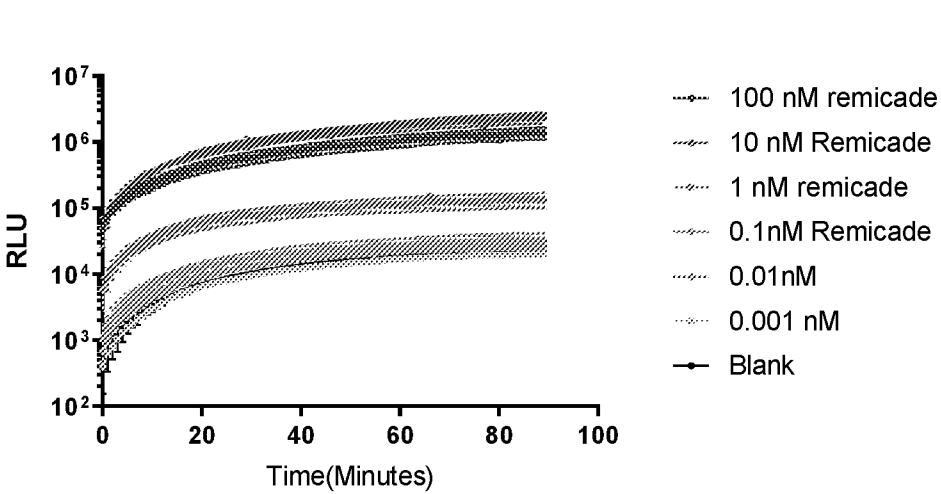


FIG. 72A:
Formulated, split-cakes
Yellow Vial: LgBiT-TNFα and furimazine
White Vial: SmBiT – Protein G

FIG. 72B:
Combining separate cakes manually

FIG. 72C:
Reconstituted cakes in opti-mem buffer containing analyte of interest

FIG. 72D:
Light output of Split NanoBiT cakes after reconstitution in the presence of increasing amounts of Remicade

FIGS. 72A-72D

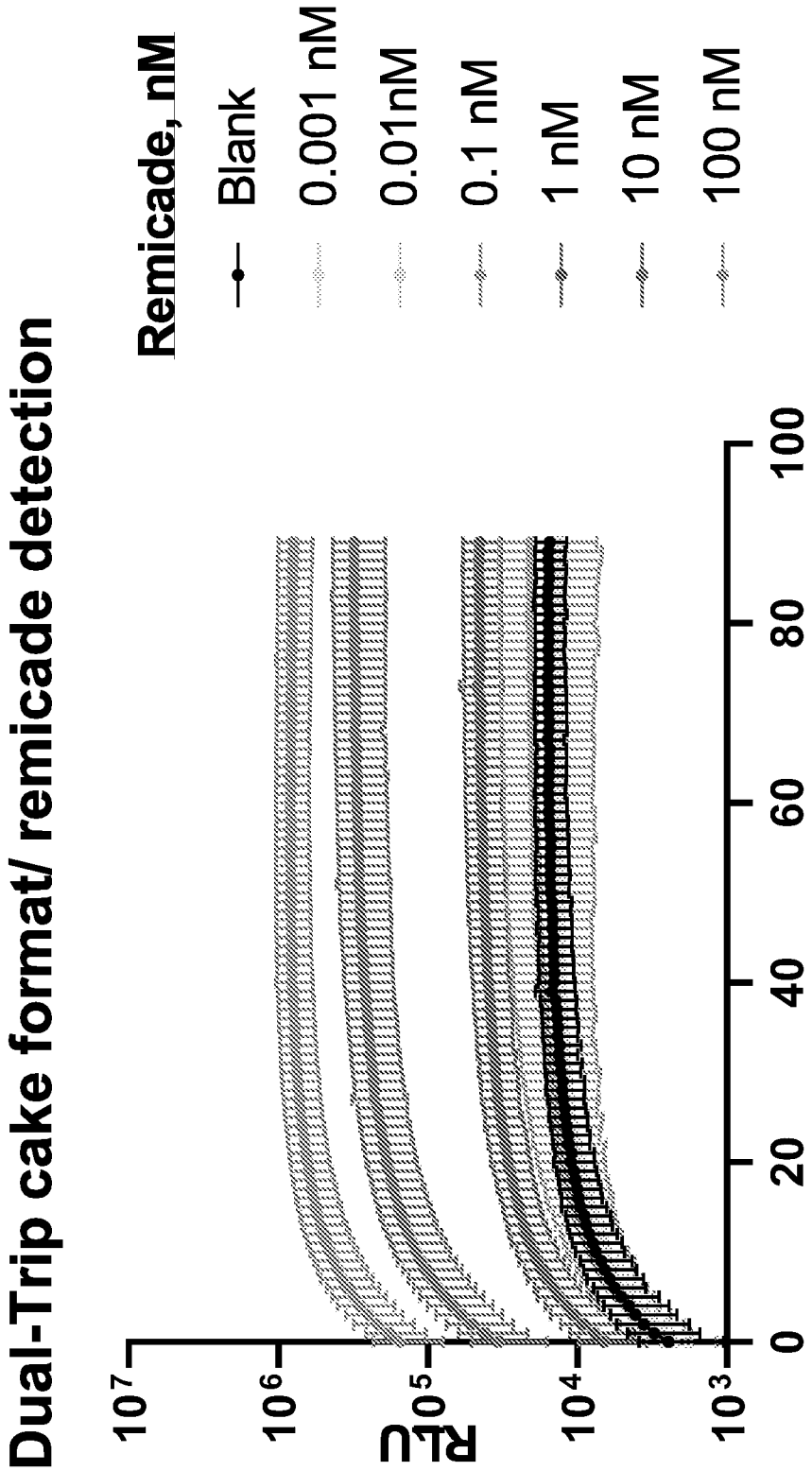

Dual-Trip cake format/ remicade detection
Remicade, nM
Blank
0.001 nM
0.01nM
0.1 nM
1 nM
10 nM
100 nM
RLU
10^7
10^6
10^5
10^4
10^3
0
20
40
60
80
100
Time(minutes)

FIG. 73

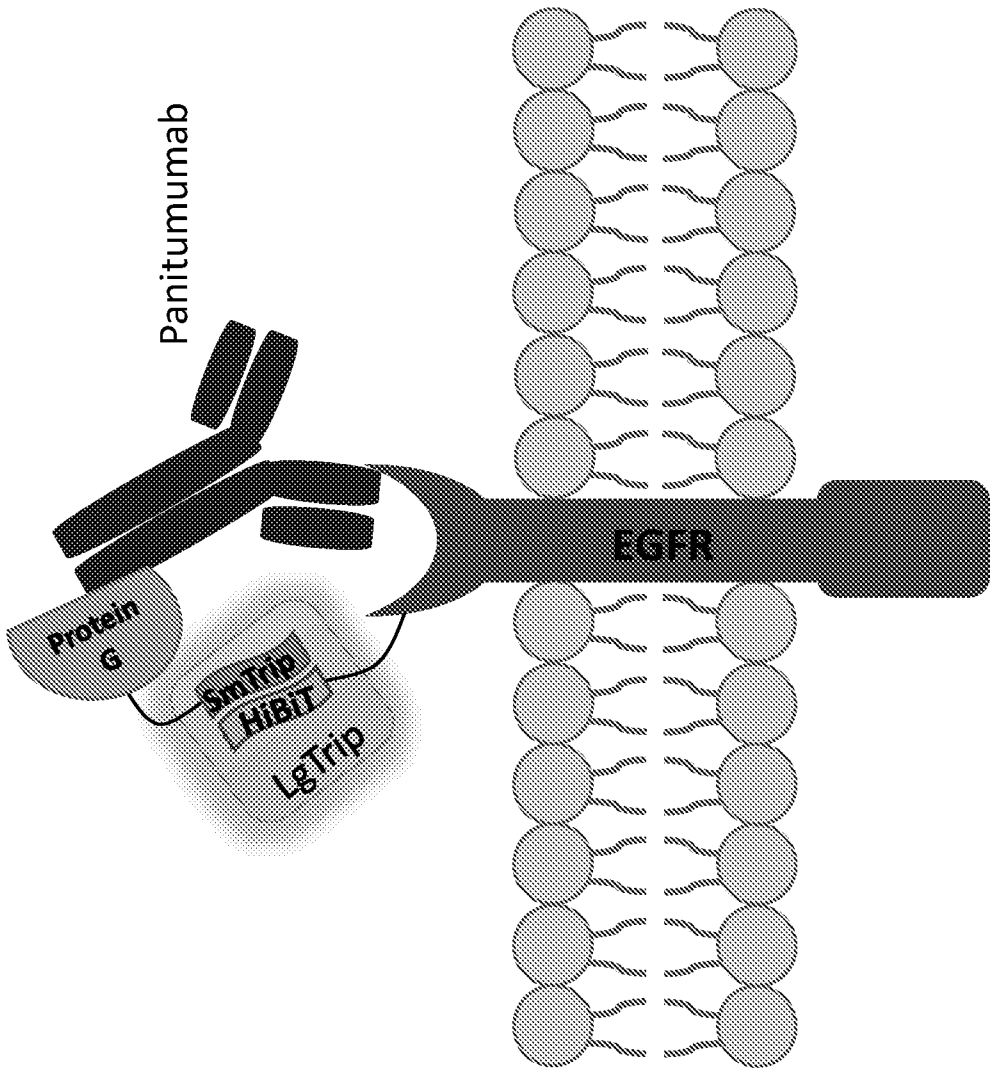


FIG. 74

- 5nM SmTrip9-Protein G
- 20,000 SmTrip10-EGFR expressing cells/well
- 1uM LgTrip
- 1 hour incubation at 37C
- Opti-MEM Assay Buffer
- 10uM final LCS
- *n = 3 independent experiments*

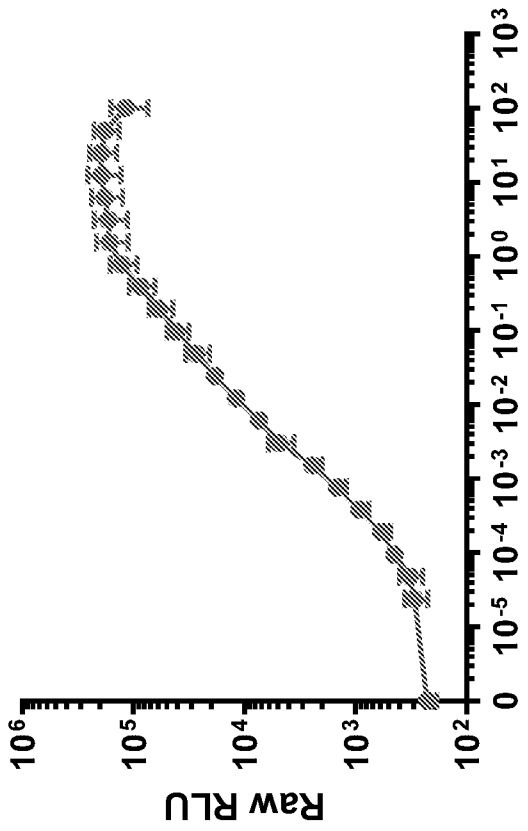


FIG. 75

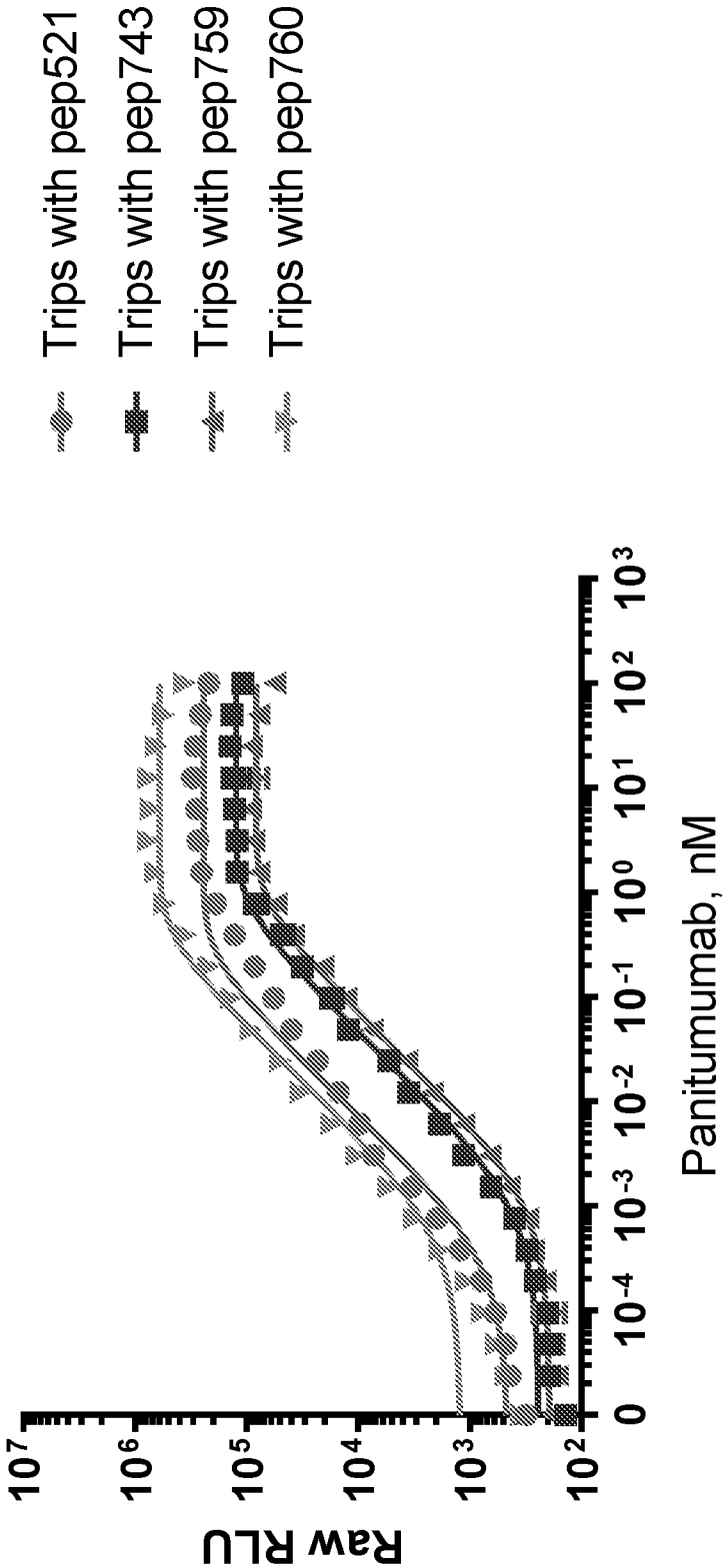
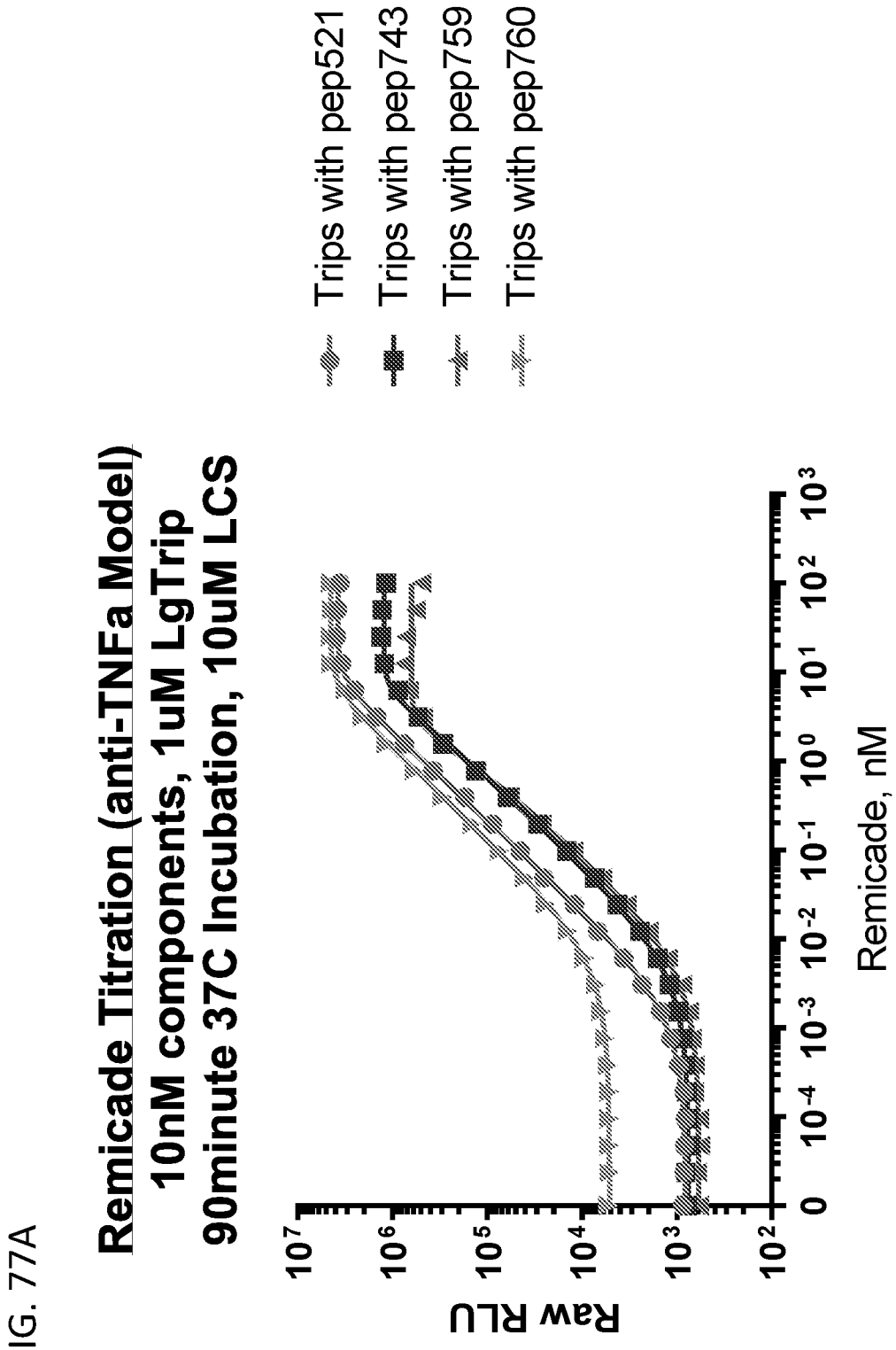

Raw RLU
Trips with pep521
Trips with pep743
Trips with pep759
Trips with pep760
Raw RLU
10^7
10^6
10^5
10^4
10^3
10^2
0
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^0
10^1
10^2
10^3
Panitumumab, nM

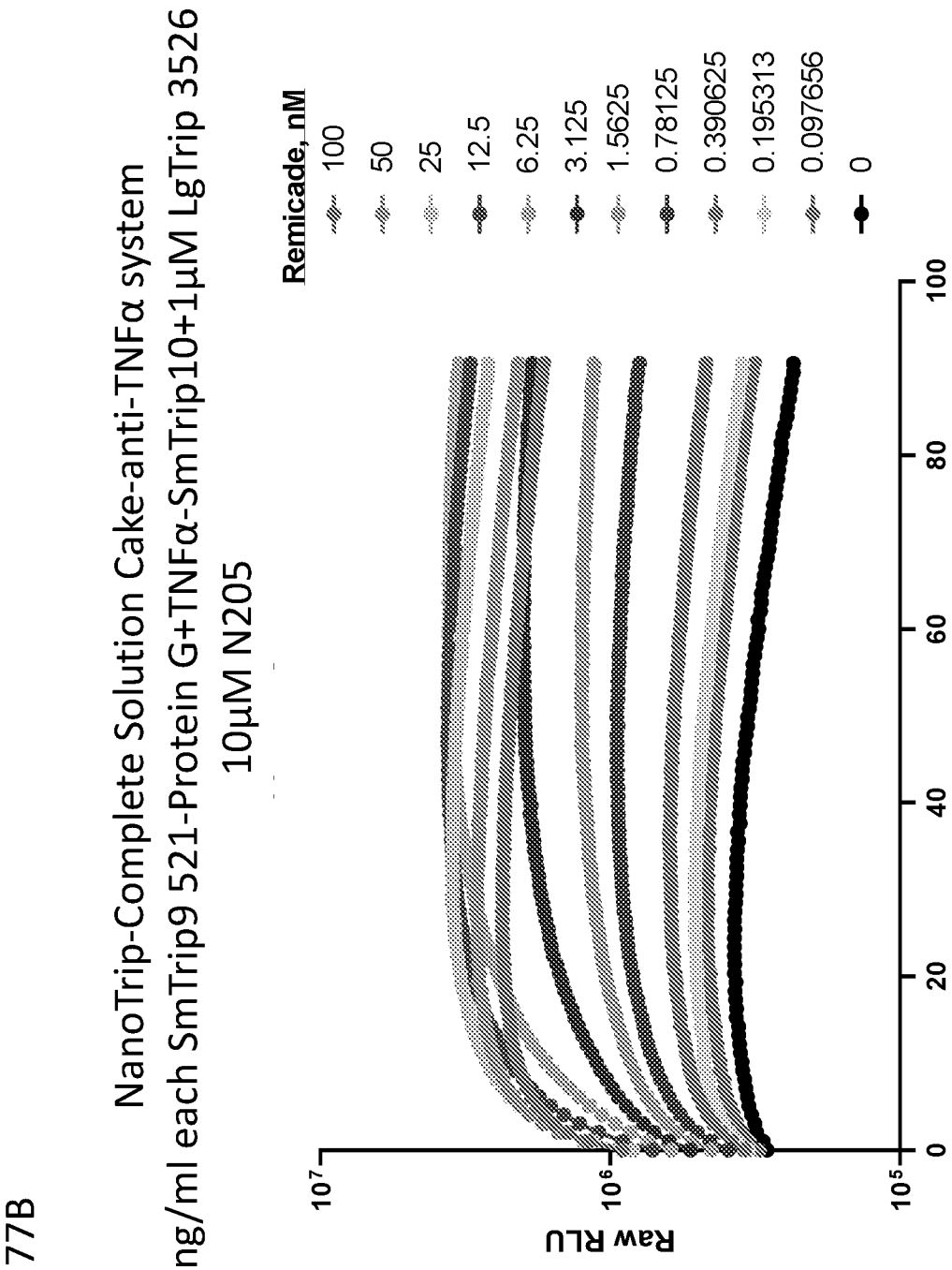
FIG. 76

FIG. 77A



FIGS. 77A-77B

FIG. 77B



FIGS. 77A-77B

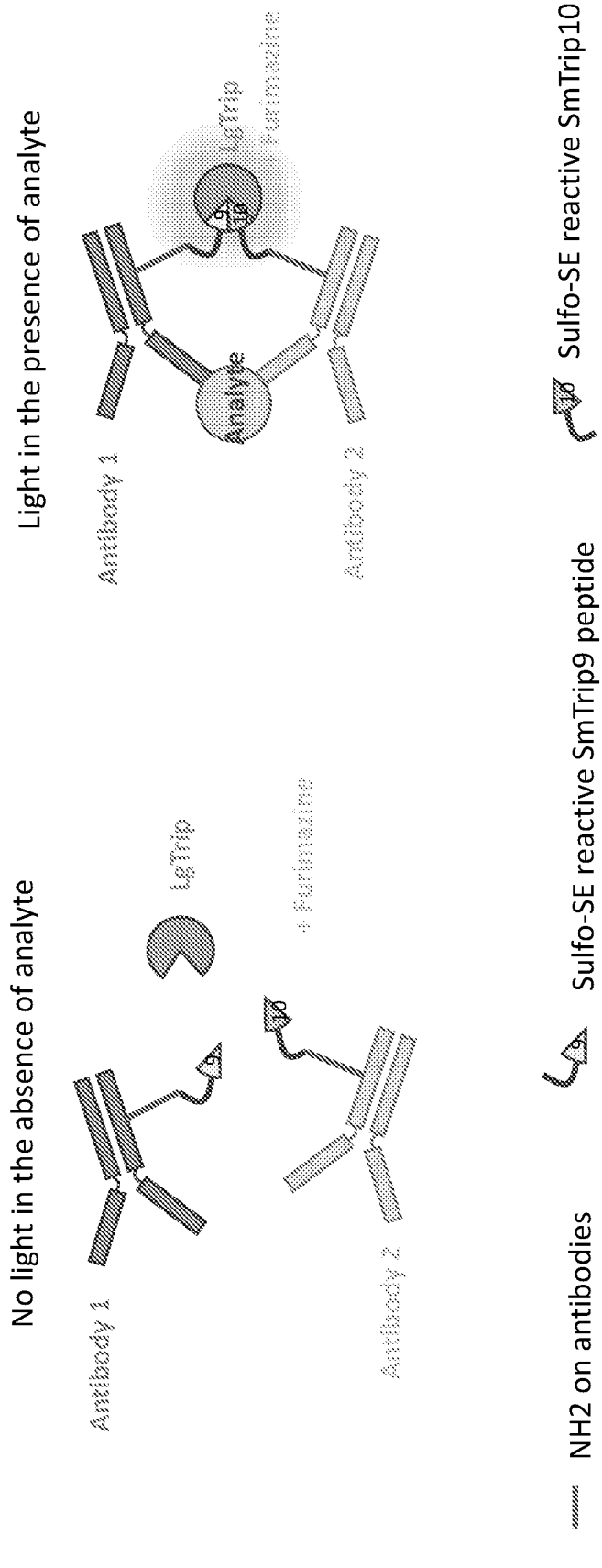

No light in the absence of analyte
Antibody 1
9
LgTrip
+ Furimazine
10
Antibody 2
Light in the presence of analyte
Antibody 1
LgTrip
Furimazine
9
10
Analyte
Antibody 2
NH2 on antibodies
Sulfo-SE reactive SmTrip9 peptide
Sulfo-SE reactive SmTrip10 peptide

FIG. 78

FIG. 79A

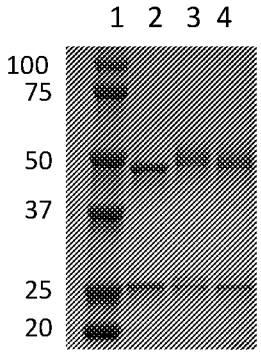


FIG. 79B

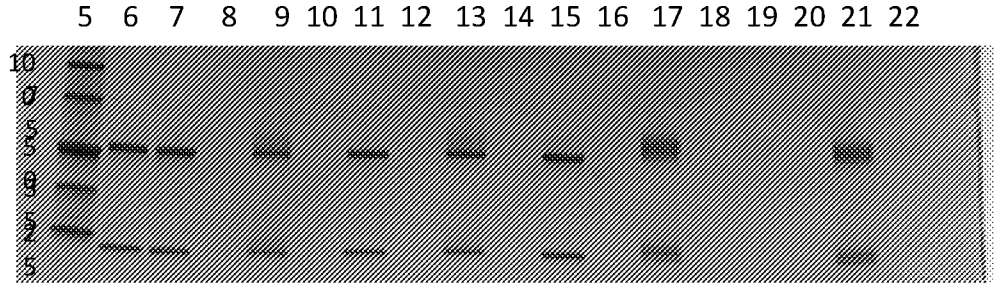
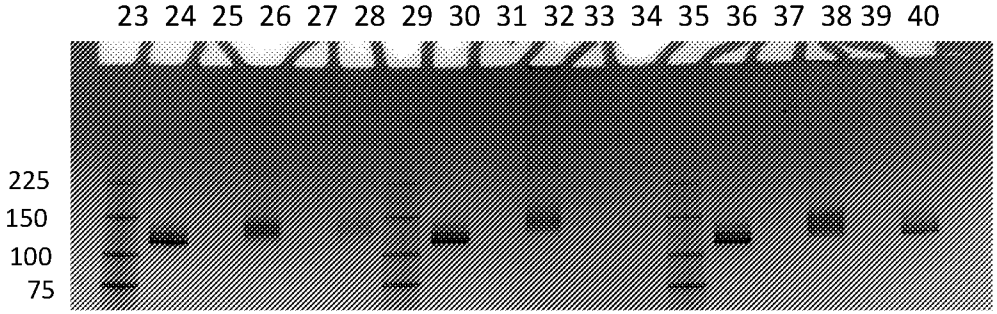


FIG. 79C



Lane	Sample
1	PP Dual Color Ladder
2	505E Ab ctrl
3	HW-0977 + 505E #2
4	HW-1053 + 505E
5	Promega Ladder
6	5IL6 Ab ctrl
7	5IL6 Ab ctrl
8	HW-1010 only
9	5IL6 + HW-1010 rxn
10	HW-1010 only – post clean
11	5IL6 + HW-1010 rxn – post clean
12	HW-1043 only
13	5IL6 + HW-1043 rxn
14	HW-1043 only – post clean
15	5IL6 + HW-1043 – post clean
16	HW-1052 only
17	5IL6 + HW-1052 rxn
18	HW-1052 only – post clean
19	5IL6 + HW-1052 rxn – post clean
20	HW-1055 only
21	5IL6 + HW-1055 rxn
22	5IL6 + HW-1055 rxn – post clean
23	Promega Ladder
24	5IL6 Ab ctrl
25	HW-0984 only
26	5IL6 +HW-0984 rxn
27	HW-0984 only – post clean
28	5IL6 + HW-0984 rxn – post clean
29	Promega Ladder
30	5IL6 Ab ctrl
31	HW-1042 only
32	5IL6 + HW-1042 rxn
33	HW-1042 only – post clean
34	5IL6 + HW-1042 rxn – post clean
35	Promega Ladder
36	5IL6 Ab ctrl
37	HW-1050 only
38	5IL6 +HW-1050 rxn
39	HW-1050 only – post clean
40	5IL6 + HW-1050 rxn – post clean

FIGS. 79A-79C

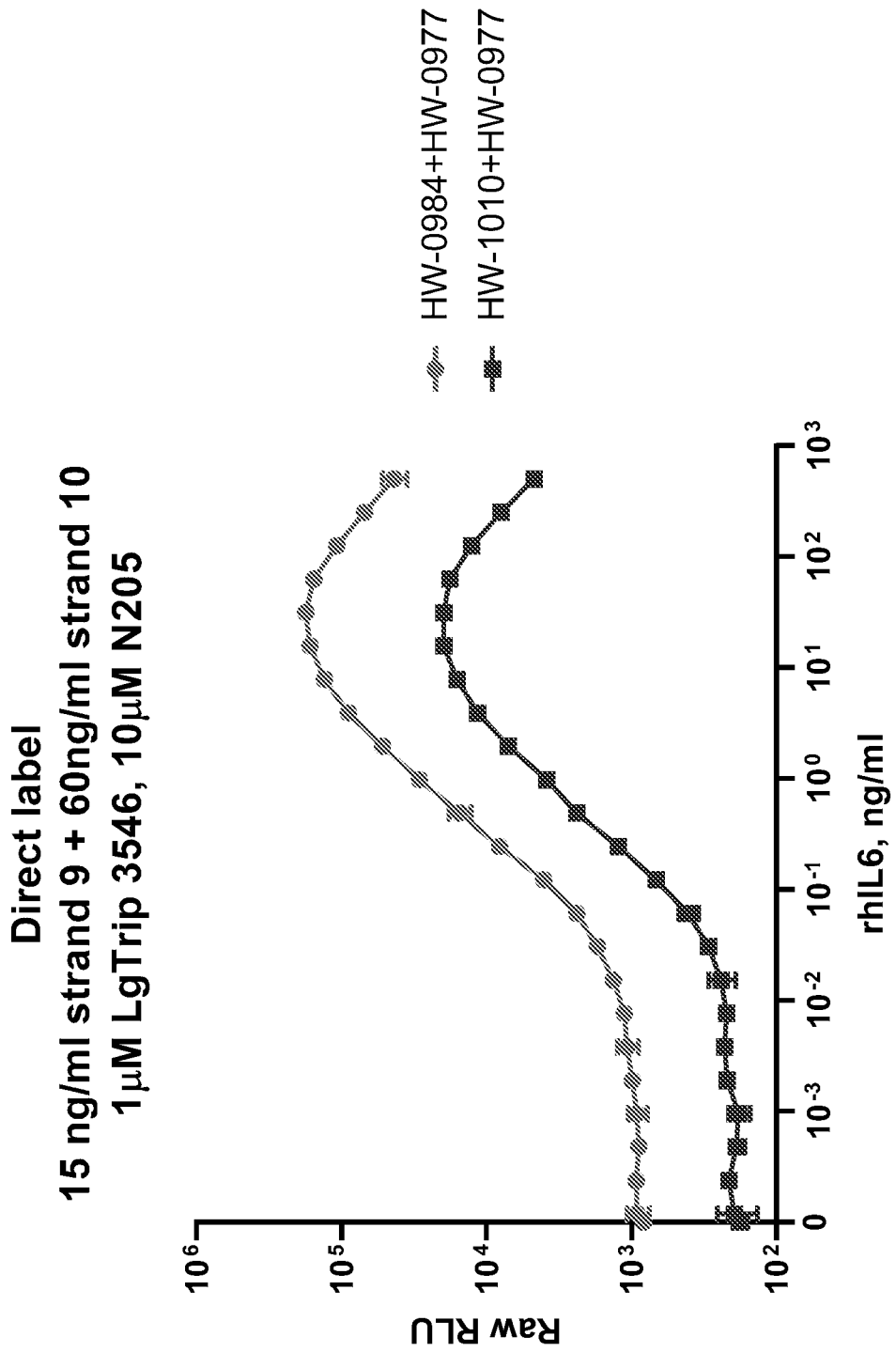

Direct label
15 ng/ml strand 9 + 60ng/ml strand 10
1μM LgTrip 3546, 10μM N205
HW-0984+HW-0977
HW-1010+HW-0977
Raw RLU
rhIL6, ng/ml
10^6
10^5
10^4
10^3
10^2
0
10^-3
10^-2
10^-1
10^0
10^1
10^2
10^3

FIG. 80

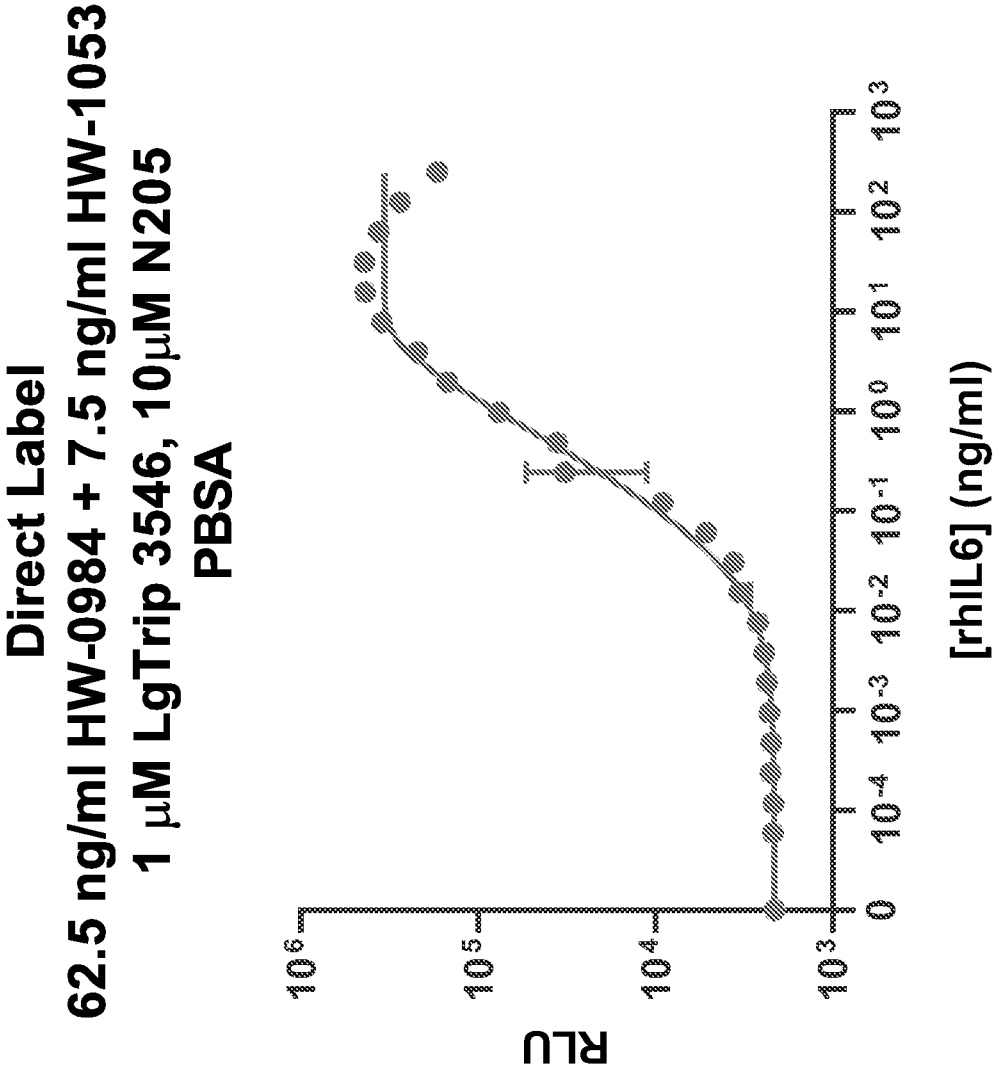

Direct Label
62.5 ng/ml HW-0984 + 7.5 ng/ml HW-1053
1 μM LgTrip 3546, 10μM N205
PBSA
RLU
10^6
10^5
10^4
10^3
0
10^-4
10^-3
10^-2
10^-1
10^0
10^1
10^2
10^3
[rhIL6] (ng/ml)

FIG. 81

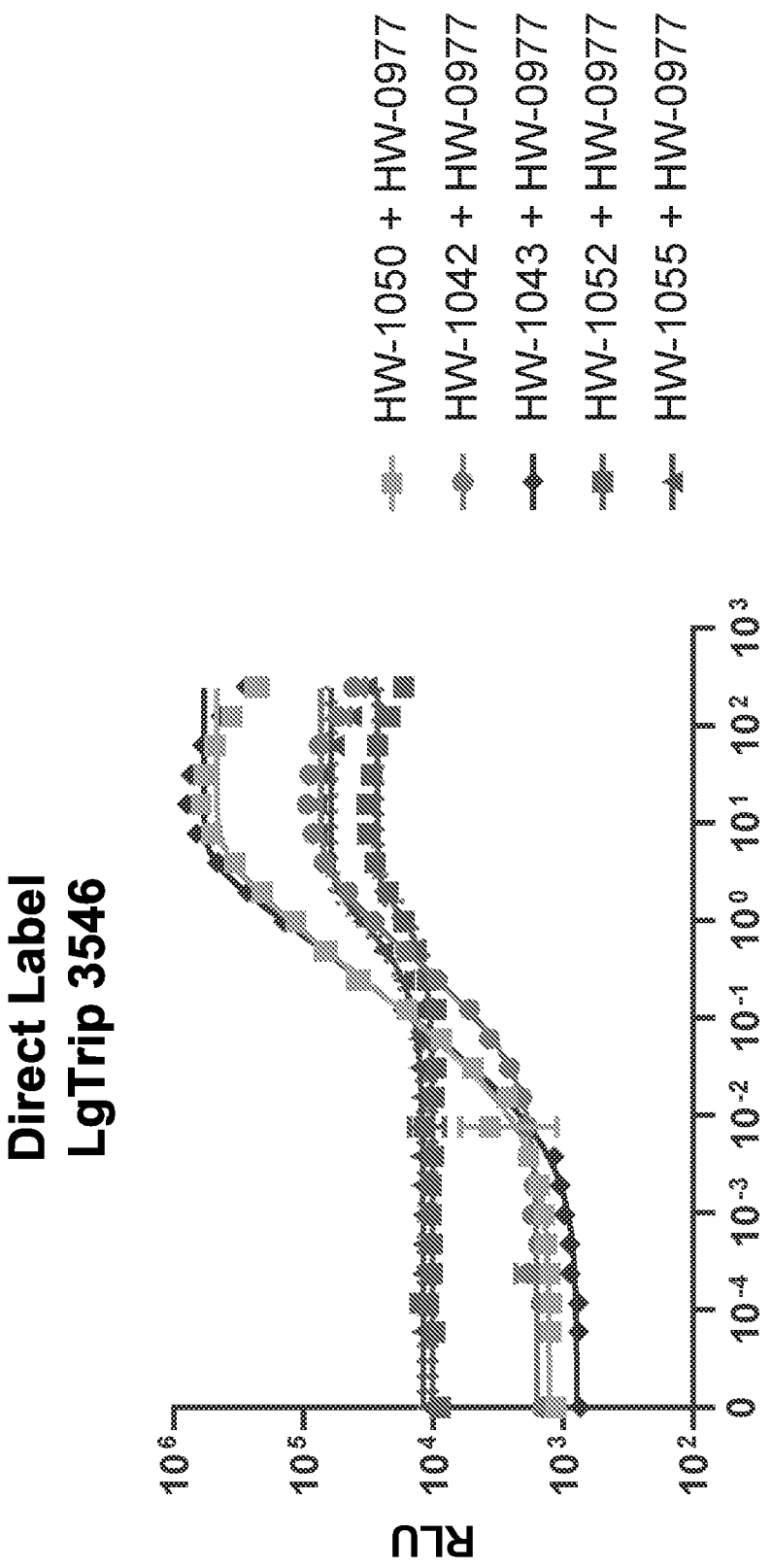

Direct Label
LgTrip 3546
HW-1050 + HW-0977
HW-1042 + HW-0977
HW-1043 + HW-0977
HW-1052 + HW-0977
HW-1055 + HW-0977
RLU
10^6
10^5
10^4
10^3
10^2
0
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^0
10^1
10^2
10^3
[rhIL6] (ng/ml)

FIG. 82

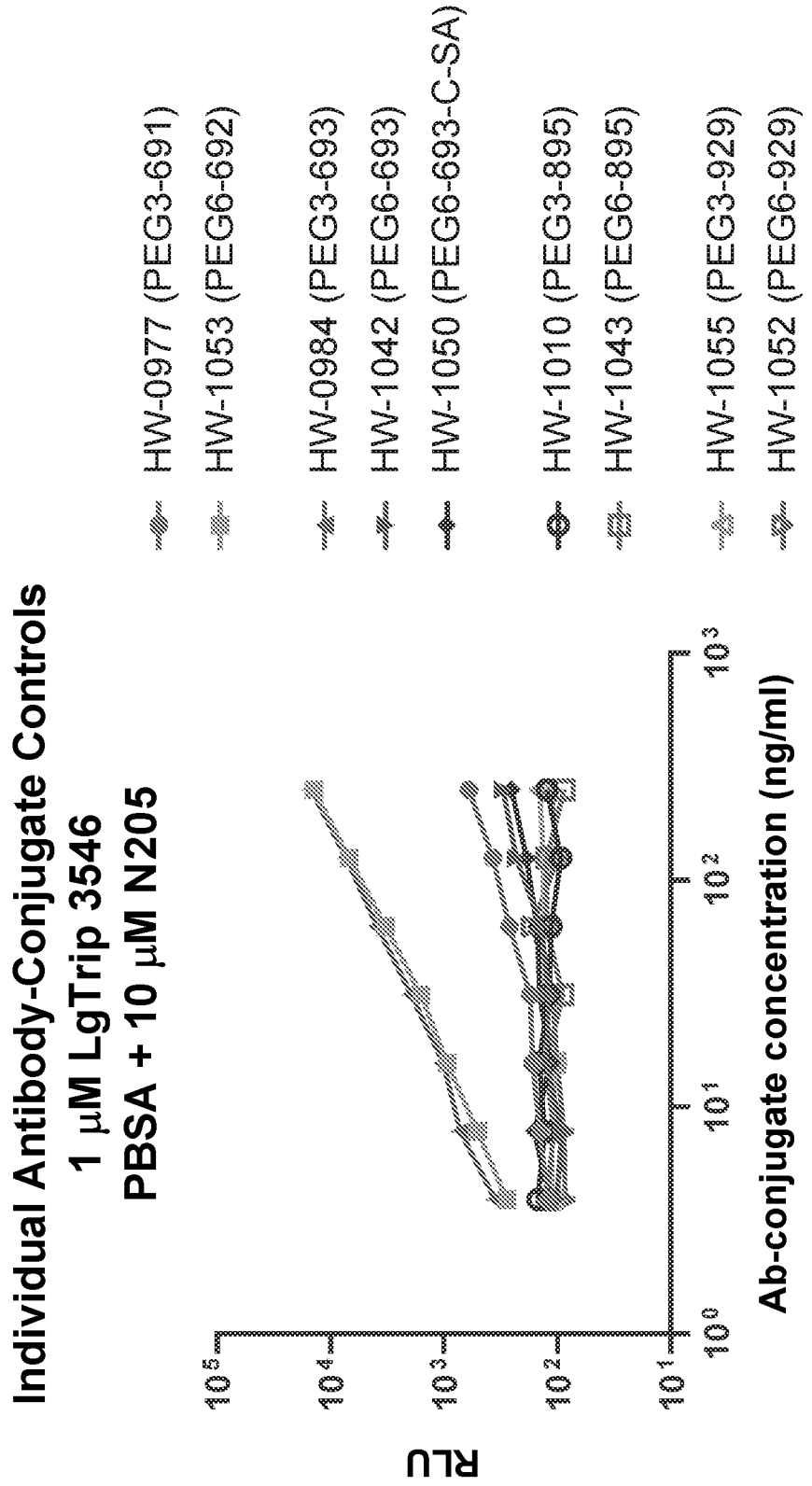

Individual Antibody-Conjugate Controls
1 μM LgTrip 3546
PBSA + 10 μM N205
HW-0977 (PEG3-691)
HW-1053 (PEG6-692)
HW-0984 (PEG3-693)
HW-1042 (PEG6-693)
HW-1050 (PEG6-693-C-SA)
HW-1010 (PEG3-895)
HW-1043 (PEG6-895)
HW-1055 (PEG3-929)
HW-1052 (PEG6-929)
RLU
10^5
10^4
10^3
10^2
10^1
10^0
10^1
10^2
10^3
Ab-conjugate concentration (ng/ml)

FIG. 83

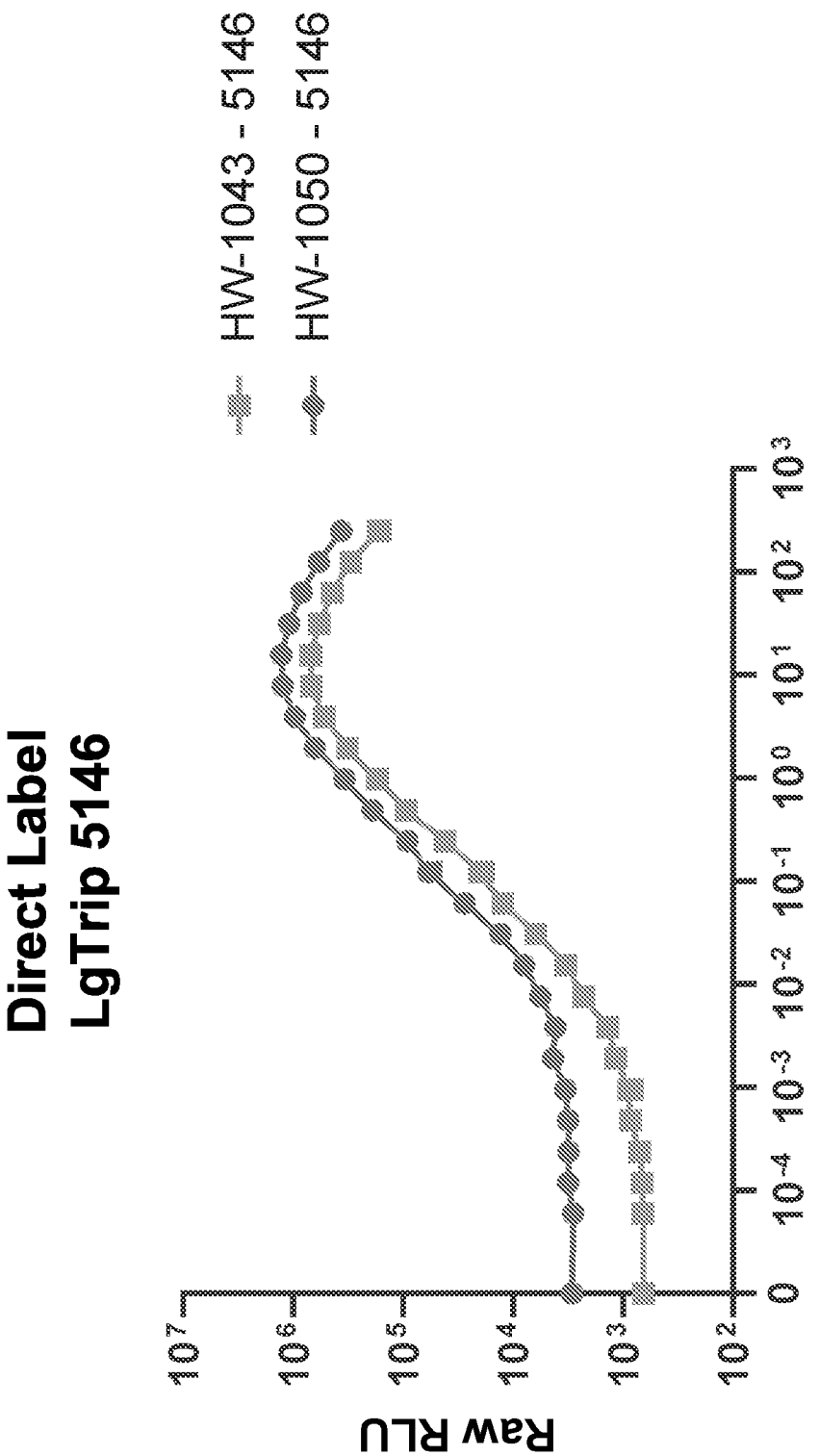

Direct Label
LgTrip 5146
HW-1043 - 5146
HW-1050 - 5146
Raw RLU
[rhIL6] (ng/ml)

FIG. 84

No light in the absence of analyte
Antibody 1
LgTrip
+ furimazine
Antibody 2
Light in the presence of analyte
Antibody 1
LgTrip
Furimazine
Analyte
Antibody 2
NH2 on antibodies
Sulfo-SE reactive SmTrip9 peptide with fluorescent BRET acceptor label
Sulfo-SE reactive SmTrip10 peptide with fluorescent BRET acceptor label

FIG. 85

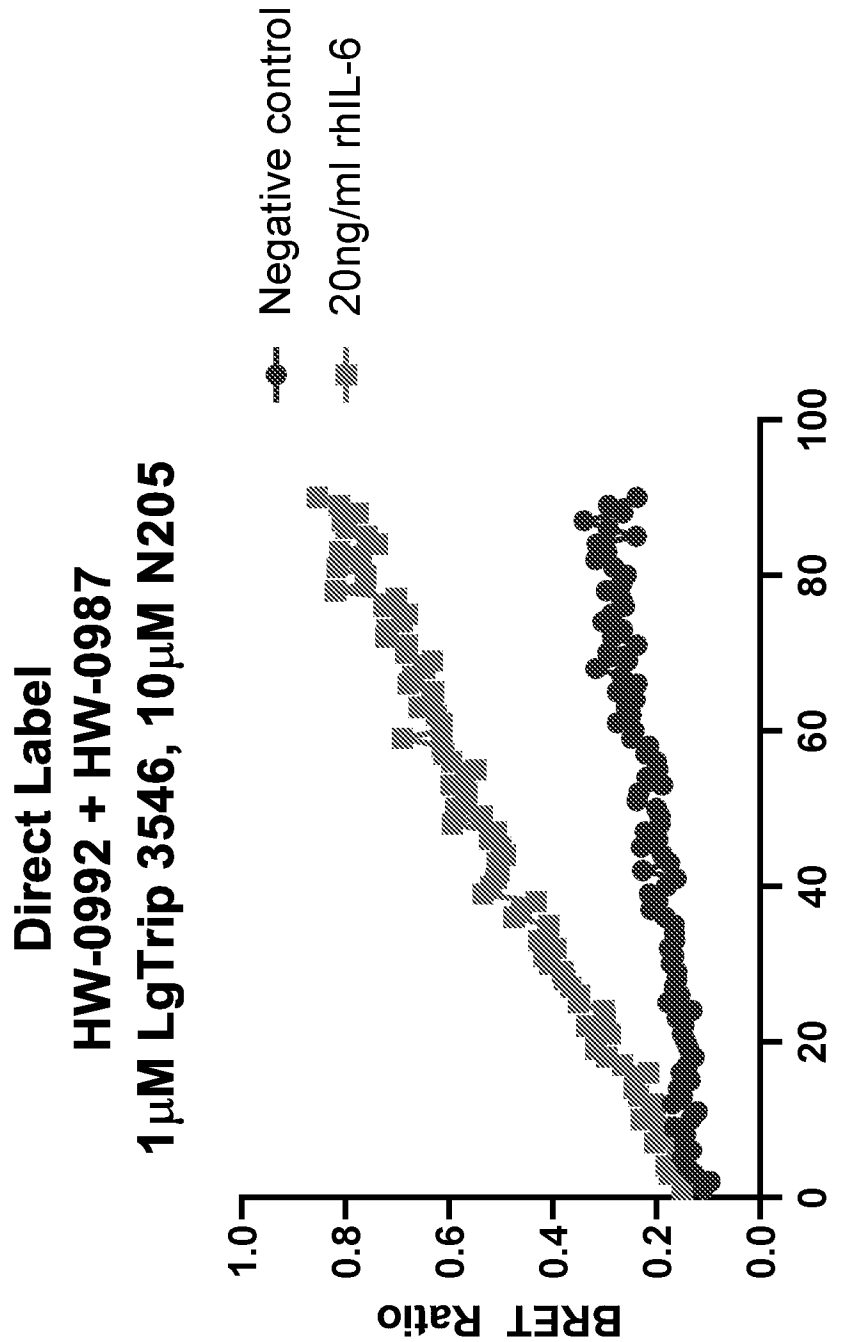

Direct Label
HW-0992 + HW-0987
1μM LgTrip 3546, 10μM N205
Negative control
20ng/ml rhIL-6
BRET Ratio
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
Time, minutes

FIG. 86

FIG. 87A

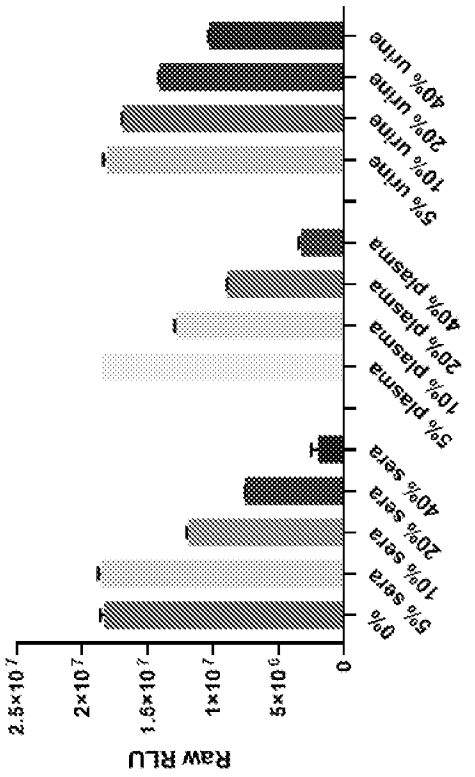


FIG. 87B

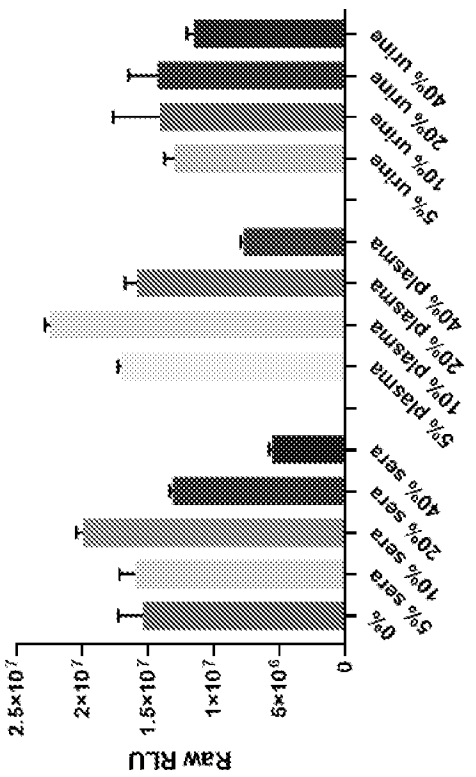
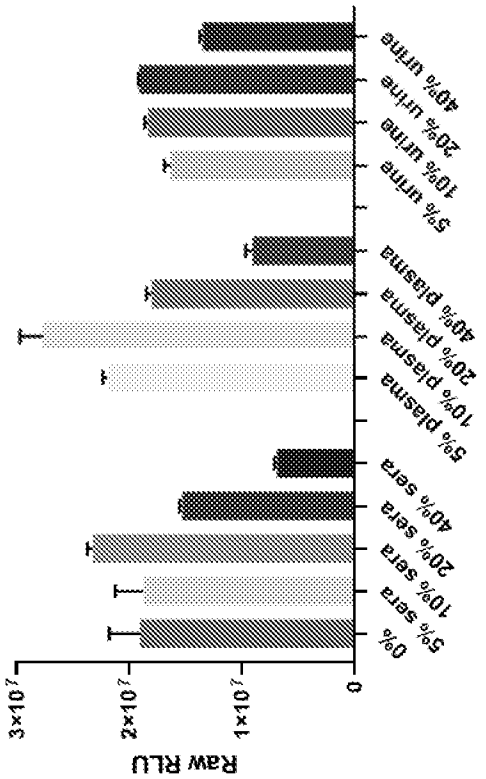


FIG. 87C



FIGS. 87A-87C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 20/27711

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C07K 14/435; C12N 9/02; C07K 7/08; G01N 33/542; C12Q 1/66; A61J 1/20 (2020.01)

CPC - C07K 14/43504; C12N 15/1055; C07K 7/08; G01N 33/542; C07K 2319/00; C12N 9/0069; A61J 1/2093

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	US 2018/0095074 A1 (PROMEGA CORPORATION) 5 April 2018 (05.04.2018) para [0004], [0008], [0235], [0357], [0387], [0595], [0596], claims 42, 44, 47-49, SEQ ID NOs: 390, 440, 836, 2150, 2157, 2271.	1-4, 6, 157-159 ---------- 5, 69-77, 89-119
Y	US 6,045,254 A (INBAR et al.) 4 April 2000 (04.04.2000) claims 1, 19	5
Y	US 7,829,347 B2 (SONG) 9 November 2010 (09.11.2010) pg 32 Table 1 col 12 ln 63-65, claims 1-2.	69-77, 89-119
Y	US 2004/0096924 A1 (HAWKINS et al.) 20 May 2004 (20.05.2004) para [0153], [0154]	94-95, 102,103, 118, 119
Y	US 2017/0328896 A1 (INSIGHT INSTRUMENTS, INC.) 16 November 2017 (16.11.2017) para [0183]	98, (99,101-103)/98 ,113
Y	US 2014/0194325 A1 (PROMEGA CORP) 10 July 2014 (10.07.2014) para [0029]	111-112

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 August 2020	18 SEP 2020
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Lee Young Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 20/27711

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 7-9, 22-33, 40-68, 78-88, 149-156, 160-177
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
----Go to Extra Sheet for continuation------

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
Claims 1-6, 69-77, 89-119, 157-159

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 20/27711

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:
GenCore ver 6.4.1 SEQ ID NOs: 5, 6, 10-14 were searched.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US 20/27711

Continuation of Box III: Observations where Unity of Invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I: Claims 1-6, 157-159, drawn to a composition comprising a luminogenic substrate.
Group II: Claims 10-18, 39, drawn to a composition that is a dry formulation.
Group III: Claims 19-21, 34-38 drawn to a composition that includes a liquid formulation.
Group IV: Claims 69-77, 89-119, drawn to a lateral flow detection system of a solid phase detection platform.
Group V: Claims 120-133, drawn to a solution phase detection platform.
Group VI: Claims 134-145, drawn to a method of detecting an analyte in a sample.
Group VII: Claims 146-148, drawn to a method of producing a substrate for use in a bioluminescent assay.

The inventions listed as Groups I-VII do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features:

Group I has the special technical feature of a composition comprising a luminogenic substrate, not required by Groups II-VII.

Group II has the special technical feature of a composition where all binding agents and complementary peptides and/or polypeptides are in a dry formulation, not required by Groups I, III-VII.

Group III has the special technical feature of a composition in two parts, comprising a dry formulation and a liquid formulation, where one or more of a binding agent, complementary peptide and complementary polypeptide are in the liquid formulation, not required by Groups I, II, IV-VII.

Group IV has the special technical feature of a lateral flow detection system or solid phase detection platform comprises a conjugate pad, an analytical membrane, and/or detection region, not required by Groups I-III, V-VII.

Group V has the special technical feature of a solution phase detection platform comprising a detection receptacle and a lyophilized table (lyocake), not required by Groups I-IV, VI, VII.

Group VI has the special technical feature of method steps used in detecting an analyte in a sample, not required by Groups I-V, VII.

Group VII has the special technical feature of a method of making a substrate for a bioluminescent assay, not required by Groups I-VI.

Common Technical Features:

1. Groups I-V share the common technical feature of a target binding agent comprising a target binding element and a polypeptide component or a peptide component [see instant application para [0248] for a definition of the difference between a peptide and a polypeptide].

2. Groups II-III share the common technical feature of a first target binding agent and a second target binding agent.

3. Groups II, III, IV share the common technical feature of a peptide component having at least 60% identity to SEQ ID NOs: 10, 11, 13, 14 and 15.

4. Groups II, III, IV share the common technical feature of a polypeptide component having at least 60% sequence identity to SEQ ID NOs 5, 9 and 12.

5. Group VI claim 134 depends from Group I claim 1.

6. Group VI claim 140 depends from Group III claim 34.

7. Groups II and III share a dry formulation

However, said common technical features do not represent a contribution over the prior art, and is disclosed by US 2018/0095074 A1 to Promega Corporation (hereinafter "Promega"), in view of US 6,045,254 A to Inbar et al. (hereinafter "Inbar").

-----continued on next sheet----

----continued from previous sheet-----

As to common technical feature #1, Promega discloses a target binding agent comprising a target binding element and a polypeptide component or a peptide component (claim 42; "A kit comprising:
(a) a peptide comprising a peptide amino acid sequence having greater than 45% but not greater than 90% sequence identity with SEQ ID NO: 2, wherein the peptide amino acid sequence is not a naturally occurring protein or a fragment thereof, and wherein the peptide is conjugated to a first binding moiety;
(b) a polypeptide comprising a polypeptide amino acid sequence having greater than 40% but not greater than 95% sequence identity with SEQ ID NO: 440, wherein the polypeptide amino acid sequence is not a naturally occurring protein or a fragment thereof, and wherein the polypeptide is conjugated to a second binding moiety;
wherein a bioluminescent signal produced in the presence of a furimazine substrate is substantially increased when the peptide contacts the polypeptide when compared to a bioluminescent signal produced by either: (i) the peptide and furimazine substrate alone and (ii) the polypeptide and furimazine substrate alone").

As to common technical feature #2, Promega discloses a first target binding agent and a second target binding agent (clam 42).

As to common technical feature #3, Promega discloses a peptide component having at least 60% identity to SEQ ID NOs: 10, 11, 13, 14 and 15 [note: SEQ ID NO: 11 and 15 are identical]:

SEQ ID NO: 10 (Table 1 SEQ ID NO: 2271; 1-VTGYRLFEEIL-11 100% sequence identity)
SEQ ID NOs: 11 and 15 (Table 1 SEQ ID NO: 390; 1-VSGWRLFKKIS-11 100% sequence identity)
SEQ ID NO: 13 (Table 1 SEQ ID NO: 2130; 148-GSMLFRVTINS-158 100% sequence identity)
SEQ ID NO: 14 (Table 1 SEQ ID NO: 406 6-RVTINpVSGWRLFKKIS-22 72.8% sequence identity)

As to common technical feature #4, Promega discloses a polypeptide component having at least 60% sequence identity to SEQ ID NOs 5, 9 and 12.

SEQ ID NO: 5 (SEQ ID NO: 440; AA 1-158 100% sequence identity)
SEQ ID NO: 9 (SEQ ID NO: 440; AA 1-158; 90.5 % sequence identity)
SEQ ID NO: 12 (SEQ ID NO: 440: AA 1-155 87.8 % sequence identity)

As to common technical feature #5, discloses (claim 1) a composition comprising:
a luminogenic substrate (claim 42); and a target analyte binding agent comprising a target analyte binding element (claim 42) and one of a polypeptide component of a bioluminescent complex (claim 42), or a peptide component of a bioluminescent complex (claim 42).

As to common technical feature #6, Inbar, in view of Promega discloses (instant application claim 34) a composition comprising:
(a) a dried formulation (Inbar claims 1 and 19; "1. A two-compartment container for separately storing two components, mixing the components and dispensing a formulation formed by mixing the two components"; "19. A container according to claim 1, wherein a dry formulation is stored in the bottom compartment and a liquid is stored in the top compartment") comprising a first target analyte binding agent comprising a target analyte binding element and a polypeptide component (Promega claim 42) having at least 60% sequence identity with SEQ ID NO: 12 (Promega SEQ ID NO: 440: AA 1-155 87.8 % sequence identity); and (b) a liquid formulation (Inbar; claims 1 and 19) comprising a second target analyte binding agent comprising a target analyte binding element and a complementary peptide component (claim 42) having at least 60% sequence identity with SEQ ID NO: 13 (Promega Table 1 SEQ ID NO: 2130; 148-GSMLFRVTINS-158 100% sequence identity); and
a second complementary peptide component (claim 42) having at least 60% sequence identity with SEQ ID NO: 15 (Promega Table 1 SEQ ID NO: 390; 1-VSGWRLFKKIS-11 100% sequence identity).

As to common technical feature #7, Inbar discloses a dry formulation (claims 1, 19).

As the common technical features were known in the art at the time of the invention, they cannot be considered common special technical features that would otherwise unify the groups. The inventions lack unity with one another.

Therefore, Groups I-VII lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Item 4 (cont.): Claims 7-9, 22-33, 40-68, 78-88, 149-156, 160-177 are held unsearchable because they are not drafted according to the second and third sentences of PCT Rule 6.4(a).