



(51) International Patent Classification:

G01N 33/74 (2006.01)

A61K 39/00 (2006.01)

C07K 16/26 (2006.01)

(21) International Application Number:

PCT/US2019/025726

(22) International Filing Date:

04 April 2019 (04.04.2019)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/652,701

04 April 2018 (04.04.2018)

US

(71) Applicant (for all designated States except AL, AT, BE, BG, CH, CN, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IN, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR): **GENENTECH, INC.** [US/US]; 1 DNA Way, South San Francisco, CA 94080-4990 (US).

(71) Applicant (for AL, AT, BE, BG, CH, CN, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IN, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR)

only): **F. HOFFMANN-LA ROCHE AG** [CH/CH]; Grenzacherstrasse 124, 4070 Basel (CH).

(72) Inventors: **LOWE, John, Hok Nin**; c/o Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080 (US). **SON-ODA, Junichiro**; c/o Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080 (US).

(74) Agent: **LENDARIS, Steven, P.** et al.; Baker Botts LLP, 30 Rockefeller Plaza, New York, NY 10112-4498 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ,

(54) Title: METHODS FOR DETECTING AND QUANTIFYING FGF21

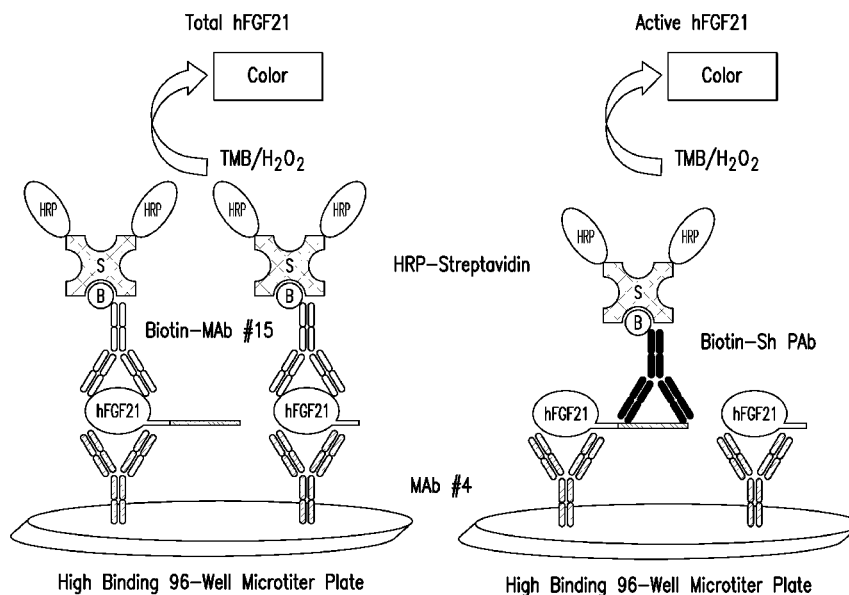


FIGURE 5

(57) Abstract: The presently disclosed subject matter provides antibodies that bind FGF21 and methods of using the same. In particular, the present disclosure provides immunoassay methods for detecting and quantifying active and total FGF21 levels in a sample and kits for performing such methods.

UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- *of inventorship (Rule 4.17(iv))*

Published:

- *with international search report (Art. 21(3))*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
- *with sequence listing part of description (Rule 5.2(a))*

METHODS FOR DETECTING AND QUANTIFYING FGF21

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No.
5 62/652,701, filed April 4, 2018, the disclosure of which is incorporated herein by
reference in its entirety.

SEQUENCE LISTING

The present application contains a Sequence Listing which has been
submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its
10 entirety. Said ASCII copy, created on April 2, 2019, is named 00B206_0809_SL.txt and
is 105,595 bytes in size.

FIELD OF THE INVENTION

The present invention relates to antibodies that bind to FGF21 as well as
immunoassay methods and kits using the same.

BACKGROUND

Fibroblast growth factor 21 (FGF21) is an endocrine member of the FGF
superfamily and plays a role in the regulation of glucose and lipid metabolism. FGF21
requires FGF-receptor (FGFR) isoforms and the membrane-bound co-receptor Klotho-
beta (KLB) for signaling (Ogawa et al. *Proc. Natl. Acad. Sci. USA* 104(18):7432-37
20 (2007); US 2010/0184665). FGF21 is a potent disease-modifying protein that has
beneficial effects on glucose homeostasis and insulin sensitivity, and has been shown to
reverse obesity and type 2 diabetes in animal disease models (Kharitononkov et al. *J.*
Clin. Invest. 115(6): 1627-35 (2005)). The administration of recombinant FGF21 has
been shown to reduce hepatic lipids, improve insulin sensitivity, and normalize glycemic
25 control in leptin-signaling-deficient (ob/ob or db/db) mice or high-fat diet (HFD)-fed
mice (Dunshee et al. *J. Biol. Chem.* 291(11):5986-96 (2016); US 2015/0218276).
Reduction in blood glucose and improvements in various cardiovascular risk factors have
also been observed in obese and diabetic rhesus monkeys treated daily with recombinant
FGF21.

30 FGF21 can be proteolytically cleaved at both the N-terminus and the C-
terminus, and such cleavage has been shown to affect the activity of FGF21. At the N-

terminus, the first four amino acids, which have the sequence His-Pro-Ile-Pro (HPIP (SEQ ID NO: 76)) in human FGF21 can be cleaved by a dipeptidyl peptidase (Dunshee et al. (2016)). At the C-terminus, the endopeptidase fibroblast activation protein (FAP) cleaves the most terminal 10 amino acids, which have the amino acid sequence Ser-Gln-Gly-Arg-Ser-Pro-Ser-Tyr-Ala-Ser (SQGRSPSYAS (SEQ ID NO: 77)) in human FGF21 (Dunshee et al. (2016)). FGF21 that lacks the four N-terminal amino acids is fully active; whereas, FGF21 that lacks the last ten C-terminal amino acids cannot bind the co-receptor KLB and is inactive (Yie et al. *FEBS Letters* 583:19-24 (2009)).

Circulating FGF21 has been proposed to be a biomarker for metabolic disorders such as diabetes as increased serum levels of FGF21 were observed in obese subjects, in subjects with nonalcoholic fatty liver disease (NAFLD) and in subjects with type 2 diabetes (Zhang et al. *Diabetes* 57(5):1246-1253 (2008); Li et al. *Diabetes Res. Clin. Pract.* 93(1):10-16 (2011)). Given the significant role for FGF21 in the treatment and development of metabolic disorders, there remains a need in the art for assays for determining the amount of FGF21 protein in an individual.

SUMMARY

The present disclosure provides antibodies that bind Fibroblast growth factor 21 (FGF21) and use of such antibodies in immunoassay methods for the detection and quantification of FGF21 protein, *e.g.*, total and/or active FGF21 protein, in a sample.

In certain embodiments, the present disclosure provides immunoassays for determining the amount of total FGF21 protein in a sample. For example, but not by way of limitation, the method to determine the amount of total FGF21 protein in a sample can include contacting a capture antibody that binds to an epitope present within amino acid residues 5-172 of FGF21 with the sample to generate a sample-capture antibody combination material, (b) contacting the sample-capture antibody combination material with a detector antibody that binds to an epitope present within amino acid residues 5-172 of FGF21, (c) detecting the detector antibody bound to the sample-capture antibody combination material and (d) calculating an amount of total FGF21 protein present in the sample based on the level of the detector antibody bound. In certain embodiments, the capture antibody and the detector antibody bind to different epitopes within amino acid residues 5-172 of FGF21.

In certain embodiments, the present disclosure provides immunoassays for determining the amount of active FGF21 protein in a sample. For example, but not by

way of limitation, the method to determine the amount of active FGF21 protein in a sample can include (a) contacting a capture antibody that binds to an epitope present within amino acid residues 5-172 of FGF21 with the sample to generate a sample-capture antibody combination material, (b) contacting the sample-capture antibody combination material with a detector antibody that binds to an epitope present within amino acid residues 173-182 of FGF21, (c) detecting the detector antibody bound to the sample-capture antibody combination material and (d) calculating an amount of active FGF21 protein present in the sample based on the level of the detector antibody bound.

In certain embodiments, the present disclosure provides immunoassays for determining the ratio of active FGF21 protein to total FGF21 protein in a sample. For example, but not by way of limitation, the method can include (i) contacting a first capture antibody that binds to an epitope present within amino acid residues 5-172 of FGF21 with the sample to generate a first sample-capture antibody combination material, (ii) contacting the first sample-capture antibody combination material with a first detector antibody that binds to an epitope present within amino acid residues 5-172 of FGF21, (iii) detecting the first detector antibody bound to the sample-capture antibody combination material and (iv) calculating an amount of total FGF21 protein present in the sample based on the level of the first detector antibody bound. In certain embodiments, the method can further include (i) contacting a second capture antibody that binds to an epitope present within amino acid residues 5-172 of FGF21 with the sample to generate a second sample-capture antibody combination material, (ii) contacting the second sample-capture antibody combination material with a second detector antibody that binds to an epitope present within amino acid residues 173-182 of FGF21, (iii) detecting the second detector antibody bound to the sample-capture antibody combination material and (iv) calculating an amount of active FGF21 protein present in the sample based on the level of the second detector antibody bound. In certain embodiments, the method can include comparing the calculated amount of total FGF21 protein with the calculated amount of active FGF21 protein to determine the ratio of active FGF21 protein to total FGF21 protein in the sample. In certain embodiments, the first capture antibody and second capture antibody are the same antibody. In certain embodiments, the first capture antibody and the first detector antibody bind to different epitopes within amino acid residues 5-172 of FGF21.

In certain embodiments, the immunoassay method is an enzyme-linked immunosorbent assay (ELISA). In certain embodiments, the immunoassay method

detects the amount of total or active FGF21 protein in the sample at an in-well sensitivity from about 2 pg/ml to about 20 pg/ml.

In certain embodiments, the immunoassay method is a single molecule detection assay, *e.g.*, that uses the Quanterix Simoa HD-1 Analyzer™. In certain
5 embodiments, the immunoassay method detects the amount of total or active FGF21 protein in the sample at an in-well sensitivity from about 0.2 pg/ml to about 0.5 pg/ml.

The present disclosure further provides kits for performing immunoassay methods for the detection and quantification of FGF21 protein. In certain embodiments, the present disclosure provides kits for determining the amount of total FGF21 protein in
10 a sample. For example, but not by way of limitation, the kit for quantifying the amount of total FGF21 protein includes (a) a capture antibody that binds to an epitope present within amino acid residues 5-172 of FGF21, (b) a detector antibody that binds to an epitope present within amino acid residues 5-172 of FGF21 and (c) a detection agent. In certain embodiments, the capture antibody and the detector antibody bind to different
15 epitopes within amino acid residues 5-172 of FGF21.

In certain embodiments, the present disclosure provides kits for determining the amount of active FGF21 protein in a sample. For example, but not by way of limitation, the kit for quantifying the amount of active FGF21 protein includes (a) a capture antibody that binds to an epitope present within amino acid residues 5-172 of
20 FGF21, (b) a detector antibody that binds to an epitope present within amino acid residues 173-182 of FGF21 and (c) a detection agent.

In certain embodiments, the present disclosure provides kits for determining the amount of active FGF21 protein in a sample. For example, but not by way of limitation, the kit for determining the ratio of active FGF21 protein to total FGF21
25 protein in a sample can include (a) a first capture antibody that binds to an epitope present within amino acid residues 5-172 of FGF21, (b) a first detector antibody that binds to an epitope present within amino acid residues 5-172 of FGF21, (c) a second capture antibody that binds to an epitope present within amino acid residues 5-172 of FGF21, (d) a second detector antibody that binds to an epitope present within amino acid
30 residues 173-182 of FGF21 and (e) one or more detection agents. In certain embodiments, the first capture antibody and second capture antibody are the same antibody. In certain embodiments, the first capture antibody and the first detector antibody bind to different epitopes within amino acid residues 5-172 of FGF21.

In certain embodiments, the detection agent for detecting the detector antibody, first detector antibody and/or second detector antibody can be selected from a group consisting of a streptavidin- β -D-galactopyranose conjugate, a streptavidin-horseradish peroxidase conjugate and a combination thereof. In certain embodiments, the streptavidin- β -D-galactopyranose conjugate has a concentration from about 100 pM to about 400 pM.

In certain embodiments, a kit of the present disclosure can further include resorufin β -D-galactopyranoside, tetramethylbenzidine, hydrogen peroxide or combinations thereof. For example, but not by way of limitation, a kit of the present disclosure can include a streptavidin- β -D-galactopyranose conjugate as the detection agent and can further include resorufin β -D-galactopyranoside. In certain embodiments, a kit of the present disclosure can include a streptavidin-horseradish peroxidase conjugate as the detection agent and can further include tetramethylbenzidine and hydrogen peroxide.

In certain embodiments, a kit disclosed herein detects the amount of total or active FGF21 protein in the sample at an in-well sensitivity from about 2 pg/ml to about 20 pg/ml. In certain embodiments, a kit disclosed herein detects the amount of total or active FGF21 protein in the sample at an in-well sensitivity from about 0.2 pg/ml to about 0.5 pg/ml.

In certain embodiments, the capture antibody, first capture antibody or second capture antibody is immobilized to a paramagnetic bead. In certain embodiments, the capture antibody, first capture antibody and/or second capture antibody binds to FGF21 with a K_d from about 10^{-10} M to 10^{-13} M. In certain embodiments, the detector antibody, first detector antibody and second detector antibody is conjugated to biotin. In certain embodiments, the detector antibody and/or first detector antibody binds to FGF21 with a K_d from about 10^{-10} M to 10^{-13} M. In certain embodiments, the detector antibody and/or first detector antibody for use in determining the amount of total FGF21 protein has a concentration from about 0.1 μ g/ml to about 1 μ g/ml. In certain embodiments, the detector antibody and/or second detector antibody for use in determining the amount of active FGF21 protein has a concentration from about 1 μ g/ml to about 3 μ g/ml.

In certain embodiments, the capture antibody, first capture antibody and/or second capture antibody includes or competitively binds to an antibody that includes: (a) a heavy chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 26 and 27, *e.g.*, 26, and conservative substitutions

thereof, (b) a heavy chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 30 and 31, *e.g.*, 30, and conservative substitutions thereof, (c) a heavy chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 34 and 35, *e.g.*, 34, and conservative substitutions thereof, (d) a light chain variable region CDR1 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 38 and 39, *e.g.*, 38, and conservative substitutions thereof, (e) a light chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 42 and 43, *e.g.*, 42, and conservative substitutions thereof and (f) a light chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 46 and 47, *e.g.*, 46, and conservative substitutions thereof.

In certain embodiments, the capture antibody, first capture antibody and/or second capture antibody includes or competitively binds to an antibody that includes: (a) a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 54, 55, 74 and 75, *e.g.*, 54, and conservative substitutions thereof; and (b) a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 50, 51, 70 and 71, *e.g.*, 50, and conservative substitutions thereof. In certain embodiments, the capture antibody, first capture antibody and/or second capture antibody includes or competitively binds to an antibody that includes: (a) a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 22, 23, 66 and 67, *e.g.*, 22, and conservative substitutions thereof; and (b) a light chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 18, 19, 62 and 63, *e.g.*, 18, and conservative substitutions thereof.

In certain embodiments, the detector antibody and/or first detector antibody includes or competitively binds to an antibody that includes: (a) a heavy chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 28 and 29, *e.g.*, 29, and conservative substitutions thereof, (b) a heavy chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 32 and 33, *e.g.*, 33, and conservative substitutions thereof, (c) a heavy chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 36 and 37, *e.g.*, 37, and conservative substitutions thereof, (d) a light chain variable region CDR1 domain

comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 40 and 41, *e.g.*, 41, and conservative substitutions thereof, (e) a light chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 44 and 45, *e.g.*, 45, and conservative substitutions thereof and (f) a light chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 48 and 49, *e.g.*, 49, and conservative substitutions thereof.

In certain embodiments, the detector antibody and/or first detector antibody includes or competitively binds to an antibody that includes: (a) a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 56, 57, 72 and 73, *e.g.*, 57, and conservative substitutions thereof; and (b) light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 52, 53, 68 and 69, *e.g.*, 53, and conservative substitutions thereof. In certain embodiments, the detector antibody and/or first detector antibody includes or competitively binds to an antibody that includes: (a) a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 24, 25, 64 and 65, *e.g.*, 25, and conservative substitutions thereof; and (b) a light chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 20, 21, 60 and 61, *e.g.*, 21, and conservative substitutions thereof.

In certain embodiments, an antibody used in the disclosed immunoassay methods can be a monoclonal antibody, a chimeric antibody, a humanized antibody or a human antibody. In certain embodiments, an antibody used in the disclosed immunoassay methods can be an antibody fragment, *e.g.*, a Fv, Fab, Fab', scFv, diabody or F(ab')₂ fragment.

In certain embodiments, the sample being analyzed is a blood sample obtained from a subject. In certain embodiments, the sample is a plasma sample obtained from a subject.

The present disclosure further provides isolated anti-FGF21 antibodies. In certain embodiments, an isolated anti-FGF21 antibody, or an antigen-binding portion thereof, comprises: (a) a heavy chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 26-29, and conservative substitutions thereof; (b) a heavy chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 30-33, and conservative substitutions thereof; (c) a heavy chain variable region CDR3 domain

comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 34-37, and conservative substitutions thereof; (d) a light chain variable region CDR1 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 38-41, and conservative substitutions thereof; (e) a light chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 42-45, and conservative substitutions thereof; and (f) a light chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 46-49, and conservative substitutions thereof.

In certain embodiments, an isolated anti-FGF21 antibody, or an antigen-binding portion thereof, comprises: (a) a heavy chain variable domain (VH) sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 54-57 and 72-75; and (b) a light chain variable domain (VH) sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 50-53 and 68-71. In certain embodiments, an isolated anti-FGF21 antibody, or an antigen-binding portion thereof, comprises: (a) a heavy chain sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 22-25 and 64-67; and (b) a light chain sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 18-21 and 60-63.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Depicts the results of an ELISA screen of 80 hybridoma supernatants expressing anti-FGF21 antibodies.

Figure 2: Depicts the dose response of intact versus cleaved FGF21 detection by sandwich ELISA using mAb4 or mAb9 capture antibodies and the mAb11 detector antibody.

Figure 3: Depicts the BIACORE® surface plasmon resonance analysis of anti-FGF21 antibodies mAb4, mAb9, mAb11 and mAb15.

Figure 4: Depicts a schematic diagram showing anti-FGF21 antibody binding to FGF21 (FGF19 is used as negative control).

Figure 5: Depicts a schematic diagram of a non-limiting embodiment of colorimetric ELISA methods for detecting total FGF21 and active FGF21.

Figure 6: Depicts a non-limiting embodiment of a protocol for performing total and active FGF21 ELISA assays.

Figure 7: Depicts the results of ELISA assays using either mAb4 or mAb11 capture antibodies and various detection antibodies.

Figure 8: Depicts a comparison of the sensitivity of detecting wild-type and cleaved human FGF21 using exemplary total and active FGF21 ELISA assays.

5 Figure 9: Depicts detection of human FGF21 using an exemplary total FGF21 ELISA assay.

Figure 10: Depicts an ELISA assay indicating that exemplary anti-FGF21 antibodies do not cross-react with mouse FGF21.

10 Figure 11: Depicts a comparison of the sensitivities of capture antibodies mAb4 and mAb9 in exemplary total and active FGF21 ELISA assays.

Figure 12: Depicts the effect of coat buffer and concentration on the sensitivity of an exemplary total FGF21 ELISA assay using mAb4 as the capture antibody and mAb15 as the detector antibody.

15 Figure 13: Depicts the effect of coat buffer and concentration on the sensitivity of an exemplary active FGF21 ELISA assay using mAb4 as the capture antibody and the sheep C-terminal pAb as the detector antibody.

Figure 14: Depicts the effect of biotin-conjugated detector antibody and HRP concentration on the sensitivity of an exemplary total FGF21 ELISA assay using mAb4 as the capture antibody and mAb15 as the detector antibody.

20 Figure 15: Depicts a schematic diagram of a non-limiting embodiment of single molecule detection methods for detecting total FGF21 and active FGF21 using the Quanterix Simoa HD-1 Analyzer™ (“Quanterix Simoa”).

Figure 16: Depicts a non-limiting embodiment of a two-step assay protocol for exemplary total FGF21 and active FGF21 assays using the Quanterix Simoa.

25 Figure 17: Depicts the dose response of intact versus cleaved FGF21 detection by exemplary total FGF21 and active FGF21 assays using the Quanterix Simoa.

Figure 18: Depicts a non-limiting embodiment of a protocol for performing exemplary total and active FGF21 assays using the Quanterix Simoa.

30 Figure 19: Depicts the standard curves in exemplary total and active FGF21 assays using the Quanterix Simoa.

Figure 20: Depicts the standard curve performance in exemplary total and active FGF21 assays using the Quanterix Simoa.

Figure 21: Depicts a comparison of the sensitivity of detecting total and active FGF21 in the presence of BA010 and IL-12 buffers in exemplary total and active FGF21 assays using the Quanterix Simoa.

5 Figure 22: Depicts the effect of high bead (HB) and low bead (LB) concentrations on the sensitivity of exemplary total and active FGF21 assays using the Quanterix Simoa.

Figure 23: Depicts a comparison of the sensitivity of detecting total and active FGF21 using three capture paramagnetic bead lots in exemplary total and active FGF21 assays using the Quanterix Simoa.

10 Figure 24: Depicts a comparison of the sensitivity of detecting total and active FGF21 using various detection antibodies in an exemplary total FGF21 assay using the Quanterix Simoa.

Figure 25: Depicts an analysis of the hook effect in an exemplary total FGF21 assay using mAb4 as the capture antibody and mAb15 as the detector antibody using the
15 Quanterix Simoa.

Figure 26: Depicts the detection of total FGF21 and active FGF21 in plasma and serum samples from a healthy donor using exemplary total and active FGF21 ELISA assays.

20 Figure 27: Depicts the detection of total FGF21 and active FGF21 in plasma samples or plasma samples treated with MS-SAFE from donors that are hypertensive and donors that are not on medication using exemplary total and active FGF21 ELISA assays.

Figure 28A: Depicts the detection of total FGF21 and active FGF21 in plasma samples from healthy and type 2 diabetic patients using exemplary total and active
25 FGF21 assays (Day 1) using the Quanterix Simoa.

Figure 28B: Depicts the detection of total FGF21 and active FGF21 in plasma samples from healthy and type 2 diabetic patients using exemplary total and active FGF21 assays (Day 2) using the Quanterix Simoa.

30 Figure 29: Depicts the reproducibility of exemplary total and active FGF21 assays used for the detection of total FGF21 and active FGF21 in plasma samples from healthy and type 2 diabetic patients using the Quanterix Simoa.

Figure 30: Depicts the linearity of dilution of exemplary total and active FGF21 assays used for the detection of total FGF21 and active FGF21 in plasma samples from type 2 diabetic patients using the Quanterix Simoa.

Figure 31: Depicts the determination of lower limit of quantification (LLOQ) in exemplary total and active FGF21 assays used for the detection of total FGF21 and active FGF21 in plasma samples from type 2 diabetic patients using the Quanterix Simoa.

5 Figure 32: Depicts the specificity of exemplary total and active FGF21 assays used for the detection of total FGF21 and active FGF21 in plasma samples from type 2 diabetic patients using the Quanterix Simoa.

 Figure 33: Depicts the detection of total FGF21 and active FGF21 in plasma samples prepared using P800 or K₂-EDTA using exemplary total and active FGF21
10 assays using the Quanterix Simoa.

 Figure 34: Depicts an analysis of total FGF21 and active FGF21 detected in P800 and K₂-EDTA plasma samples from the GC29819 study in exemplary total and active FGF21 assays using the Quanterix Simoa.

 Figure 35: Depicts a correlation between the amount of total FGF21 and
15 active FGF21 detected in P800 and K₂-EDTA plasma samples (GC29819 clinical Study) quantitated using an exemplary total FGF21 assay using the Quanterix Simoa.

 Figure 36: Depicts a correlation between the amount of total FGF21 and active FGF21 detected in P800 and K₂-EDTA plasma samples (GC29819 Study) quantitated using an exemplary active FGF21 assay using the Quanterix Simoa.

20 Figure 37: Depicts an evaluation of the stability of the P800 plasma samples from the GC29819 study using exemplary total and active FGF21 assays using the Quanterix Simoa.

 Figure 38: Depicts the effect of assay diluent containing 10 µg/ml of mouse or sheep IgG on the total and active assays using the Quanterix Simoa.

25 Figure 39: Depicts the effect of assay diluent containing 10 µg/ml of mouse and sheep IgG on the total and active assays using the Quanterix Simoa.

 Figure 40: Depicts the effect of assay diluent containing 10 µg/ml of mouse or sheep IgG on the standard curves on the total and active assays using the Quanterix Simoa.

30 Figure 41A: Depicts the sequences of the light chain variable regions of exemplary anti-FGF21 antibodies. Light chain variable region sequences are disclosed as SEQ ID NOs: 50, 51, 52, 53, 71, 70, 69 and 68, respectively, in order of appearance. CDR-L1 sequences are disclosed as SEQ ID NOs: 38, 39, 40, 41, 38, 39, 40 and 41, respectively, in order of appearance; CDR-L2 sequences are disclosed as SEQ ID NOs:

42, 43, 44, 45, 42, 43, 44 and 45, respectively, in order of appearance; and CDR-L3 sequences are disclosed as SEQ ID NOs: 46, 47, 48, 49, 46, 47, 48 and 49, respectively, in order of appearance.

Figure 41B: Depicts the sequences of the heavy chain variable regions of exemplary anti-FGF21 antibodies. Heavy chain variable region sequences are disclosed as SEQ ID NOs: 54, 55, 56, 57, 75, 74, 73 and 72, respectively, in order of appearance. CDR-H1 sequences are disclosed as SEQ ID NOs: 26, 27, 28, 29, 26, 27, 28 and 29, respectively, in order of appearance; CDR-H2 sequences are disclosed as SEQ ID NOs: 30, 31, 32, 33, 30, 31, 32 and 33, respectively, in order of appearance; and CDR-H3 sequences are disclosed as SEQ ID NOs: 34, 35, 36, 37, 34, 35, 36 and 37, respectively, in order of appearance.

DETAILED DESCRIPTION

For clarity, but not by way of limitation, the detailed description of the presently disclosed subject matter is divided into the following subsections:

- I. Definitions;
- II. Immunoassays;
- III. Antibodies;
- IV. Kits; and
- V. Exemplary Embodiments.

I. DEFINITIONS

Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. The following references provide one of skill with a general definition of many of the terms used in this invention: Singleton et al., Dictionary of Microbiology and Molecular Biology (2nd ed. 1994); The Cambridge Dictionary of Science and Technology (Walker ed., 1988); The Glossary of Genetics, 5th Ed., R. Rieger et al. (eds.), Springer Verlag (1991); and Hale & Marham, The Harper Collins Dictionary of Biology (1991). As used herein, the following terms have the meanings ascribed to them below, unless specified otherwise.

The term “about” or “approximately,” as used herein, can mean within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *e.g.*, the

limitations of the measurement system. For example, “about” can mean within 1 or more than 1 standard deviation, per the practice in the given value. Where particular values are described in the application and claims, unless otherwise stated the term “about” can mean an acceptable error range for the particular value, such as $\pm 10\%$ of the value modified by the term “about.”

The terms “polypeptide” and “protein,” as used interchangeably herein, refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), as well as other modifications known in the art.

The terms “polypeptide” and “protein” as used herein specifically encompass antibodies.

The term “Fibroblast growth factor 21” or “FGF21,” as used herein, refers to any native FGF21 from any vertebrate source, including mammals such as primates (*e.g.*, humans) and rodents (*e.g.*, mice and rats), unless otherwise indicated. The term encompasses “full-length,” unprocessed FGF21 as well as any form of FGF21 that results from processing in the cell. The term also encompasses naturally occurring variants of FGF21, *e.g.*, splice variants or allelic variants, unless otherwise indicated. A non-limiting example of a full-length human FGF21 amino acid is shown below:

HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQSPESLLQL
KALKPGVIQILGVKTSRFLCQRPDGALYGS LHFDP EACSFRELLLEDGYNVYQSE
AHGLPLHLPGNKSPHRDPAPRGPARFLPLPGLPPALPEPPGILAPQPPDVGSSDPL
SMVGPSQGRSPSYAS (SEQ ID NO: 1).

The term “total FGF21,” as used herein, includes unprocessed forms of FGF21 as well as all forms of FGF21 that result from cellular processing, *e.g.*, N-terminally-cleaved FGF21 and C-terminally-cleaved FGF21. A non-limiting example of a human FGF21 amino acid that lacks the ten C-terminal amino acids is:

HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQSPESLLQL
KALKPGVIQILGVKTSRFLCQRPDGALYGS LHFDP EACSFRELLLEDGYNVYQSE
AHGLPLHLPGNKSPHRDPAPRGPARFLPLPGLPPALPEPPGILAPQPPDVGSSDPL

SMVGP (SEQ ID NO: 58). A non-limiting example of a human FGF21 amino acid that lacks the 4 N-terminal amino acids is:

DSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQSPESLLQLKAL
KPGVIQILGVKTSRFLCQRPDGALYGSLHFDPEACSFRELLLEDGYNVYQSEAHG

5 LPLHLPGNKSPHRDPAPRGPAPRFLPLPGLPPALPEPPGILAPQPPDVGSSDPLSMV
GPSQGRSPSYAS (SEQ ID NO: 59). For example, but not by way of limitation, the term “total FGF21” includes FGF21 proteins that have the amino acid sequence set forth in SEQ ID NO: 1, SEQ ID NO: 58 or SEQ ID NO: 59.

The term “active FGF21,” as used herein, refers to an FGF21 protein that
10 retains its C-terminal fragment. In certain embodiments, the term includes processed forms of FGF21, such as those where the N-terminal fragment of FGF21, *e.g.*, amino acid residues 1-4 of SEQ ID NO: 1, has been cleaved. For example, but not by way of limitation, the term “active FGF21” includes FGF21 proteins that have the amino acid sequence set forth in SEQ ID NO: 1 or the amino acid sequence set forth in SEQ ID NO:
15 59.

The term “antibody” herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity.

20 An “antibody fragment” refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include, but are not limited to, Fv, Fab, Fab’, Fab’-SH, F(ab’)₂; diabodies; linear antibodies; single-chain antibody molecules (*e.g.*, scFv); and multispecific antibodies formed from antibody fragments.

25 An antibody “which binds” an antigen of interest, *e.g.*, a FGF21 protein, is one that binds the antigen with sufficient affinity such that the antibody is useful as an assay reagent, *e.g.*, as a capture antibody or as a detection antibody. Typically, such an antibody does not significantly cross-react with other polypeptides. With regard to the binding of a polypeptide to a target molecule, the term “specific binding” or “specifically
30 binds to” or is “specific for” a particular polypeptide or an epitope on a particular polypeptide target means binding that is measurably different from a non-specific interaction. Specific binding can be measured, for example, by determining binding of a target molecule compared to binding of a control molecule, which generally is a molecule of similar structure that does not have binding activity.

The term “anti-FGF21 antibody” refers to an antibody that is capable of binding FGF21 with sufficient affinity such that the antibody is useful as an agent in targeting FGF21, *e.g.*, as an agent in the assays described herein. In certain embodiments, the extent of binding of an anti-FGF21 antibody to an unrelated, non-FGF21 protein is less than about 10% of the binding of the antibody to FGF21 as measured, *e.g.*, by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to FGF21 has a dissociation constant (K_d) of $\leq 1 \text{ M}$, $\leq 100 \text{ mM}$, $\leq 10 \text{ mM}$, $\leq 1 \text{ mM}$, $\leq 100 \text{ }\mu\text{M}$, $\leq 10 \text{ }\mu\text{M}$, $\leq 1 \text{ }\mu\text{M}$, $\leq 100 \text{ nM}$, $\leq 10 \text{ nM}$, $\leq 1 \text{ nM}$, $\leq 0.1 \text{ nM}$, $\leq 0.01 \text{ nM}$ or $\leq 0.001 \text{ nM}$. In certain embodiments, the K_d of an antibody that binds to FGF21, disclosed herein, can be 10^{-3} M or less or 10^{-8} M or less, *e.g.*, from 10^{-8} M to 10^{-13} M , *e.g.*, from 10^{-9} M to 10^{-13} M . In certain embodiments, the K_d of an antibody that binds to FGF21, disclosed herein, can be 10^{-10} M to 10^{-13} M . In certain embodiments, an anti-FGF21 antibody binds to an epitope of FGF21 that is conserved among FGF21 from different species.

An “acceptor human framework” for the purposes herein is a framework comprising the amino acid sequence of a light chain variable domain (VL) framework or a heavy chain variable domain (VH) framework derived from a human immunoglobulin framework or a human consensus framework, as defined below. An acceptor human framework “derived from” a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain amino acid sequence changes. In certain embodiments, the number of amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. In certain embodiments, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

“Affinity” refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (*e.g.*, an antibody) and its binding partner (*e.g.*, an antigen). Unless indicated otherwise, as used herein, “binding affinity” refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (*e.g.*, antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K_d). Affinity can be measured by common methods known in the art, including those described herein. Specific illustrative and exemplary embodiments for measuring binding affinity are described in the following.

An “affinity matured” antibody refers to an antibody with one or more alterations in one or more hypervariable regions (CDRs), compared to a parent antibody which does not possess such alterations, such alterations resulting in an improvement in the affinity of the antibody for antigen.

5 An “antibody that competes for binding” with a reference antibody refers to an antibody that blocks binding of the reference antibody to its antigen in a competition assay by 50% or more, and conversely, the reference antibody blocks binding of the antibody to its antigen in a competition assay by 50% or more. An exemplary competition assay is described in “Antibodies,” Harlow and Lane (Cold Spring Harbor
10 Press, Cold Spring Harbor, NY).

A “capture antibody,” as used herein, refers to an antibody that specifically binds a target molecule, *e.g.*, a form of FGF21, in a sample. Under certain conditions, the capture antibody forms a complex with the target molecule such that the antibody-target molecule complex can be separated from the rest of the sample. In certain embodiments,
15 such separation may include washing away substances or material in the sample that did not bind the capture antibody. In certain embodiments, a capture antibody may be attached to a solid support surface, such as, for example but not limited to, a plate or a bead, *e.g.*, a paramagnetic bead.

A “detection antibody,” as used herein, refers to an antibody that specifically
20 binds a target molecule in a sample or in a sample-capture antibody combination material. Under certain conditions, the detection antibody forms a complex with the target molecule or with a target molecule-capture antibody complex. A detection antibody is capable of being detected either directly through a label, which may be amplified, or indirectly, *e.g.*, through use of another antibody that is labeled and that
25 binds the detection antibody. For direct labeling, the detection antibody is typically conjugated to a moiety that is detectable by some means, for example, including but not limited to, biotin or ruthenium.

The term “chimeric” antibody refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the
30 remainder of the heavy and/or light chain is derived from a different source or species.

The “class” of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG₁, IgG₂, IgG₃, IgG₄, IgA₁, and IgA₂. The heavy chain constant

domains that correspond to the different classes of immunoglobulins are called α , δ , ϵ , γ , and μ , respectively.

The term “cytotoxic agent” as used herein refers to a substance that inhibits or prevents a cellular function and/or causes cell death or destruction. Cytotoxic agents include, but are not limited to, radioactive isotopes (*e.g.*, At²¹¹, I¹³¹, I¹²⁵, Y⁹⁰, Re¹⁸⁶, Re¹⁸⁸, Sm¹⁵³, Bi²¹², P³², Pb²¹² and radioactive isotopes of Lu); chemotherapeutic agents or drugs (*e.g.*, methotrexate, adriamycin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents); growth inhibitory agents; enzymes and fragments thereof such as nucleolytic enzymes; antibiotics; toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof; and the various antitumor or anticancer agents disclosed below.

“Effector functions” refer to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (*e.g.*, B cell receptor); and B cell activation.

The term “Fc region” herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. In certain embodiments, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991.

“Framework” or “FR” refers to variable domain residues other than hypervariable region (CDR) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the CDR and FR sequences generally appear in the following sequence in VH (or VL): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

The terms “full-length antibody,” “intact antibody” and “whole antibody” are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or having heavy chains that contain an Fc region as defined herein.

5 A “human antibody” is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

10 A “human consensus framework” is a framework which represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat et al., *Sequences of Proteins of*
15 *Immunological Interest*, Fifth Edition, NIH Publication 91-3242, Bethesda MD (1991), Vols. 1-3. In certain embodiments, for the VL, the subgroup is subgroup kappa I as in Kabat et al., *supra*. In certain embodiments, for the VH, the subgroup is subgroup III as in Kabat et al., *supra*.

A “humanized” antibody refers to a chimeric antibody comprising amino acid
20 residues from non-human CDRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDRs (*e.g.*, CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may
25 comprise at least a portion of an antibody constant region derived from a human antibody. A “humanized form” of an antibody, *e.g.*, a non-human antibody, refers to an antibody that has undergone humanization.

The term “hypervariable region” or “CDR,” as used herein, refers to each of the regions of an antibody variable domain which are hypervariable in sequence (also
30 referred to herein as “complementarity determining regions” or “CDRs”) and/or form structurally defined loops (“hypervariable loops”) and/or contain the antigen-contacting residues (“antigen contacts”). Unless otherwise indicated, CDR residues and other residues in the variable domain (*e.g.*, FR residues) are numbered herein according to

Kabat et al., *supra*. Generally, antibodies comprise six CDRs: three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). Exemplary CDRs herein include:

- (a) hypervariable loops occurring at amino acid residues 26-32 (L1), 50-52 (L2), 91-96 (L3), 26-32 (H1), 53-55 (H2), and 96-101 (H3) (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987));
- (b) CDRs occurring at amino acid residues 24-34 (L1), 50-56 (L2), 89-97 (L3), 31-35b (H1), 50-65 (H2), and 95-102 (H3) (Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991));
- (c) antigen contacts occurring at amino acid residues 27c-36 (L1), 46-55 (L2), 89-96 (L3), 30-35b (H1), 47-58 (H2), and 93-101 (H3) (MacCallum et al. *J. Mol. Biol.* 262: 732-745 (1996)); and
- (d) combinations of (a), (b), and/or (c), including CDR amino acid residues 46-56 (L2), 47-56 (L2), 48-56 (L2), 49-56 (L2), 26-35 (H1), 26-35b (H1), 49-65 (H2), 93-102 (H3), and 94-102 (H3).

An “immunoconjugate” refers to an antibody conjugated to one or more heterologous molecule(s), including but not limited to a cytotoxic agent.

An “isolated” antibody is one which has been separated from a component of its natural environment. In certain embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (*e.g.*, SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (*e.g.*, ion exchange or reverse phase HPLC). For review of methods for assessment of antibody purity, see, *e.g.*, Flatman et al., *J. Chromatogr. B* 848:79-87 (2007).

An “isolated” nucleic acid refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

“Isolated nucleic acid encoding an antibody” (including references to a specific antibody, *e.g.*, an anti-FGF21 antibody) refers to one or more nucleic acid molecules encoding antibody heavy and light chains (or fragments thereof), including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

The term “monoclonal antibody,” as used herein, refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, *e.g.*, containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, the modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the presently disclosed subject matter may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein.

A “naked antibody” refers to an antibody that is not conjugated to a heterologous moiety (*e.g.*, a cytotoxic moiety) or radiolabel. The naked antibody may be present in a pharmaceutical formulation.

“Native antibodies” refer to naturally occurring immunoglobulin molecules with varying structures. For example, native IgG antibodies are heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light chains and two identical heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CH1, CH2, and CH3). Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable domain, followed by a constant light (CL) domain. The light chain of an antibody may be assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain.

“Purified” polypeptide (*e.g.*, antibody), as used herein, refers to a polypeptide that has been increased in purity, such that it exists in a form that is more pure than it exists in its natural environment and/or when initially synthesized and/or amplified under

laboratory conditions. Purity is a relative term and does not necessarily mean absolute purity.

The term “package insert,” as used herein, refers to instructions customarily included in commercial packages that contain information concerning the use of the components of the package.

“Percent (%) amino acid sequence identity” with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, California, or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

$$100 \text{ times the fraction } X/Y$$

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program’s alignment of A and B, and

where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

The term “variable region” or “variable domain” refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (CDRs). (See, *e.g.*, Kindt et al. *Kuby Immunology*, 6th ed., W.H. Freeman and Co., page 91 (2007).) A single VH or VL domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively. See, *e.g.*, Portolano et al., *J. Immunol.* 150:880-887 (1993); Clarkson et al., *Nature* 352:624-628 (1991).

The terms “host cell,” “host cell line,” and “host cell culture” as used interchangeably herein, refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include “transformants” and “transformed cells,” which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

The term “vector,” as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as “expression vectors.”

The terms “label” or “detectable label,” as used herein, refers to any chemical group or moiety that can be linked to a substance that is to be detected or quantitated, *e.g.*, an antibody. A label is a detectable label that is suitable for the sensitive detection

or quantification of a substance. Non-limiting examples of detectable labels include, but are not limited to, luminescent labels, *e.g.*, fluorescent, phosphorescent, chemiluminescent, bioluminescent and electrochemiluminescent labels, radioactive labels, enzymes, particles, magnetic substances, electroactive species and the like.

- 5 Alternatively, a detectable label may signal its presence by participating in specific binding reactions. Non-limiting examples of such labels include haptens, antibodies, biotin, streptavidin, his-tag, nitrilotriacetic acid, glutathione S-transferase, glutathione and the like.

10 The term “detection means,” as used herein, refers to a moiety or technique used to detect the presence of the detectable antibody through signal reporting that is then read out in an assay. Typically, a detection means employ reagents, *e.g.*, a detection agent, that amplify an immobilized label such as the label captured onto a microtiter plate, *e.g.*, avidin, streptavidin-HRP or streptavidin- β -D-galactopyranose.

15 The term “detecting,” is used herein, to include both qualitative and quantitative measurements of a target molecule, *e.g.*, FGF21 or processed forms thereof. In certain embodiments, detecting includes identifying the mere presence of the target molecule in a sample as well as determining whether the target molecule is present in the sample at detectable levels.

20 An “individual” or “subject,” as used interchangeably herein, is a mammal. Mammals include, but are not limited to, domesticated animals (*e.g.*, cows, sheep, cats, dogs, and horses), primates (*e.g.*, humans and non-human primates such as monkeys), rabbits, and rodents (*e.g.*, mice and rats). In certain embodiments, the individual or subject is a human.

25 A “sample,” as used herein, refers to a small portion of a larger quantity of material. In certain embodiments, a sample includes, but is not limited to, cells in culture, cell supernatants, cell lysates, serum, blood plasma, biological fluid (*e.g.*, blood, plasma, serum, stool, urine, lymphatic fluid, ascites, ductal lavage, saliva and cerebrospinal fluid) and tissue samples. The source of the sample may be solid tissue (*e.g.*, from a fresh, frozen, and/or preserved organ, tissue sample, biopsy or aspirate),
30 blood or any blood constituents, bodily fluids (such as, *e.g.*, urine, lymph, cerebral spinal fluid, amniotic fluid, peritoneal fluid or interstitial fluid), or cells from the individual, including circulating cells.

II. IMMUNOASSAYS

The presently disclosed subject matter provides methods for the detection and quantification of FGF21 protein. In certain embodiments, the present disclosure provides immunoassays for determining the amount of total FGF21 and/or active FGF21 protein in a sample. The present disclosure further provides immunoassay methods for determining the ratio of active FGF21 protein to total FGF21 protein in a sample. In certain embodiments, the immunoassay methods of the present disclosure use the anti-FGF21 antibodies disclosed herein. Non-limiting examples of anti-FGF21 antibodies for use in the presently disclosed methods are provided in Tables 8-13 and 16-19.

In certain embodiments, the present disclosure provides immunoassay methods for the detection and quantification of human FGF21 protein. For example, the immunoassay methods can be used for the detection and quantification of FGF21, *e.g.*, total human FGF21 and/or active human FGF21 protein, in a sample. The immunoassay methods of the present disclosure can incorporate strategies known in the art, including but not limited to, sandwich assay, enzyme-linked immunosorbent assay (ELISA) assay, a digital form of ELISA, electrochemical assay (ECL) assay and magnetic immunoassay. In certain embodiments, the immunoassay method is a single molecule immunoassay, *e.g.*, using a single molecule array. For example, but not by way of limitation, the immunoassay method can be performed using a Quanterix instrument, *e.g.*, a Simoa HD-1 Analyzer™.

In certain embodiments, the methods of the present disclosure comprise contacting a sample obtained from a subject with a capture anti-FGF21 antibody, such as those described herein, under conditions permissive for the binding of the capture anti-FGF21 antibody to FGF21 protein in the sample. For example, but not by way of limitation, the sample can be incubated with a capture antibody that binds to an epitope present on FGF21 to generate a sample-capture antibody combination material. The conditions for the incubation of the sample and the capture antibody can be selected to maximize the sensitivity of the assay and/or to minimize dissociation, as well as to ensure that the FGF21 protein present in the sample binds to the capture antibody.

In certain embodiments, the capture antibodies used in the immunoassay methods disclosed herein can be used at a concentration from about 0.1 µg/ml to about 5.0 µg/ml. For example, but not by way of limitation, the capture antibodies can be used at a concentration from about 0.1 µg/ml to about 0.5 µg/ml, from about 0.1 µg/ml to

about 1.0 µg/ml, from about 0.1 µg/ml to about 1.5 µg/ml, from about 0.1 µg/ml to about 2.0 µg/ml, from about 0.1 µg/ml to about 2.5 µg/ml, from about 0.1 µg/ml to about 3.0 µg/ml, from about 0.1 µg/ml to about 3.5 µg/ml, from about 0.1 µg/ml to about 4.0 µg/ml, from about 0.1 µg/ml to about 4.5 µg/ml, from about 0.5 µg/ml to about 5.0 µg/ml, from about 1.0 µg/ml to about 5.0 µg/ml, from about 1.5 µg/ml to about 5.0 µg/ml, from about 2.0 µg/ml to about 5.0 µg/ml, from about 2.5 µg/ml to about 5.0 µg/ml, from about 3.0 µg/ml to about 5.0 µg/ml, from about 3.5 µg/ml to about 5.0 µg/ml, from about 4.0 µg/ml to about 5.0 µg/ml, from about 4.5 µg/ml to about 5.0 µg/ml, from about 0.5 µg/ml to about 2.0 µg/ml or from about 0.5 µg/ml to about 1.0 µg/ml, *e.g.*, about 0.5 µg/ml.

In certain embodiments, the capture antibody can be diluted in a coating buffer. Non-limiting examples of coating buffers include PBS, a carbonate buffer, a bicarbonate buffer or combinations thereof. In certain embodiments, the coating buffer is sodium bicarbonate. In certain embodiments, the coating buffer is PBS. In certain embodiments, the coating buffer can be used at a concentration from about 10 mM to about 1 M. For example, but not by way of limitation, the coating buffer can be used at a concentration from about 10 mM to about 100 mM, from about 10 mM to about 200 mM, from about 10 mM to about 300 mM, from about 10 mM to about 400 mM, from about 10 mM to about 500 mM, from about 10 mM to about 600 mM, from about 10 mM to about 700 mM, from about 10 mM to about 800 mM, from about 10 mM to about 900 mM, from about 100 mM to about 1 M, from about 200 mM to about 1 M, from about 300 mM to about 1 M, from about 400 mM to about 1 M, from about 500 mM to about 1 M, from about 600 mM to about 1 M, from about 700 mM to about 1 M, from about 800 mM to about 1 M or from about 900 mM to about 1 M.

Capture antibodies, as used herein, can be immobilized on a solid phase. For example, but not by way of limitation, the solid phase can be any inert support or carrier that is useful in immunometric assays, including supports in the form of, *e.g.*, surfaces, particles, porous matrices, beads and the like. Non-limiting examples of commonly used supports include small sheets, SEPHADEX®, gels, polyvinyl chloride, plastic beads and assay plates or test tubes manufactured from polyethylene, polypropylene, polystyrene, and the like, including 96-well microtiter plates, as well as particulate materials such as filter paper, agarose, cross-linked dextran, and other polysaccharides. In certain embodiments, the solid phase used for immobilization can be beads. For example, but not by way of limitation, a capture antibody disclosed herein is immobilized to

paramagnetic beads. In certain embodiments, the immobilized capture antibodies are coated on a microtiter plate that can be used to analyze several samples at one time.

In certain embodiments, the paramagnetic beads coupled to the capture antibody can be used at a concentration from about 0.1×10^7 beads/ml to about 10.0×10^7 beads/ml, *e.g.*, from about 0.1×10^7 beads/ml to about 0.5×10^7 beads/ml, from about 0.1×10^7 beads/ml to about 1.0×10^7 beads/ml, from about 0.1×10^7 beads/ml to about 2.0×10^7 beads/ml, from about 0.1×10^7 beads/ml to about 3.0×10^7 beads/ml, from about 0.1×10^7 beads/ml to about 4.0×10^7 beads/ml, from about 0.1×10^7 beads/ml to about 5.0×10^7 beads/ml, from about 0.1×10^7 beads/ml to about 6.0×10^7 beads/ml, from about 0.1×10^7 beads/ml to about 7.0×10^7 beads/ml, from about 0.1×10^7 beads/ml to about 8.0×10^7 beads/ml, from about 0.1×10^7 beads/ml to about 9.0×10^7 beads/ml, from about 0.5×10^7 beads/ml to about 10.0×10^7 beads/ml, from about 1.0×10^7 beads/ml to about 10.0×10^7 beads/ml, from about 2.0×10^7 beads/ml to about 10.0×10^7 beads/ml, from about 3.0×10^7 beads/ml to about 10.0×10^7 beads/ml, from about 4.0×10^7 beads/ml to about 10.0×10^7 beads/ml, from about 5.0×10^7 beads/ml to about 10.0×10^7 beads/ml, from about 6.0×10^7 beads/ml to about 10.0×10^7 beads/ml, from about 7.0×10^7 beads/ml to about 10.0×10^7 beads/ml, from about 8.0×10^7 beads/ml to about 10.0×10^7 beads/ml, from about 9.0×10^7 beads/ml to about 10.0×10^7 beads/ml, from about 0.5×10^7 beads/ml to about 1.0×10^7 beads/ml, from about 0.5×10^7 beads/ml to about 2.0×10^7 beads/ml or from about 0.5×10^7 beads/ml to about 3.0×10^7 beads/ml. In certain embodiments, the paramagnetic beads can be used at a concentration from about 0.5×10^7 beads/ml to about 2.0×10^7 beads/ml. In certain embodiments, the paramagnetic beads can be used at a concentration of about 1.0×10^7 beads/ml, *e.g.*, about 1.22×10^7 beads/ml, or at a concentration of about 0.5×10^7 beads/ml, *e.g.*, about 0.59×10^7 beads/ml.

The immunoassay methods disclosed herein can further include contacting a sample-capture antibody combination material with a detector antibody. In certain embodiments, the detector antibody binds to an epitope present on FGF21. In certain embodiments, the detector antibody binds to an epitope present on the sample-capture antibody combination material, but not on the capture antibody in the absence of FGF21. In certain embodiments, the detector antibody bound to the sample-capture antibody combination is subsequently measured or quantified using a detection means, *e.g.*, one or more detection agents, for the detection antibody to determine the amount of FGF21 protein, *e.g.*, total FGF21 or active FGF21 protein, bound by the detector antibody.

In certain embodiments, the detector antibody can be used in a concentration from about 0.1 µg/ml to about 5.0 µg/ml. For example, but not by way of limitation, the detector antibody can be used at a concentration from about 0.1 µg/ml to about 0.5 µg/ml, from about 0.1 µg/ml to about 1.0 µg/ml, from about 0.1 µg/ml to about 1.5 µg/ml, from about 0.1 µg/ml to about 2.0 µg/ml, from about 0.1 µg/ml to about 2.5 µg/ml, from about 0.1 µg/ml to about 3.0 µg/ml, from about 0.1 µg/ml to about 3.5 µg/ml, from about 0.1 µg/ml to about 4.0 µg/ml, from about 0.1 µg/ml to about 4.5 µg/ml, from about 0.5 µg/ml to about 5.0 µg/ml, from about 1.0 µg/ml to about 5.0 µg/ml, from about 1.5 µg/ml to about 5.0 µg/ml, from about 2.0 µg/ml to about 5.0 µg/ml, from about 2.5 µg/ml to about 5.0 µg/ml, from about 3.0 µg/ml to about 5.0 µg/ml, from about 3.5 µg/ml to about 5.0 µg/ml, from about 4.0 µg/ml to about 5.0 µg/ml, from about 4.5 µg/ml to about 5.0 µg/ml, from about 1.0 µg/ml to about 3.0 µg/ml, from about 0.5 µg/ml to about 3.0 µg/ml or from about 0.5 µg/ml to about 2.0 µg/ml. In certain embodiments, an immunoassay for detecting total FGF21 protein can use a detector antibody at a concentration between about 0.1 µg/ml to about 1.0 µg/ml, *e.g.*, about 0.4 µg/ml or about 0.8 µg/ml. In certain embodiments, an immunoassay for detecting active FGF21 protein can use a detector antibody at a concentration between about 1.0 µg/ml to about 3.0 µg/ml, *e.g.*, about 1.1 µg/ml or about 2.1 µg/ml.

In certain embodiments, the anti-FGF21 antibodies for use in the disclosed methods can be labeled. Labels include, but are not limited to, labels or moieties that are detected directly, such as fluorescent, chromophoric, electron-dense, chemiluminescent, and radioactive labels, as well as moieties, such as enzymes or ligands, that are detected indirectly, *e.g.*, through an enzymatic reaction or molecular interaction. Non-limiting examples of labels include the radioisotopes ³²P, ¹⁴C, ¹²⁵I, ³H and ¹³¹I, fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luciferases, *e.g.*, firefly luciferase and bacterial luciferase (*see* U.S. Patent No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, β-galactosidase, glucoamylase, lysozyme, saccharide oxidases, *e.g.*, glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals and the like. In certain embodiments, the detector antibody is labeled with biotin, *e.g.*, the detector antibody is conjugated to biotin.

In certain embodiments, the detection agent for the biotinylated detector antibody is avidin, streptavidin-HRP or streptavidin- β -D-galactopyranose (SBG). In certain embodiments, the readout of the detection agent is fluorimetric or colorimetric. For example, but not by way of limitation, tetramethylbenzidine and hydrogen peroxide can be used as the readout. In certain embodiments, if the detection agent is streptavidin-HRP, the readout can be colorimetric by using tetramethylbenzidine and hydrogen peroxide. Alternatively, in certain embodiments, resorufin β -D-galactopyranoside can be used as the readout. For example, but not by way of limitation, if the detection agent is SBG, the readout can be fluorimetric by using resorufin β -D-galactopyranoside.

In certain embodiments, the detection agent, *e.g.*, SBG, can be used at a concentration from about 50 to about 500 pM. For example, but not by way of limitation, the detection agent can be used at a concentration from about 50 to about 100 pM, from about 50 to about 150 pM, from about 50 to about 200 pM, from about 50 to about 250 pM, from about 50 to about 300 pM, from about 50 to about 350 pM, from about 50 to about 400 pM, from about 50 to about 450 pM, from about 100 to about 500 pM, from about 150 to about 500 pM, from about 200 to about 500 pM, from about 250 to about 500 pM, from about 300 to about 500 pM, from about 350 to about 500 pM, from about 400 to about 500 pM, from about 450 to about 500 pM, from about 100 to about 400 pM or from about 200 to about 400 pM. In certain embodiments, the detection agent can be used at a concentration from about 100 pM to about 400 pM, *e.g.*, SBG can be used at a concentration of about 110 pM, about 155 pM or about 310 pM. In certain embodiments, SBG is used at a concentration of about 310 pM. In certain embodiments, the detection agent, *e.g.*, HRP, can be used at a dilution from about 1/10 to about 1/1000. For example, but not by way of limitation, the detection agent can be used at a dilution from about 1/10 to about 1/100, from about 1/10 to about 1/500, from about 1/100 to about 1/1000 or from about 1/500 to about 1/1000. In certain embodiments, the detection agent can be used at a dilution from about 1/100 to about 1/1000, *e.g.*, HRP can be used at a dilution of about 1/100 or about 1/500.

In certain embodiments, the methods of the present disclosure can include blocking the capture antibody with a blocking buffer. In certain embodiments, the blocking buffer can include PBS, bovine serum albumin (BSA) and/or a biocide, *e.g.*, ProClin™ (Sigma-Aldrich, Saint Louis, MO). In certain embodiments, the method can include multiple washing steps. In certain embodiments, the solution used for washing is generally a buffer (*e.g.*, a “washing buffer”) such as, but not limited to, a PBS buffer that

includes a detergent, *e.g.*, Tween 20. For example, but not by way of limitation, the capture antibody can be washed after blocking and/or the sample can be separated from the capture antibody to remove uncaptured material, *e.g.*, by washing.

The immunoassay methods of the present disclosure can be used, in certain
5 embodiments, to detect the amount of total FGF21 protein in a sample, *e.g.*, by detecting
full-length and processed forms of FGF21. For example, but not by way of limitation, an
immunoassay method for the detection of total FGF21 protein can use one or more
antibodies that bind to an epitope present within amino acid residues 5-172 of FGF21,
e.g., amino acid residues 5-172 of SEQ ID NO: 1. In certain embodiments, the capture
10 antibody is an antibody that binds to an epitope present within amino acid residues 5-172
of FGF21 and the detector antibody is an antibody that binds to an epitope present within
amino acid residues 5-172 of FGF21. In certain embodiments, the capture antibody and
the detector antibody are the same antibody, while in other embodiments, the capture
antibody and the detector antibody are different antibodies but both bind to an epitope
15 present within amino acid residues 5-172 of FGF21. In certain embodiments, the capture
antibody and the detector antibody bind to different epitopes within amino acid residues
5-172 of FGF21. For example, but not by way of limitation, the capture antibody and the
detector antibody bind to epitopes within amino acid residues 5-172 of FGF21 that do
not overlap. In certain embodiments, the capture antibody and the detector antibody bind
20 to epitopes within amino acid residues 5-172 of FGF21 that partially overlap.

In certain embodiments, an immunoassay for determining the amount of total
FGF21 protein in a sample can include (a) contacting a capture antibody that binds to an
epitope present within amino acid residues 5-172 of FGF21 with the sample to generate a
sample-capture antibody combination material; (b) contacting the sample-capture
25 antibody combination material with a detector antibody that binds to an epitope present
within amino acid residues 5-172 of FGF21; (c) detecting the detector antibody bound to
the sample-capture antibody combination material; and (d) calculating an amount of total
FGF21 protein present in the sample based on the level of the detector antibody bound.

In certain embodiments, an immunoassay method of the present disclosure
30 can be used to detect the amount of active FGF21 protein in a sample, *e.g.*, by detecting
FGF21 protein that retains its C-terminal fragment. In certain embodiments, an
immunoassay method for the detection of total FGF21 protein can use one or more
antibodies that bind to an epitope present within amino acid residues 173-182 of FGF21,
e.g., amino acid residues 173-182 of SEQ ID NO: 1, and one or more antibodies that

bind to an epitope present within amino acid residues 5-172 of FGF21. For example, but not by way of limitation, an immunoassay method to detect the amount of active FGF21 protein can use a capture antibody that binds to an epitope present within amino acid residues 5-172 of FGF21 and a detector antibody that binds to an epitope present within amino acid residues 173-182 of FGF21. In certain embodiments, the detector antibody that binds to amino acid residues 173-182 of FGF21 can be the anti-FGF21 antibody from Epitope Diagnostics, Inc., San Diego, CA, sold under catalog number 31002. In certain embodiments, the detector antibody that binds to amino acid residues 173-182 of FGF21 can be the anti-FGF21 antibody from Epitope Diagnostics, Inc., San Diego, CA, sold under catalog number 30661. In certain embodiments, an immunoassay method for determining the amount of active FGF21 protein in a sample can include (a) contacting a capture antibody that binds to an epitope present within amino acid residues 5-172 of FGF21 with the sample to generate a sample-capture antibody combination material; (b) contacting the sample-capture antibody combination material with a detector antibody that binds to an epitope present within amino acid residues 173-182 of FGF21; (c) detecting the detector antibody bound to the sample-capture antibody combination material; and (d) calculating an amount of active FGF21 protein present in the sample based on the level of the detector antibody bound.

The present disclosure further provides immunoassay methods for determining the ratio of active FGF21 protein to total FGF21 protein in a sample. For example, but not by way of limitation, such methods can involve combining an immunoassay for detecting total FGF21 protein with an immunoassay for detecting active FGF21 protein. In certain embodiments, the immunoassay methods for determining the ratio of active FGF21 protein to total FGF21 protein in a sample can include (a)(i) contacting a first capture antibody that binds to an epitope present within amino acid residues 5-172 of FGF21 with the sample to generate a first sample-capture antibody combination material; (ii) contacting the first sample-capture antibody combination material with a first detector antibody that binds to an epitope present within amino acid residues 5-172 of FGF21; (iii) detecting the first detector antibody bound to the sample-capture antibody combination material; and (iv) calculating an amount of total FGF21 protein present in the sample based on the level of the first detector antibody bound; and (b)(i) contacting a second capture antibody that binds to an epitope present within amino acid residues 5-172 of FGF21 with the sample to generate a second sample-capture antibody combination material; (ii) contacting the second sample-

capture antibody combination material with a second detector antibody that binds to an epitope present within amino acid residues 173-182 of FGF21; (iii) detecting the second detector antibody bound to the sample-capture antibody combination material; and (iv) calculating an amount of active FGF21 protein present in the sample based on the level of the second detector antibody bound. The methods can further include comparing the amount of total FGF21 protein as determined by step (a) with the amount of active FGF21 protein as determined by step (b) to determine the ratio of active FGF21 protein to total FGF21 protein in the sample. In certain embodiments, the first capture antibody and second capture antibody are the same antibody. Alternatively, in certain embodiments, the first capture antibody and second capture antibody are different antibodies but both bind to an epitope present within amino acid residues 5-172 of FGF21. In certain embodiments, the first capture antibody and the first detector antibody bind to different epitopes within amino acid residues 5-172 of FGF21. For example, but not by way of limitation, the first capture antibody and the first detector antibody bind to epitopes within amino acid residues 5-172 of FGF21 that do not overlap. In certain embodiments, the first capture antibody and the first detector antibody bind to epitopes within amino acid residues 5-172 of FGF21 that partially overlap.

In certain embodiments, the immunoassay methods disclosed herein have a detection sensitivity, *e.g.*, an in-well sensitivity, from about 2 pg/ml to about 20 pg/ml. For example, but not by way of limitation, an immunoassay disclosed herein has a sensitivity from about 2 pg/ml to about 3 pg/ml, from about 2 pg/ml to about 4 pg/ml, from about 2 pg/ml to about 5 pg/ml, from about 2 pg/ml to about 6 pg/ml, from about 2 pg/ml to about 7 pg/ml, from about 2 pg/ml to about 8 pg/ml, from about 2 pg/ml to about 10 pg/ml, from about 2 pg/ml to about 11 pg/ml, from about 2 pg/ml to about 12 pg/ml, from about 2 pg/ml to about 13 pg/ml, from about 2 pg/ml to about 14 pg/ml, from about 2 pg/ml to about 15 pg/ml, from about 2 pg/ml to about 16 pg/ml, from about 2 pg/ml to about 17 pg/ml, from about 2 pg/ml to about 18 pg/ml, from about 2 pg/ml to about 19 pg/ml, from about 3 pg/ml to about 15 pg/ml, from about 3 pg/ml to about 10 pg/ml or from about 3 pg/ml to about 5 pg/ml. In certain embodiments, an immunoassay disclosed herein has a sensitivity of about 2 pg/ml or greater, 1 pg/ml or greater or 0.5 pg/ml or greater. In certain embodiments, an immunoassay disclosed herein has a detection sensitivity, *e.g.*, an in-well sensitivity, from about 0.2 pg/ml to about 2.0 pg/ml, *e.g.*, from about 0.2 pg/ml to about 0.5 pg/ml, from about 0.2 pg/ml to about 1.0 pg/ml or from about 0.2 pg/ml to about 1.5 pg/ml. For example, but not by way of limitation, an

immunoassay disclosed herein, *e.g.*, a single molecule immunoassay using the Simoa HD-1 Analyzer™, has a sensitivity, *e.g.*, an in-well sensitivity, from about 0.2 pg/ml to about 0.5 pg/ml.

The samples analyzed by the immunoassay methods of the present disclosure
5 can be clinical samples, cells in culture, cell supernatants, cell lysates, serum samples, blood plasma samples, other biological fluid (*e.g.*, lymphatic fluid) samples or tissue samples. In certain embodiments, the source of the sample may be solid tissue (*e.g.*, from a fresh, frozen and/or preserved organ, tissue sample, serum, blood plasma, biopsy or aspirate) or cells from a subject. In certain embodiments, the sample is a blood
10 sample. In certain embodiments, the sample is a plasma sample. In certain embodiments, the sample, *e.g.*, blood or plasma sample, can be obtained from a subject and treated with one or more protease, esterase, DDP-IV and/or phosphatase inhibitors. For example, but not by way of limitation, a sample can be treated with a cocktail of protease and phosphatase inhibitors, *e.g.*, MS-SAFE (Sigma-Aldrich, Saint Louis, MO).
15 In certain embodiments, the sample is treated with an anti-coagulant or collected in tube that contains an anti-coagulant, *e.g.*, K₂-EDTA. In certain embodiments, the sample can be collected using the P800 Blood Collection System (BD Biosciences, San Jose, CA).

III. ANTIBODIES

The present disclosure further provides antibodies that bind to FGF21, *e.g.*,
20 human FGF21. Antibodies of the present disclosure are useful for detecting and quantifying FGF21 protein levels in a sample. In certain embodiments, the antibodies of the present disclosure can be used in immunoassay methods for the detection and quantification of FGF21 protein, disclosed herein. For example, but not by way of limitation, antibodies of the present disclosure can be used to detect the levels of total
25 FGF21 protein and/or active FGF21 protein in a sample.

In certain embodiments, an antibody of the present disclosure can be humanized. In certain embodiments, an antibody of the present disclosure comprises an acceptor human framework, *e.g.*, a human immunoglobulin framework or a human consensus framework. In certain embodiments, an antibody of the present disclosure can
30 be a monoclonal antibody, including a chimeric, humanized or human antibody. For example, but not by way of limitation, an antibody of the present disclosure can be chimeric. In certain embodiments, an antibody of the present disclosure can be an antibody fragment, *e.g.*, a Fv, Fab, Fab', scFv, diabody or F(ab')₂ fragment. In certain

embodiments, the antibody is an IgG. In certain embodiments, the antibody is selected from IgG1, IgG2, IgG3 and IgG4. In certain embodiments, the antibody is a full-length antibody, *e.g.*, an intact IgG1 antibody, or other antibody class or isotype as defined herein. In certain embodiments, the anti-FGF21 antibodies disclosed herein can be
5 labeled, *e.g.*, conjugated to biotin. In certain embodiments, an antibody of the present disclosure can incorporate any of the features, singly or in combination, as described in Sections 1-7, detailed below.

A. Exemplary Anti-FGF21 Antibodies

The present disclosure provides isolated antibodies that bind to a FGF21
10 protein. In certain embodiments, an antibody of the present disclosure can bind to an epitope present within amino acid residues 5-172 of FGF21, *e.g.*, amino acid residues 5-172 of SEQ ID NO: 1. In certain embodiments, an antibody of the present disclosure can bind to an epitope present within amino acid residues 173-182 of FGF21, *e.g.*, amino acid residues 173-182 of SEQ ID NO: 1. In certain embodiments, an antibody of the
15 present disclosure does not bind to an epitope present within amino acid residues 1-4 of FGF21, *e.g.*, amino acid residues 1-4 of SEQ ID NO: 1. Non-limiting examples of anti-FGF21 antibodies are disclosed in Tables 8-13 and 16-19 and Figure 41A-B.

The present disclosure provides anti-FGF21 antibodies comprising, in certain embodiments, at least one, two, three, four, five or six CDRs selected from (a) CDR-H1
20 comprising an amino acid sequence of any one of SEQ ID NOs: 26-29 and conservative substitutions thereof; (b) CDR-H2 comprising an amino acid sequence of any one of SEQ ID NOs: 30-33 and conservative substitutions thereof; (c) CDR-H3 comprising an amino acid sequence of any one of SEQ ID NOs: 34-37 and conservative substitutions thereof; (d) CDR-L1 comprising an amino acid sequence of any one of SEQ ID NOs: 38-
25 41 and conservative substitutions thereof; (e) CDR-L2 comprising SEQ ID NOs: 42-45 and conservative substitutions thereof; and (f) CDR-L3 comprising an amino acid sequence of any one of SEQ ID NOs: 46-49 and conservative substitutions thereof.

The present disclosure provides anti-FGF21 antibodies that, in certain embodiments, comprise: (a) CDR-H1 comprising the amino acid sequence of SEQ ID
30 NO: 26 and conservative substitutions thereof; (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO: 30 and conservative substitutions thereof; (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO: 34 and conservative substitutions thereof; (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO: 38 and conservative substitutions thereof; (e) CDR-L2 comprising the amino acid sequence of

SEQ ID NO: 42 and conservative substitutions thereof; and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO: 46 and conservative substitutions thereof.

The present disclosure provides anti-FGF21 antibodies that, in certain embodiments, comprise: (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO: 27 and conservative substitutions thereof; (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO: 31 and conservative substitutions thereof; (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO: 35; (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO: 39 and conservative substitutions thereof; (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO: 43 and conservative substitutions thereof; and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO: 47 and conservative substitutions thereof.

The present disclosure provides anti-FGF21 antibodies that, in certain embodiments, comprise: (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO: 28 and conservative substitutions thereof; (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO: 32 and conservative substitutions thereof; (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO: 36 and conservative substitutions thereof; (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO: 40 and conservative substitutions thereof; (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO: 44 and conservative substitutions thereof; and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO: 48 and conservative substitutions thereof.

The present disclosure provides anti-FGF21 antibodies that, in certain embodiments, comprise: (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO: 29 and conservative substitutions thereof; (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO: 33 and conservative substitutions thereof; (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO: 37 and conservative substitutions thereof; (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO: 41 and conservative substitutions thereof; (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO: 45 and conservative substitutions thereof; and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO: 49 and conservative substitutions thereof.

In certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to an amino acid sequence of any one of SEQ ID NOs: 54-57 and 72-75. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%

identity contains substitutions (*e.g.*, conservative substitutions), insertions or deletions relative to the reference sequence, but an anti-FGF21 antibody comprising that sequence retains the ability to bind to FGF21. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted. In certain embodiments, substitutions, insertions or deletions occur in regions outside the CDRs (*i.e.*, in the FRs). In certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a VH sequence comprising an amino acid sequence of any one of SEQ ID NOs: 54-57 and 72-75.

In certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a light chain variable domain (VL) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to an amino acid sequence of any one of SEQ ID NOs: 50-53 and 68-71. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity contains substitutions (*e.g.*, conservative substitutions), insertions or deletions relative to the reference sequence, but an anti-FGF21 antibody comprising that sequence retains the ability to bind to FGF21. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted. In certain embodiments, substitutions, insertions or deletions occur in regions outside the CDRs (*i.e.*, in the FRs). In certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a VL sequence comprising an amino acid sequence of any one of SEQ ID NOs: 50-53 and 68-71.

In certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 54. In certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 50. In certain embodiments, the VH comprises one, two or three CDRs selected from: (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO: 26, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO: 30, and (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO: 34. In certain embodiments, the VL comprises one, two or three CDRs selected from: (a) CDR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (b) CDR-L2 comprising the amino acid sequence of SEQ ID NO: 42, and (c) CDR-L3 comprising the amino acid sequence of SEQ ID NO: 46.

In certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 55. In certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a VL
5 sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 51. In certain embodiments, the VH comprises one, two or three CDRs selected from: (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO: 27, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO: 31, and (c) CDR-H3 comprising the amino acid
10 sequence of SEQ ID NO: 35. In certain embodiments, the VL comprises one, two or three CDRs selected from: (a) CDR-L1 comprising the amino acid sequence of SEQ ID NO: 39, (b) CDR-L2 comprising the amino acid sequence of SEQ ID NO: 43, and (c) CDR-L3 comprising the amino acid sequence of SEQ ID NO: 47.

In certain embodiments, an anti-FGF21 antibody of the present disclosure
15 comprises a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 56. In certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 52. In certain
20 embodiments, the VH comprises one, two or three CDRs selected from: (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO: 28, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO: 32, and (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO: 36. In certain embodiments, the VL comprises one, two or three CDRs selected from: (a) CDR-L1 comprising the amino acid sequence of SEQ ID
25 NO: 40, (b) CDR-L2 comprising the amino acid sequence of SEQ ID NO: 44, and (c) CDR-L3 comprising the amino acid sequence of SEQ ID NO: 48.

In certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 57. In
30 certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 53. In certain embodiments, the VH comprises one, two or three CDRs selected from: (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO: 29, (b) CDR-H2 comprising the

amino acid sequence of SEQ ID NO: 33, and (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO: 37. In certain embodiments, the VL comprises one, two or three CDRs selected from: (a) CDR-L1 comprising the amino acid sequence of SEQ ID NO: 41, (b) CDR-L2 comprising the amino acid sequence of SEQ ID NO: 45, and (c) CDR-L3 comprising the amino acid sequence of SEQ ID NO: 49.

In certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 75. In certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 71. In certain embodiments, the VH comprises one, two or three CDRs selected from: (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO: 26, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO: 30, and (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO: 34. In certain embodiments, the VL comprises one, two or three CDRs selected from: (a) CDR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (b) CDR-L2 comprising the amino acid sequence of SEQ ID NO: 42, and (c) CDR-L3 comprising the amino acid sequence of SEQ ID NO: 46.

In certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 74. In certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 70. In certain embodiments, the VH comprises one, two or three CDRs selected from: (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO: 27, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO: 31, and (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO: 35. In certain embodiments, the VL comprises one, two or three CDRs selected from: (a) CDR-L1 comprising the amino acid sequence of SEQ ID NO: 39, (b) CDR-L2 comprising the amino acid sequence of SEQ ID NO: 43, and (c) CDR-L3 comprising the amino acid sequence of SEQ ID NO: 47.

In certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 73. In

certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 69. In certain embodiments, the VH comprises one, two or three CDRs selected from: (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO: 28, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO: 32, and (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO: 36. In certain embodiments, the VL comprises one, two or three CDRs selected from: (a) CDR-L1 comprising the amino acid sequence of SEQ ID NO: 40, (b) CDR-L2 comprising the amino acid sequence of SEQ ID NO: 44, and (c) CDR-L3 comprising the amino acid sequence of SEQ ID NO: 48.

In certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 72. In certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 68. In certain embodiments, the VH comprises one, two or three CDRs selected from: (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO: 29, (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO: 33, and (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO: 37. In certain embodiments, the VL comprises one, two or three CDRs selected from: (a) CDR-L1 comprising the amino acid sequence of SEQ ID NO: 41, (b) CDR-L2 comprising the amino acid sequence of SEQ ID NO: 45, and (c) CDR-L3 comprising the amino acid sequence of SEQ ID NO: 49.

In certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a full-length heavy chain (HC) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to an amino acid sequence of any one of SEQ ID NOs: 22-25 and 64-67. In certain embodiments, a HC sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity contains substitutions (*e.g.*, conservative substitutions), insertions or deletions relative to the reference sequence, but an anti-FGF21 antibody comprising that sequence retains the ability to bind to FGF21. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted. In certain embodiments, substitutions, insertions or deletions occur in regions outside the CDRs (*i.e.*, in the FRs). In certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a

HC sequence comprising an amino acid sequence of any one of SEQ ID NOs: 22-25 and 64-67.

In certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a full-length light chain (LC) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to an amino acid sequence of any one of SEQ ID NOs: 18-21 and 60-63. In certain embodiments, a LC sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity contains substitutions (*e.g.*, conservative substitutions), insertions or deletions relative to the reference sequence, but an anti-FGF21 antibody comprising that sequence retains the ability to bind to FGF21. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted. In certain embodiments, substitutions, insertions or deletions occur in regions outside the CDRs (*i.e.*, in the FRs). In certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a LC sequence comprising an amino acid sequence of any one of SEQ ID NOs: 18-21 and 60-63.

In certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a HC sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 22. In certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a LC sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 18.

In certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a HC sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 23. In certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a LC sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 19.

In certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a HC sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 24. In certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a LC sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 20.

In certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a HC sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 25. In certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a LC
5 sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 21.

In certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a HC sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 67. In
10 certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a LC sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 63.

In certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a HC sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 66. In
15 certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a LC sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 62.

In certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a HC sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 65. In
20 certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a LC sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 61.

In certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a HC sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 64. In
25 certain embodiments, an anti-FGF21 antibody of the present disclosure comprises a LC sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or
30 100% sequence identity to the amino acid sequence of SEQ ID NO: 60.

In certain embodiments, an anti-FGF21 antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above. In certain embodiments, an anti-FGF21 antibody is provided, wherein the antibody comprises a full-length HC as in any of the

embodiments provided above, and a full-length LC as in any of the embodiments provided above.

1. Antibody Affinity

In certain embodiments, an anti-FGF21 antibody of the present disclosure can have a dissociation constant (K_d) of ≤ 1 M, ≤ 100 mM, ≤ 10 mM, ≤ 1 mM, ≤ 100 μ M, ≤ 10 μ M, ≤ 1 μ M, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM or ≤ 0.001 nM. In certain embodiments, an antibody of the present disclosure can have a K_d of about 10^{-3} or less or 10^{-8} M or less, *e.g.*, from 10^{-8} M to 10^{-13} M, *e.g.*, from 10^{-9} M to 10^{-13} M. In certain embodiments, an anti-FGF21 antibody, disclosed herein, can have a K_d of about 10^{-10} M to 10^{-13} M. For example, but not by way of limitation, a capture antibody or a detector antibody of the present disclosure binds to FGF21 with a K_d from about 10^{-10} M to 10^{-13} M.

In certain embodiments, K_d can be measured by a radiolabeled antigen binding assay (RIA). In certain embodiments, an RIA can be performed with a Fab version of an antibody of interest and its antigen. For example, but not by way of limitation, a solution binding affinity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of (^{125} I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (see, *e.g.*, Chen et al., *J. Mol. Biol.* 293:865-881(1999)). To establish conditions for the assay, MICROTITER[®] multi-well plates (Thermo Scientific) are coated overnight with 5 μ g/ml of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23°C). In a non-adsorbent plate (Nunc #269620), 100 pM or 26 pM [^{125} I]-antigen are mixed with serial dilutions of a Fab of interest (*e.g.*, consistent with assessment of the anti-VEGF antibody, Fab-12, in Presta et al., *Cancer Res.* 57:4593-4599 (1997)). The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (*e.g.*, about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature (*e.g.*, for one hour). The solution is then removed and the plate washed eight times with 0.1% polysorbate 20 (TWEEN-20[®]) in PBS. When the plates have dried, 150 μ l/well of scintillant (MICROSCINT-20[™], Packard) is added, and the plates are counted on a TOPCOUNT[™] gamma counter (Packard) for ten minutes. Concentrations of each Fab

that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

In certain embodiments, K_d can be measured using a BIACORE® surface plasmon resonance assay. For example, but not by way of limitation, an assay using a BIACORE®-2000, a BIACORE®-3000, a BIACORE X100 or a BIACORE T200 processing unit (Biacore, Inc., Piscataway, NJ) is performed at 25°C with immobilized antigen CM5 chips at ~10 response units (RU). In certain embodiments, carboxymethylated dextran biosensor chips (CM5, Biacore, Inc.) are activated with *N*-ethyl-*N*'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 µg/ml (~0.2 µM) before injection at a flow rate of 5 µl/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% polysorbate 20 (TWEEN-20™) surfactant (PBST) at 25°C at a flow rate of approximately 25 µl/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIACORE® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (K_d) can be calculated as the ratio k_{off}/k_{on} . See, e.g., Chen et al., *J. Mol. Biol.* 293:865-881 (1999). If the on-rate exceeds $10^6 \text{ M}^{-1} \text{ s}^{-1}$ by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25°C of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophotometer (Aviv Instruments) or a 8000-series SLM-AMINCO™ spectrophotometer (ThermoSpectronic) with a stirred cuvette.

2. Antibody Fragments

In certain embodiments, an antibody of the present disclosure is an antibody fragment. Antibody fragments include, but are not limited to, Fab, Fab', Fab'-SH, F(ab')₂, Fv, and scFv fragments, and other fragments described below. For a review of certain antibody fragments, see Hudson et al. *Nat. Med.* 9:129-134 (2003). For a review

of scFv fragments, see, *e.g.*, Pluckthün, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds., (Springer-Verlag, New York), pp. 269-315 (1994); see also WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. For a discussion of Fab and F(ab')₂ fragments comprising salvage receptor binding epitope residues and
5 having increased *in vivo* half-life, see U.S. Patent No. 5,869,046.

In certain embodiments, an antibody of the present disclosure can be a diabody. Diabodies are antibody fragments comprising two antigen-binding sites that may be bivalent or bispecific. See, for example, EP 404,097; WO 1993/01161; Hudson et al., *Nat. Med.* 9:129-134 (2003); and Hollinger et al., *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993). Triabodies and tetrabodies, which are additional antibody fragments within the scope of the antibodies of the present disclosure, are also described in Hudson et al., *Nat. Med.* 9:129-134 (2003).
10

In certain embodiments, an antibody of the present disclosure can be a single-domain antibody. Single-domain antibodies are antibody fragments that comprise all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; see, *e.g.*, U.S. Patent No. 6,248,516 B1).
15

Antibody fragments can be made by various techniques including, but not limited to, proteolytic digestion of an intact antibody as well as production by recombinant host cells (*e.g.*, *E. coli* or phage), as described herein.
20

3. Chimeric and Humanized Antibodies

In certain embodiments, an antibody of the present disclosure is a chimeric antibody. Certain chimeric antibodies are described in the art, *e.g.*, in U.S. Patent No. 4,816,567; and Morrison et al., *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). In certain embodiments, a chimeric antibody of the present disclosure comprises a non-human variable region (*e.g.*, a variable region derived from a mouse, rat, hamster, rabbit or non-human primate, such as a monkey) and a human constant region. In a further example, a chimeric antibody can be a "class switched" antibody in which the class or subclass has been changed from that of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.
25
30

In certain embodiments, a chimeric antibody of the present disclosure can be a humanized antibody. Typically, a non-human antibody is humanized to reduce

immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which CDRs, *e.g.*, CDRs, (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In certain embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (*e.g.*, the antibody from which the CDR residues are derived), *e.g.*, to restore or improve antibody specificity or affinity.

Humanized antibodies and methods of making them are reviewed, *e.g.*, in Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008), and are further described, *e.g.*, in Riechmann et al., *Nature* 332:323-329 (1988); Queen et al., *Proc. Nat'l Acad. Sci. USA* 86:10029-10033 (1989); US Patent Nos. 5, 821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri *et al.*, *Methods* 36:25-34 (2005) (describing specificity determining region (SDR) grafting); Padlan, *Mol. Immunol.* 28:489-498 (1991) (describing "resurfacing"); Dall'Acqua et al., *Methods* 36:43-60 (2005) (describing "FR shuffling"); and Osbourn et al., *Methods* 36:61-68 (2005) and Klimka et al., *Br. J. Cancer*, 83:252-260 (2000) (describing the "guided selection" approach to FR shuffling).

Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the "best-fit" method (see, *e.g.*, Sims et al. *J. Immunol.* 151:2296 (1993)); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (see, *e.g.*, Carter et al. *Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); and Presta et al. *J. Immunol.*, 151:2623 (1993)); human mature (somatically mutated) framework regions or human germline framework regions (see, *e.g.*, Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008)); and framework regions derived from screening FR libraries (see, *e.g.*, Baca et al., *J. Biol. Chem.* 272:10678-10684 (1997) and Rosok et al., *J. Biol. Chem.* 271:22611-22618 (1996)).

4. Human Antibodies

In certain embodiments, an antibody of the present disclosure can be a human antibody. Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, *Curr. Opin. Pharmacol.* 5: 368-74 (2001) and Lonberg, *Curr. Opin. Immunol.* 20:450-459 (2008).

Human antibodies can be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic mice, the endogenous immunoglobulin loci have generally been inactivated. For review of methods for obtaining human antibodies from transgenic animals, see Lonberg, *Nat. Biotech.* 23:1117-1125 (2005). See also, e.g., U.S. Patent Nos. 6,075,181 and 6,150,584 describing XENOMOUSE™ technology; U.S. Patent No. 5,770,429 describing HUMAB® technology; U.S. Patent No. 7,041,870 describing K-M MOUSE® technology, and U.S. Patent Application Publication No. US 2007/0061900, describing VELOCIMOUSE® technology). Human variable regions from intact antibodies generated by such animals may be further modified, e.g., by combining with a different human constant region.

Human antibodies can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described. (See, e.g., Kozbor *J. Immunol.*, 133: 3001 (1984); Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner et al., *J. Immunol.*, 147: 86 (1991).) Human antibodies generated via human B-cell hybridoma technology are also described in Li et al., *Proc. Natl. Acad. Sci. USA*, 103:3557-3562 (2006). Additional methods include those described, for example, in U.S. Patent No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines) and Ni, *Xiandai Mianyixue*, 26(4):265-268 (2006) (describing human-human hybridomas). Human hybridoma technology (Trioma technology) is also described in Vollmers and Brandlein, *Histology and Histopathology*, 20(3):927-937 (2005) and Vollmers and Brandlein, *Methods and Findings in Experimental and Clinical Pharmacology*, 27(3):185-91 (2005).

Human antibodies may also be generated by isolating Fv clone variable domain sequences selected from human-derived phage display libraries. Such variable domain sequences may then be combined with a desired human constant domain. Techniques for selecting human antibodies from antibody libraries are described below.

5. Library-Derived Antibodies

Antibodies of the present disclosure can be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, *e.g.*, in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, 2001) and further described, *e.g.*, in the McCafferty et al., *Nature* 348:552-554; Clackson et al., *Nature* 352: 624-628 (1991); Marks et al., *J. Mol. Biol.* 222: 581-597 (1992); Marks and Bradbury, in *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, NJ, 2003); Sidhu et al., *J. Mol. Biol.* 338(2): 299-310 (2004); Lee et al., *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee et al., *J. Immunol. Methods* 284(1-2): 119-132 (2004).

In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter et al., *Ann. Rev. Immunol.*, 12: 433-455 (1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (*e.g.*, from human) to provide a single source of antibodies to a wide range of non-self and also self antigens without any immunization as described by Griffiths et al., *EMBO J*, 12: 725-734 (1993). In certain embodiments, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement *in vitro*, as described by Hoogenboom and Winter, *J. Mol. Biol.*, 227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: US Patent No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

Antibodies or antibody fragments isolated from human antibody libraries are considered human antibodies or human antibody fragments herein.

6. Multispecific Antibodies

In certain embodiments, an antibody of the present disclosure can be a multispecific antibody, *e.g.*, a bispecific antibody. Multispecific antibodies are monoclonal antibodies that have binding specificities for at least two different epitopes.

5 In certain embodiments, one of the binding specificities is for an epitope present on FGF21 and the other is for any other antigen. Bispecific antibodies can be prepared as full-length antibodies or antibody fragments.

Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs
10 having different specificities (*see* Milstein and Cuello, *Nature* 305: 537 (1983)), WO 93/08829, and Traunecker et al., *EMBO J.* 10: 3655 (1991)), and “knob-in-hole” engineering (*see, e.g.*, U.S. Patent No. 5,731,168). Multi-specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004A1); cross-linking two or more antibodies or fragments
15 (*see, e.g.*, US Patent No. 4,676,980, and Brennan et al., *Science*, 229: 81 (1985)); using leucine zippers to produce bi-specific antibodies (*see, e.g.*, Kostelny et al., *J. Immunol.*, 148(5):1547-1553 (1992)); using “diabody” technology for making bispecific antibody fragments (*see, e.g.*, Hollinger et al., *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993)); and using single-chain Fv (sFv) dimers (*see, e.g.*, Gruber et al., *J. Immunol.*, 152:5368
20 (1994)); and preparing trispecific antibodies as described, *e.g.*, in Tutt et al. *J. Immunol.* 147: 60 (1991).

Engineered antibodies with three or more functional antigen binding sites, including “Octopus antibodies,” are also included herein (*see, e.g.*, US 2006/0025576A1).

25 7. Antibody Variants

The presently disclosed subject matter further provides amino acid sequence variants of the disclosed antibodies. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody can be prepared by introducing appropriate modifications into the
30 nucleotide sequence encoding the antibody or by peptide synthesis. Such modifications include, but are not limited to, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided

that the final antibody, *i.e.*, modified, possesses the desired characteristics, *e.g.*, antigen-binding.

a) Substitution, Insertion, and Deletion Variants

Antibody variants can have one or more amino acid substitutions, insertions
 5 and/or deletions. Sites of interest for such variation include, but are not limited to, the CDRs, and FRs. Non-limiting examples of conservative substitutions are shown in Table 1 under the heading of “preferred substitutions.” Non-limiting examples of more substantial changes are provided in Table 1 under the heading of “exemplary
 10 substitutions,” and as further described below in reference to amino acid side chain classes. Amino acid substitutions can be introduced into an antibody of interest and the products screened for a desired activity, *e.g.*, retained/improved antigen binding, decreased immunogenicity or improved complement dependent cytotoxicity (CDC) or antibody-dependent cell-mediated cytotoxicity (ADCC).

15 Table 1

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr

Original Residue	Exemplary Substitutions	Preferred Substitutions
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

Amino acids may be grouped according to common side-chain properties:

- (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;
- (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;
- (3) acidic: Asp, Glu;
- (4) basic: His, Lys, Arg;
- (5) residues that influence chain orientation: Gly, Pro;
- (6) aromatic: Trp, Tyr, Phe.

In certain embodiments, non-conservative substitutions will entail exchanging a member of one of these classes for another class.

In certain embodiments, a type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody, *e.g.*, a humanized or human antibody. Generally, the resulting variant(s) selected for further study will have modifications, *e.g.*, improvements, in certain biological properties such as, but not limited to, increased affinity, reduced immunogenicity, relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. A non-limiting example of a substitutional variant is an affinity matured antibody, which may be conveniently generated, *e.g.*, using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more CDR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (*e.g.*, binding affinity).

In certain embodiments, alterations (*e.g.*, substitutions) can be made in CDRs, *e.g.*, to improve antibody affinity. Such alterations may be made in CDR “hotspots,” *i.e.*, residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (see, *e.g.*, Chowdhury, *Methods Mol. Biol.* 207:179-196 (2008)), and/or residues that contact antigen, with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, *e.g.*, in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O’Brien et al., ed., Human Press, Totowa, NJ, (2001)). In certain

embodiments of affinity maturation, diversity can be introduced into the variable genes chosen for maturation by any of a variety of methods (*e.g.*, error-prone PCR, chain shuffling or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity.

- 5 Another method to introduce diversity involves CDR-directed approaches, in which several CDR residues (*e.g.*, 4-6 residues at a time) are randomized. CDR residues involved in antigen binding can be specifically identified, *e.g.*, using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

- 10 In certain embodiments, substitutions, insertions and/or deletions can occur within one or more CDRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (*e.g.*, conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in CDRs. Such alterations may, for example, be outside of antigen contacting residues in the CDRs. In certain embodiments of the variant VH and VL
15 sequences provided above, each CDR either is unaltered or contains no more than one, two or three amino acid substitutions.

- A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called “alanine scanning mutagenesis” as described by Cunningham and Wells (1989) *Science*, 244:1081-1085. In this method, a residue or
20 group of target residues (*e.g.*, charged residues such as arg, asp, his, lys, and glu) are identified and replaced by a neutral or negatively charged amino acid (*e.g.*, alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively or
25 additionally, a crystal structure of an antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

- Amino acid sequence insertions include amino- and/or carboxyl-terminal
30 fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N-

or C-terminus of the antibody to an enzyme (*e.g.*, for Antibody-directed enzyme prodrug therapy (ADEPT)) or a polypeptide which increases the serum half-life of the antibody.

b) Glycosylation variants

Antibodies of the present disclosure can, in certain embodiments, be altered to increase or decrease the extent to which the antibody is glycosylated. For example, but not by way of limitation, the addition or deletion of glycosylation sites of an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

Where the antibodies of the present disclosure comprise an Fc region, the carbohydrate attached thereto, if present, can be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. See, *e.g.*, Wright et al. *TIBTECH* 15:26-32 (1997). The oligosaccharide may include various carbohydrates, *e.g.*, mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the “stem” of the biantennary oligosaccharide structure. In certain embodiments, modifications of the oligosaccharide in an antibody of the present disclosure can be made in order to create antibody variants with certain improved properties.

In certain embodiments, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody can be from about 1% to about 80%, from about 1% to about 65%, from about 5% to about 65% or from about 20% to about 40% and values in between.

In certain embodiments, the amount of fucose can be determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (*e.g.*, complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (Eu numbering of Fc region residues); however, Asn297 can also be located about ± 3 amino acids upstream or downstream of position 297, *i.e.*, between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. See, *e.g.*, US Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko

Kogyo Co., Ltd). Examples of publications related to “defucosylated” or “fucose-deficient” antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki et al. *J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004).

Defucosylated antibodies can be produced in any cell line that are deficient in protein fucosylation. Non-limiting examples of cell lines include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. *Arch. Biochem. Biophys.* 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams *et al.*, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, *FUT8*, knockout CHO cells (see, *e.g.*, Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004); Kanda, Y. et al., *Biotechnol. Bioeng.*, 94(4):680-688 (2006); and WO2003/085107).

Antibodies variants are further provided with bisected oligosaccharides, *e.g.*, in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function. Non-limiting examples of such antibody variants are described, *e.g.*, in WO 2003/011878 (Jean-Mairet et al.); US Patent No. 6,602,684 (Umana et al.); and US 2005/0123546 (Umana et al.). Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants can have improved CDC function. Such antibody variants are described, *e.g.*, in WO 1997/30087 (Patel et al.); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

c) Fc region variants

In certain embodiments, one or more amino acid modifications can be introduced into the Fc region of an antibody provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (*e.g.*, a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (*e.g.*, a substitution) at one or more amino acid positions.

In certain embodiments, the present disclosure provides antibody variants that possess some but not all effector functions. Such limited effector function can make the

antibody variants desirable candidates for applications in which the half life of the antibody *in vivo* is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991). Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Patent No. 5,500,362 (see, *e.g.*, Hellstrom, I. et al. *Proc. Nat'l Acad. Sci. USA* 83:7059-7063 (1986)) and Hellstrom, I et al., *Proc. Nat'l Acad. Sci. USA* 82:1499-1502 (1985); 5,821,337 (see Bruggemann, M. et al., *J. Exp. Med.* 166:1351-1361 (1987)). Alternatively, non-radioactive assays methods can be employed (see, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (Cell Technology, Inc. Mountain View, CA; and CYTOTOX 96® non-radioactive cytotoxicity assay (Promega, Madison, WI). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, *e.g.*, in an animal model such as that disclosed in Clynes et al. *Proc. Nat'l Acad. Sci. USA* 95:652-656 (1998). C1q binding assays can also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. See, *e.g.*, C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay can be performed (see, for example, Gazzano-Santoro et al., *J. Immunol. Methods* 202:163 (1996); Cragg, M.S. et al., *Blood* 101:1045-1052 (2003); and Cragg, M.S. and M.J. Glennie, *Blood* 103:2738-2743 (2004)). FcRn binding and *in vivo* clearance/half life determinations can also be performed using methods known in the art (see, *e.g.*, Petkova, S.B. et al., *Int'l. Immunol.* 18(12):1759-1769 (2006)). In certain embodiments, alterations can be made in the Fc region that result in altered (*i.e.*, either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), *e.g.*, as described in US Patent No. 6,194,551, WO 99/51642, and Idusogie et al. *J. Immunol.* 164: 4178-4184 (2000).

Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).

Certain antibody variants with improved or diminished binding to FcRs are described. *See, e.g.*, U.S. Patent No. 6,737,056; WO 2004/056312, and Shields et al., *J. Biol. Chem.* 9(2): 6591-6604 (2001).

In certain embodiments, antibody variants of the present disclosure comprise an Fc region with one or more amino acid substitutions that improve ADCC, *e.g.*, substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues).

In certain embodiments, alteration made in the Fc region of an antibody, *e.g.*, a bispecific antibody, disclosed herein, can produce a variant antibody with an increased half-life and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., *J. Immunol.* 117:587 (1976) and Kim et al., *J. Immunol.* 24:249 (1994)), are described in US2005/0014934A1 (Hinton et al.). Those antibodies comprise an Fc region with one or more substitutions therein, which improve binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, *e.g.*, substitution of Fc region residue 434 (US Patent No. 7,371,826).

See also Duncan & Winter, *Nature* 322:738-40 (1988); U.S. Patent No. 5,648,260; U.S. Patent No. 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

d) Cysteine engineered antibody variants

In certain embodiments, it may be desirable to create cysteine engineered antibodies, *e.g.*, "thioMAbs," in which one or more residues of an antibody are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate, as described further herein. In certain

embodiments, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain; A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region. Cysteine engineered antibodies can be generated as described, *e.g.*, in U.S. Patent No. 7,521,541.

5

e) Antibody Derivatives

In certain embodiments, antibodies of the present disclosure can be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propylene glycol homopolymers, 15 polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (*e.g.*, glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer are attached, they can 20 be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

In certain embodiments, conjugates of an antibody and nonproteinaceous 25 moiety that may be selectively heated by exposure to radiation are provided. In one embodiment, the nonproteinaceous moiety is a carbon nanotube (Kam et al., *Proc. Natl. Acad. Sci. USA* 102: 11600-11605 (2005)). In certain embodiments, the radiation can be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which 30 cells proximal to the antibody-nonproteinaceous moiety are killed.

B. Methods of Antibody Production

The antibodies disclosed herein can be produced using any available or known technique in the art. For example, but not by way of limitation, antibodies can be produced using recombinant methods and compositions, *e.g.*, as described in U.S. Patent No. 4,816,567. Detailed procedures to generate antibodies are described in the Examples below.

The presently disclosed subject matter further provides an isolated nucleic acid encoding an antibody disclosed herein. For example, the isolated nucleic acid can encode an amino acid sequence that includes the VL and/or an amino acid sequence comprising the VH of the antibody, *e.g.*, the light and/or heavy chains of the antibody. In certain embodiments, the isolated nucleic acid can include a nucleotide sequence that encodes a heavy chain variable region amino acid sequence having the sequence set forth in SEQ ID NO: 54, and/or a nucleotide sequence that encodes a light chain variable region amino acid sequence having the sequence set forth in SEQ ID NO: 50. In certain embodiments, the isolated nucleic acid can include a nucleotide sequence that encodes a heavy chain variable region amino acid sequence having the sequence set forth in SEQ ID NO: 57, and/or a nucleotide sequence that encodes a light chain variable region amino acid sequence having the sequence set forth in SEQ ID NO: 53.

In certain embodiments, the nucleic acid can be present in one or more vectors, *e.g.*, expression vectors. As used herein, the term “vector” refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a “plasmid,” which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, where additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors, expression vectors, are capable of directing the expression of genes to which they are operably linked. In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids (vectors). However, the disclosed subject matter is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*,

replication defective retroviruses, adenoviruses and adeno-associated viruses) that serve equivalent functions.

In certain embodiments, the nucleic acid encoding an antibody of the present disclosure and/or the one or more vectors including the nucleic acid can be introduced
5 into a host cell. In certain embodiments, the introduction of a nucleic acid into a cell can be carried out by any method known in the art including, but not limited to, transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. In certain embodiments, a host cell can
10 include, *e.g.*, has been transformed with: (1) a vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and an amino acid sequence comprising the VH of the antibody, or (2) a first vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and a second vector comprising a nucleic acid that encodes an amino acid sequence comprising
15 the VH of the antibody. In certain embodiments, the host cell is eukaryotic, *e.g.*, a Chinese Hamster Ovary (CHO) cell or lymphoid cell (*e.g.*, Y0, NS0, Sp20 cell).

In certain embodiments, the methods of making a disclosed anti-FGF21 antibody can include culturing a host cell, in which a nucleic acid encoding the antibody has been introduced, under conditions suitable for expression of the antibody, and
20 optionally recovering the antibody from the host cell and/or host cell culture medium. In certain embodiments, the antibody is recovered from the host cell through chromatography techniques.

For recombinant production of an antibody of the present disclosure, a nucleic acid encoding an antibody, *e.g.*, as described above, can be isolated and inserted
25 into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acid may be readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody).

Suitable host cells for cloning or expression of antibody-encoding vectors
30 include prokaryotic or eukaryotic cells described herein. For example, antibodies can be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria, *see, e.g.*, U.S. Patent Nos. 5,648,237, 5,789,199, and 5,840,523. (*See also* Charlton, *Methods in Molecular Biology*, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, NJ, 2003), pp.

245-254, describing expression of antibody fragments in *E. coli*.) After expression, the antibody may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been “humanized,” resulting in the production of an antibody with a partially or fully human glycosylation pattern. See Gerngross, *Nat. Biotech.* 22:1409-1414 (2004), and Li et al., *Nat. Biotech.* 24:210-215 (2006). Suitable host cells for the expression of glycosylated antibody can also be derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of *Spodoptera frugiperda* cells.

Suitable host cells for the expression of glycosylated antibody are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of *Spodoptera frugiperda* cells.

In certain embodiments, plant cell cultures can be utilized as host cells. See, e.g., US Patent Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIESTM technology for producing antibodies in transgenic plants).

In certain embodiments, vertebrate cells can also be used as hosts. For example, but not by way of limitation, mammalian cell lines that are adapted to grow in suspension can be useful. Non-limiting examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293 cells as described, e.g., in Graham et al., *J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK); mouse sertoli cells (TM4 cells as described, e.g., in Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK; buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TRI cells, as described, e.g., in Mather et al., *Annals N.Y. Acad. Sci.* 383:44-68 (1982); MRC 5 cells; and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO)

cells, including DHFR⁺ CHO cells (Urlaub et al., *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); and myeloma cell lines such as Y0, NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, *see, e.g.*, Yazaki and Wu, *Methods in Molecular Biology*, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, NJ),
5 pp. 255-268 (2003).

In certain embodiments, techniques for making bispecific and/or multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (*see* Milstein and Cuello, *Nature* 305: 537 (1983)), PCT Patent Application No. WO 93/08829, and
10 Traunecker et al., *EMBO J.* 10: 3655 (1991)), and “knob-in-hole” engineering (*see, e.g.*, U.S. Patent No. 5,731,168). Bispecific antibodies can also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004A1); cross-linking two or more antibodies or fragments (*see, e.g.*, US Patent No. 4,676,980, and Brennan et al., *Science*, 229: 81 (1985)); using leucine zippers
15 to produce bispecific antibodies (*see, e.g.*, Kostelny et al., *J. Immunol.*, 148(5):1547-1553 (1992)); using “diabody” technology for making bispecific antibody fragments (*see, e.g.*, Hollinger et al., *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993)); and using single-chain Fv (sFv) dimers (*see, e.g.*, Gruber et al., *J. Immunol.*, 152:5368 (1994)); and preparing trispecific antibodies as described, *e.g.*, in Tutt et al. *J. Immunol.* 147: 60
20 (1991).

Bispecific and multispecific molecules of the present disclosure can also be made using chemical techniques (*see, e.g.*, Kranz (1981) *Proc. Natl. Acad. Sci. USA* 78:5807), “polydoma” techniques (*see, e.g.*, U.S. Patent 4,474,893) or recombinant DNA techniques. Bispecific and multispecific molecules of the presently disclosed subject
25 matter can also be prepared by conjugating the constituent binding specificities, *e.g.*, a first epitope and a second epitope binding specificities, using methods known in the art and as described herein. For example, but not by way of limitation, each binding specificity of the bispecific and multispecific molecule can be generated separately and then conjugated to one another. When the binding specificities are proteins or peptides,
30 a variety of coupling or cross-linking agents can be used for covalent conjugation. Non-limiting examples of cross-linking agents include protein A, carbodiimide, N-succinimidyl-S-acetyl-thioacetate (SATA), N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), and sulfosuccinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (sulfo-SMCC) (*see, e.g.*, Karpovsky (1984) *J. Exp. Med.*

160:1686; Liu (1985) *Proc. Natl. Acad. Sci. USA* 82:8648). Other methods include those described by Paulus (*Behring Ins. Mitt.* (1985) No. 78, 118-132; Brennan (1985) *Science* 229:81-83), Glennie (1987) *J. Immunol.* 139: 2367-2375). When the binding specificities are antibodies (*e.g.*, two humanized antibodies), they can be conjugated via
5 sulfhydryl bonding of the C-terminus hinge regions of the two heavy chains. In certain embodiments, the hinge region can be modified to contain an odd number of sulfhydryl residues, *e.g.*, one, prior to conjugation.

In certain embodiments, both binding specificities of a bispecific antibody can be encoded in the same vector and expressed and assembled in the same host cell.

10 This method is particularly useful where the bispecific and multispecific molecule is a MAb x MAb, MAb x Fab, Fab x F(ab')₂ or ligand x Fab fusion protein. In certain embodiments, a bispecific antibody of the present disclosure can be a single chain molecule, such as a single chain bispecific antibody, a single chain bispecific molecule comprising one single chain antibody and a binding determinant or a single chain
15 bispecific molecule comprising two binding determinants. Bispecific and multispecific molecules can also be single chain molecules or can comprise at least two single chain molecules. Methods for preparing bi- and multispecific molecules are described, for example, in U.S. Patent No. 5,260,203; U.S. Patent No. 5,455,030; U.S. Patent No. 4,881,175; U.S. Patent No. 5,132,405; U.S. Patent No. 5,091,513; U.S. Patent No.
20 5,476,786; U.S. Patent No. 5,013,653; U.S. Patent No. 5,258,498; and U.S. Patent No. 5,482,858. Engineered antibodies with three or more functional antigen binding sites (*e.g.*, epitope binding sites) including "Octopus antibodies," are also included herein (see, *e.g.*, US 2006/0025576A1).

In certain embodiments, an animal system can be used to produce an antibody
25 of the present disclosure. One animal system for preparing hybridomas is the murine system. Hybridoma production in the mouse is a very well established procedure. Immunization protocols and techniques for isolation of immunized splenocytes for fusion are known in the art. Fusion partners (*e.g.*, murine myeloma cells) and fusion procedures are also known (*see, e.g.*, Harlow and Lane (1988), *Antibodies, A Laboratory*
30 *Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor New York).

C. Binding Competition Assays

The anti-FGF21 antibodies of the present disclosure provided herein can be identified, screened for or characterized for their physical/chemical properties and/or

biological activities by various assays known in the art and provided herein.

1. Binding assays and other assays

An antibody of the present disclosure can be tested for its antigen binding activity by known methods, such enzyme-linked immunosorbent assay (ELISA), a
5 radioimmunoassay (RIA) or a Western Blot Assay. Each of these assays generally detects the presence of protein-antibody complexes of particular interest by employing a labeled reagent (*e.g.*, an antibody) specific for the complex of interest. For example, the FGF21-antibody complexes can be detected using, *e.g.*, an enzyme-linked antibody or antibody fragment which recognizes and specifically binds to the antibody-FGF21
10 complexes. Alternatively, the complexes can be detected using any of a variety of other immunoassays. For example, the antibody can be radioactively labeled and used in a radioimmunoassay (RIA) (*see, for example*, Weintraub, B., Principles of Radioimmunoassays, Seventh Training Course on Radioligand Assay Techniques, The Endocrine Society, March, 1986, which is incorporated by reference herein). The
15 radioactive isotope can be detected by such means as the use of a Geiger counter or a scintillation counter or by autoradiography.

In certain embodiments, competition assays can be used to identify an antibody that competes with an anti-FGF21 antibody of the present disclosure, *e.g.*, mAb4 or mAb15, for binding to FGF21. In certain embodiments, such a competing
20 antibody binds to the same epitope (*e.g.*, a linear or a conformational epitope) that is bound by mAb4 or mAb15. Detailed exemplary methods for mapping an epitope to which an antibody binds are provided in Morris (1996) "Epitope Mapping Protocols," in *Methods in Molecular Biology* vol. 66 (Humana Press, Totowa, NJ).

In a non-limiting example of a competition assay, immobilized FGF21 can be
25 incubated in a solution comprising a first labeled antibody that binds to FGF21 (*e.g.*, mAb4 or mAb15) and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to FGF21. The second antibody may be present in a hybridoma supernatant. As a control, immobilized FGF21 is incubated in a solution comprising the first labeled antibody but not the second unlabeled antibody.
30 After incubation under conditions permissive for binding of the first antibody to FGF21, excess unbound antibody is removed, and the amount of label associated with immobilized FGF21 is measured. If the amount of label associated with immobilized FGF21 is substantially reduced in the test sample relative to the control sample, then that

indicates that the second antibody is competing with the first antibody for binding to FGF21. *See* Harlow and Lane (1988) *Antibodies: A Laboratory Manual* ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

D. Immunoconjugates

5 The presently disclosed subject matter further provides immunoconjugates comprising an antibody conjugated to one or more cytotoxic agents, such as chemotherapeutic agents or drugs, growth inhibitory agents, toxins (*e.g.*, protein toxins, enzymatically active toxins of bacterial, fungal, plant or animal origin, or fragments thereof) or radioactive isotopes. For example, an antibody or antigen-binding portion of
10 the disclosed subject matter can be functionally linked (*e.g.*, by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other binding molecules, such as another antibody, antibody fragment, peptide or binding mimetic.

 In certain embodiments, an immunoconjugate is an antibody-drug conjugate (ADC) in which an antibody is conjugated to one or more drugs, including but not
15 limited to a maytansinoid (*see* U.S. Patent Nos. 5,208,020, 5,416,064 and European Patent EP 0 425 235); an auristatin such as monomethylauristatin drug moieties DE and DF (MMAE and MMAF) (*see* U.S. Patent Nos. 5,635,483 and 5,780,588, and 7,498,298); a dolastatin; a calicheamicin or derivative thereof (*see* U.S. Patent Nos. 5,712,374, 5,714,586, 5,739,116, 5,767,285, 5,770,701, 5,770,710, 5,773,001, and
20 5,877,296; Hinman et al., *Cancer Res.* 53:3336-3342 (1993); and Lode et al., *Cancer Res.* 58:2925-2928 (1998)); an anthracycline such as daunomycin or doxorubicin (*see* Kratz et al., *Current Med. Chem.* 13:477-523 (2006); Jeffrey et al., *Bioorganic & Med. Chem. Letters* 16:358-362 (2006); Torgov et al., *Bioconj. Chem.* 16:717-721 (2005); Nagy et al., *Proc. Natl. Acad. Sci. USA* 97:829-834 (2000); Dubowchik et al., *Bioorg. &*
25 *Med. Chem. Letters* 12:1529-1532 (2002); King et al., *J. Med. Chem.* 45:4336-4343 (2002); and U.S. Patent No. 6,630,579); methotrexate; vindesine; a taxane such as docetaxel, paclitaxel, larotaxel, tesetaxel, and ortataxel; a trichothecene; and CC1065.

 In certain embodiments, an immunoconjugate comprises an antibody as described herein conjugated to an enzymatically active toxin or fragment thereof,
30 including but not limited to diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor,

curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes.

In certain embodiments, an immunoconjugate comprises an antibody as described herein conjugated to a radioactive atom to form a radioconjugate. A variety of
 5 radioactive isotopes are available for the production of radioconjugates. Non-limiting examples include At²¹¹, I¹³¹, I¹²⁵, Y⁹⁰, Re¹⁸⁶, Re¹⁸⁸, Sm¹⁵³, Bi²¹², P³², Pb²¹² and radioactive isotopes of Lu. When the radioconjugate is used for detection, it can include a radioactive atom for scintigraphic studies, for example tc99m or I123, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance
 10 imaging, mri), such as iodine-123 again, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron.

Conjugates of an antibody and cytotoxic agent can be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate
 15 (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds
 20 (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., *Science* 238:1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026. The linker can be a “cleavable linker” facilitating release of a cytotoxic
 25 drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari et al., *Cancer Res.* 52:127-131 (1992); U.S. Patent No. 5,208,020) can be used.

The immunoconjugates disclosed herein expressly contemplate, but are not limited to, such conjugates prepared with cross-linker reagents including, but not limited
 30 to, BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (e.g., from Pierce Biotechnology, Inc., Rockford, IL., U.S.A).

IV. KITS

The presently disclosed subject matter further provides kits containing materials useful for performing the immunoassays disclosed herein. In certain embodiments, the kit includes a container containing an anti-FGF21 antibody disclosed
5 herein. Non-limiting examples of suitable containers include bottles, test tubes, vials and microtiter plates. The containers can be formed from a variety of materials such as glass or plastic. In certain embodiments, the kit further includes a package insert that provides instructions for using the anti-FGF21 antibody in the disclosed immunoassay methods.

In certain embodiments, the kit can include one or more containers containing
10 one or more anti-FGF21 antibodies. Non-limiting examples of anti-FGF21 antibodies are disclosed in Tables 8-13 and 16-19 and Figure 41A and B. For example, but not by way of limitation, the kit can include at least one container that includes an anti-FGF21 capture antibody and at least one container that includes an anti-FGF21 detector antibody.

15 In certain embodiments, a kit for detecting total FGF21 protein in a sample includes a first container containing a capture antibody that binds to an epitope present within amino acid residues 5-172 of FGF21, a second container containing a detector antibody that binds to an epitope present within amino acid residues 5-172 of FGF21 and a third container containing a detection agent.

20 In certain embodiments, a kit for detecting active FGF21 protein in a sample includes a first container containing a capture antibody that binds to an epitope present within amino acid residues 5-172 of FGF21, a second container containing a detector antibody that binds to an epitope present within amino acid residues 173-182 of FGF21 and a third container containing a detection agent.

25 In certain embodiments, a kit for determining the ratio of active FGF21 protein to total FGF21 protein in a sample includes a first container containing a first capture antibody that binds to an epitope present within amino acid residues 5-172 of FGF21, a second container containing a first detector antibody that binds to an epitope present within amino acid residues 5-172 of FGF21, a third container containing a
30 second capture antibody that binds to an epitope present within amino acid residues 5-172 of FGF21, a fourth container containing a second detector antibody that binds to an epitope present within amino acid residues 173-182 of FGF21 and a fifth container containing a detection agent. In certain embodiments, the first and second capture

antibodies are the same antibody and can be provided in a single container.

Alternatively, the first and second capture antibodies are different antibodies, and can be provided in separate containers.

5 In certain embodiments, the capture antibody and/or the detector antibody can be provided in a kit of the present disclosure at a concentration of about 0.1 µg/ml to about 5.0 µg/ml. In certain embodiments, the detector antibody can be labeled, *e.g.*, with biotin.

In certain embodiments, the detection agent provided in a kit of the present disclosure can be avidin, streptavidin-HRP or streptavidin-β-D-galactopyranose (SBG).
10 In certain embodiments, a kit of the present disclosure can further include tetramethylbenzidine, hydrogen peroxide and/or resorufin β-D-galactopyranoside. In certain embodiments, if the kit includes streptavidin-HRP, then the kit can further include tetramethylbenzidine and hydrogen peroxide. In certain embodiments, if the kit includes SBG, then the kit can further include resorufin β-D-galactopyranoside. In
15 certain embodiments, SBG can be provided in a kit at a concentration from about 100 pM to about 400 pM.

In certain embodiments, the capture antibody can be provided attached to solid support surface, such as, for example but not limited to, a plate or a bead, *e.g.*, a paramagnetic bead. Alternatively or additionally, the kit can further include a solid
20 support surface that can be coupled to the capture antibody. In certain embodiments, the solid support can be paramagnetic beads and can be provided at a concentration from about 0.1×10^7 beads/ml to about 10.0×10^7 beads/ml.

Alternatively or additionally, the kit can include other materials desirable from a commercial and user standpoint, including other buffers, diluents and filters. In
25 certain embodiments, the kit can include materials for collecting and/or processing blood samples.

V. EXEMPLARY EMBODIMENTS

A. In certain non-limiting embodiments, the presently disclosed subject matter provides for an immunoassay method for determining the amount of total FGF21 protein
30 in a sample comprising:

(a) contacting a capture antibody that binds to an epitope present within amino acid residues 5-172 of FGF21 with the sample to generate a sample-capture antibody combination material;

(b) contacting the sample-capture antibody combination material with a detector antibody that binds to an epitope present within amino acid residues 5-172 of FGF21;

(c) detecting the detector antibody bound to the sample-capture antibody combination material; and

5 (d) calculating an amount of total FGF21 protein present in the sample based on the level of the detector antibody bound.

A1. The foregoing immunoassay method of A, wherein the capture antibody and the detector antibody bind to different epitopes within amino acid residues 5-172 of FGF21.

10 B. In certain non-limiting embodiments, the presently disclosed subject matter provides for an immunoassay method for determining the amount of active FGF21 protein in a sample comprising:

(a) contacting a capture antibody that binds to an epitope present within amino acid residues 5-172 of FGF21 with the sample to generate a sample-capture antibody
15 combination material;

(b) contacting the sample-capture antibody combination material with a detector antibody that binds to an epitope present within amino acid residues 173-182 of FGF21;

(c) detecting the detector antibody bound to the sample-capture antibody combination material; and

20 (d) calculating an amount of active FGF21 protein present in the sample based on the level of the detector antibody bound.

C. In certain non-limiting embodiments, the presently disclosed subject matter provides for an immunoassay method for determining the ratio of active FGF21 protein to total FGF21 protein in a sample comprising:

25 (a) (i) contacting a first capture antibody that binds to an epitope present within amino acid residues 5-172 of FGF21 with the sample to generate a first sample-capture antibody combination material; (ii) contacting the first sample-capture antibody combination material with a first detector antibody that binds to an epitope present within amino acid residues 5-172 of FGF21; (iii) detecting the first detector antibody
30 bound to the sample-capture antibody combination material; and (iv) calculating an amount of total FGF21 protein present in the sample based on the level of the first detector antibody bound;

(b) (i) contacting a second capture antibody that binds to an epitope present within amino acid residues 5-172 of FGF21 with the sample to generate a second

sample-capture antibody combination material; (ii) contacting the second sample-capture antibody combination material with a second detector antibody that binds to an epitope present within amino acid residues 173-182 of FGF21; (iii) detecting the second detector antibody bound to the sample-capture antibody combination material; and (iv)

5 calculating an amount of active FGF21 protein present in the sample based on the level of the second detector antibody bound; and

(c) comparing the amount of total FGF21 protein as determined by step (a) with the amount of active FGF21 protein as determined by step (b) to determine the ratio of active FGF21 protein to total FGF21 protein in the sample.

10 C1. The foregoing immunoassay method of C, wherein the first capture antibody and second capture antibody are the same antibody.

C2. The foregoing immunoassay method of C, wherein the first capture antibody and the first detector antibody bind to different epitopes within amino acid residues 5-172 of FGF21.

15 C3. The foregoing immunoassay method of any one of A-C2, wherein the immunoassay is an enzyme-linked immunosorbent assay (ELISA).

C4. The foregoing immunoassay method of any one of A-C3, wherein one or more of the capture antibody, first capture antibody and second capture antibody is immobilized to a paramagnetic bead.

20 C5. The foregoing immunoassay method of any one of A-C4, wherein one or more of the detector antibody, first detector antibody and second detector antibody is conjugated to biotin.

C6. The foregoing immunoassay method of any one of A-C5, wherein one or more of the capture antibody, first capture antibody and second capture antibody binds to FGF21 with a K_d from about 10^{-10} M to 10^{-13} M.

25 C7. The foregoing immunoassay method of any one of A and C-C6, wherein one or more of the detector antibody and first detector antibody binds to FGF21 with a K_d from about 10^{-10} M to 10^{-13} M.

C8. The foregoing immunoassay method of any one of A-C7, wherein the sample
30 is a blood sample.

C9. The foregoing immunoassay method of any one of A-C7, wherein the sample is a plasma sample.

C10. The foregoing immunoassay method of any one of A-C9, wherein the method detects the amount of total or active FGF21 protein in the sample at an in-well sensitivity from about 2 pg/ml to about 20 pg/ml.

5 C11. The foregoing immunoassay method of any one of A-C9, wherein the immunoassay method is performed using a single molecule detection instrument.

C12. The foregoing immunoassay method of C11, wherein the single molecule detection instrument is the Quanterix Simoa HD-1 Analyzer™.

10 C13. The foregoing immunoassay method of C11 and C12, wherein the method detects the amount of total or active FGF21 protein in the sample at an in-well sensitivity from about 0.2 pg/ml to about 0.5 pg/ml.

C14. The foregoing immunoassay method of any one of A-C13, wherein one or more of the capture antibody, first capture antibody and second capture antibody comprises:

15 (a) a heavy chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 26 and 27, and conservative substitutions thereof;

(b) a heavy chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 30 and 31, and conservative substitutions thereof;

20 (c) a heavy chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 34 and 35, and conservative substitutions thereof;

25 (d) a light chain variable region CDR1 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 38 and 39, and conservative substitutions thereof;

(e) a light chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 42 and 43, and conservative substitutions thereof; and

30 (f) a light chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 46 and 47, and conservative substitutions thereof.

C15. The foregoing immunoassay of any one of A-C13, wherein one or more of the capture antibody, first capture antibody and second capture antibody comprises:

(a) a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 54, 55, 74 and 75, and conservative substitutions thereof; and

5 (b) a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 50, 51, 70 and 71, and conservative substitutions thereof.

C16. The foregoing immunoassay of any one of A-C13, wherein one or more of the capture antibody, first capture antibody and second capture antibody comprises:

10 (a) a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 22, 23, 66 and 67, and conservative substitutions thereof; and

(b) a light chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 18, 19, 62 and 63, and conservative substitutions thereof.

C17. The foregoing immunoassay method of any one of A and C-C13, wherein one or more of the detector antibody and first detector antibody comprises:

15 (a) a heavy chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 28 and 29, and conservative substitutions thereof;

(b) a heavy chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 32 and 33, and
20 conservative substitutions thereof;

(c) a heavy chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 36 and 37, and conservative substitutions thereof;

(d) a light chain variable region CDR1 domain comprising an amino acid
25 sequence selected from the group consisting of SEQ ID NOs: 40 and 41, and conservative substitutions thereof;

(e) a light chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 44 and 45, and conservative substitutions thereof; and

30 (f) a light chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 48 and 49, and conservative substitutions thereof.

C18. The foregoing immunoassay of any one of A and C-C13, wherein one or more of the detector antibody and first detector antibody comprises:

(a) a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 56, 57, 72 and 73, and conservative substitutions thereof; and

5 (b) a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 52, 53, 68 and 69, and conservative substitutions thereof.

C19. The foregoing immunoassay of any one of A and C-C13, wherein one or more of the detector antibody and first detector antibody comprises:

10 (a) a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 24, 25, 64 and 65, and conservative substitutions thereof; and

(b) a light chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 20, 21, 60 and 61, and conservative substitutions thereof.

C20. The foregoing immunoassay method of C14, wherein one or more of the capture antibody, first capture antibody and second capture antibody comprises:

15 (a) a heavy chain variable region CDR1 comprising the amino acid sequence of SEQ ID NO: 26, and conservative substitutions thereof;

(b) a heavy chain variable region CDR2 domain comprising the amino acid sequence of SEQ ID NO: 30, and conservative substitutions thereof;

20 (c) a heavy chain variable region CDR3 domain comprising the amino acid sequence of SEQ ID NO: 34, and conservative substitutions thereof;

(d) a light chain variable region CDR1 domain comprising the amino acid sequence of SEQ ID NO: 38, and conservative substitutions thereof;

(e) a light chain variable region CDR2 domain comprising the amino acid sequence of SEQ ID NO: 42, and conservative substitutions thereof; and

25 (f) a light chain variable region CDR3 domain comprising the amino acid sequence of SEQ ID NO: 46, and conservative substitutions thereof.

C21. The foregoing immunoassay of C20, wherein one or more of the capture antibody, first capture antibody and second capture antibody comprises:

30 (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 54, and conservative substitutions thereof; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 50, and conservative substitutions thereof.

C22. The foregoing immunoassay of C21, wherein one or more of the capture antibody, first capture antibody and second capture antibody comprises:

(a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 22, and conservative substitutions thereof; and

(b) a light chain comprising the amino acid sequence of SEQ ID NO: 18, and conservative substitutions thereof.

5 C23. The foregoing immunoassay method of C17, wherein one or more of the detector antibody and first detector antibody comprises:

(a) a heavy chain variable region CDR1 comprising the amino acid sequence of SEQ ID NO: 29, and conservative substitutions thereof;

10 (b) a heavy chain variable region CDR2 domain comprising the amino acid sequence of SEQ ID NO: 33, and conservative substitutions thereof;

(c) a heavy chain variable region CDR3 domain comprising the amino acid sequence of SEQ ID NO: 37, and conservative substitutions thereof;

(d) a light chain variable region CDR1 domain comprising the amino acid sequence of SEQ ID NO: 41, and conservative substitutions thereof;

15 (e) a light chain variable region CDR2 domain comprising the amino acid sequence of SEQ ID NO: 45, and conservative substitutions thereof; and

(f) a light chain variable region CDR3 domain comprising the amino acid sequence of SEQ ID NO: 49, and conservative substitutions thereof.

20 C24. The foregoing immunoassay of C23, wherein one or more of the detector antibody and first detector antibody comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 57, and conservative substitutions thereof; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 53, and conservative substitutions thereof.

25 C25. The foregoing immunoassay of C24, wherein one or more of the detector antibody and first detector antibody comprises:

(a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 25, and conservative substitutions thereof; and

30 (b) a light chain comprising the amino acid sequence of SEQ ID NO: 21, and conservative substitutions thereof.

C26. The foregoing immunoassay method of any one of A-C13, wherein one or more of the capture antibody, first capture antibody and second capture antibody competitively binds with an antibody comprising:

(a) a heavy chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 26 and 27, and conservative substitutions thereof;

5 (b) a heavy chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 30 and 31, and conservative substitutions thereof;

(c) a heavy chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 34 and 35, and conservative substitutions thereof;

10 (d) a light chain variable region CDR1 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 38 and 39, and conservative substitutions thereof;

(e) a light chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 42 and 43, and conservative substitutions thereof; and

15 (f) a light chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 46 and 47, and conservative substitutions thereof.

C27. The foregoing immunoassay method of any one of A and C-C13, wherein one or more of the detector antibody and first detector antibody competitively binds with an antibody comprising:

(a) a heavy chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 28 and 29, and conservative substitutions thereof;

25 (b) a heavy chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 32 and 33, and conservative substitutions thereof;

(c) a heavy chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 36 and 37, and conservative substitutions thereof;

30 (d) a light chain variable region CDR1 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 40 and 41, and conservative substitutions thereof;

(e) a light chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 44 and 45, and conservative substitutions thereof; and

5 (f) a light chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 48 and 49, and conservative substitutions thereof.

D. In certain non-limiting embodiments, the presently disclosed subject matter provides for a kit for detecting total FGF21 protein in a sample comprising:

10 (a) a capture antibody that binds to an epitope present within amino acid residues 5-172 of FGF21;

(b) a detector antibody that binds to an epitope present within amino acid residues 5-172 of FGF21; and

(c) a detection agent.

15 D1. The foregoing kit of D, wherein the capture antibody and the detector antibody bind to different epitopes within amino acid residues 5-172 of FGF21.

E. In certain non-limiting embodiments, the presently disclosed subject matter provides for a kit for detecting active FGF21 protein in a sample comprising:

(a) a capture antibody that binds to an epitope present within amino acid residues 5-172 of FGF21;

20 (b) a detector antibody that binds to an epitope present within amino acid residues 173-182 of FGF21; and

(c) a detection agent.

25 F. In certain non-limiting embodiments, the presently disclosed subject matter provides for a kit for determining the ratio of active FGF21 protein to total FGF21 protein in a sample comprising:

(a) (i) a first capture antibody that binds to an epitope present within amino acid residues 5-172 of FGF21 and (ii) a first detector antibody that binds to an epitope present within amino acid residues 5-172 of FGF21;

30 (b) (i) a second capture antibody that binds to an epitope present within amino acid residues 5-172 of FGF21 and (ii) a second detector antibody that binds to an epitope present within amino acid residues 173-182 of FGF21; and

(c) one or more detection agents.

F1. The foregoing kit of F, wherein the first capture antibody and second capture antibody are the same antibody.

F2. The foregoing kit of F, wherein the first capture antibody and the first detector antibody bind to different epitopes within amino acid residues 5-172 of FGF21.

5 F3. The foregoing kit of any one of D-F2, wherein one or more of the capture antibody, first capture antibody and second capture antibody is immobilized to a paramagnetic bead.

F4. The foregoing kit of any one of D-F3, wherein one or more of the detector antibody, first detector antibody and second detector antibody is conjugated to biotin.

10 F5. The foregoing kit of any one of D-F4, wherein the detection agent is selected from the group consisting of a streptavidin- β -D-galactopyranose conjugate, a streptavidin-horseradish peroxidase conjugate and a combination thereof.

F6. The foregoing kit of F5 further comprising resorufin β -D-galactopyranoside, tetramethylbenzidine, hydrogen peroxide or combinations thereof.

15 F7. The foregoing kit of any one of D-F6, wherein one or more of the capture antibody, first capture antibody and second capture antibody binds to FGF21 with a K_d from about 10^{-10} M to 10^{-13} M.

F8. The foregoing kit of any one of D and F-F7, wherein one or more of the detector antibody and first detector antibody binds to FGF21 with a K_d from about 10^{-10} M to 10^{-13} M.

20 F9. The foregoing kit of any one of D and F-F8, wherein the detector antibody or first detector antibody has a concentration from about 0.1 μ g/ml to about 1 μ g/ml.

F10. The foregoing kit of any one of E-F7, wherein one or more of the detector antibody or second detector antibody has a concentration from about 1 μ g/ml to about 3 μ g/ml.

25 F11. The foregoing kit of F5, wherein the streptavidin- β -D-galactopyranose conjugate has a concentration from about 100 pM to about 400 pM.

F12. The foregoing kit of any one of D-F11, wherein one or more of the capture antibody, first capture antibody and second capture antibody comprises:

30 (a) a heavy chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 26 and 27, and conservative substitutions thereof;

(b) a heavy chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 30 and 31, and conservative substitutions thereof;

(c) a heavy chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 34 and 35, and conservative substitutions thereof;

5 (d) a light chain variable region CDR1 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 38 and 39, and conservative substitutions thereof;

(e) a light chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 42 and 43, and conservative substitutions thereof; and

10 (f) a light chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 46 and 47, and conservative substitutions thereof.

F13. The foregoing kit of any one of D-F11, wherein one or more of the capture antibody, first capture antibody and second capture antibody comprises:

15 (a) a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 54, 55, 74 and 75, and conservative substitutions thereof; and

(b) a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 50, 51, 70 and 71, and conservative substitutions thereof.

20 F14. The foregoing kit of any one of D-F11, wherein one or more of the capture antibody, first capture antibody and second capture antibody comprises:

(a) a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 22, 23, 66 and 67, and conservative substitutions thereof; and

25 (b) a light chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 18, 19, 62 and 63, and conservative substitutions thereof.

F15. The foregoing kit of any one of D and F-F11, wherein one or more of the detector antibody and first detector antibody comprises:

30 (a) a heavy chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 28 and 29, and conservative substitutions thereof;

(b) a heavy chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 32 and 33, and conservative substitutions thereof;

(c) a heavy chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 36 and 37, and conservative substitutions thereof;

5 (d) a light chain variable region CDR1 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 40 and 41, and conservative substitutions thereof;

(e) a light chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 44 and 45, and conservative substitutions thereof; and

10 (f) a light chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 48 and 49, and conservative substitutions thereof.

F16. The foregoing kit of any one of D and F-F11, wherein one or more of the detector antibody and first detector antibody comprises:

15 (a) a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 56, 57, 72 and 73, and conservative substitutions thereof; and

(b) a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 52, 53, 68 and 69, and conservative substitutions thereof.

F17. The foregoing kit of any one of D and F-F11, wherein one or more of the detector antibody and first detector antibody comprises:

(a) a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 24, 25, 64 and 65, and conservative substitutions thereof; and

25 (b) a light chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 20, 21, 60 and 61, and conservative substitutions thereof.

F18. The foregoing kit of F12, wherein one or more of the capture antibody, first capture antibody and second capture antibody comprises:

30 (a) a heavy chain variable region CDR1 comprising the amino acid sequence of SEQ ID NO: 26, and conservative substitutions thereof;

(b) a heavy chain variable region CDR2 domain comprising the amino acid sequence of SEQ ID NO: 30, and conservative substitutions thereof;

(c) a heavy chain variable region CDR3 domain comprising the amino acid sequence of SEQ ID NO: 34, and conservative substitutions thereof;

(d) a light chain variable region CDR1 domain comprising the amino acid sequence of SEQ ID NO: 38, and conservative substitutions thereof;

(e) a light chain variable region CDR2 domain comprising the amino acid sequence of SEQ ID NO: 42, and conservative substitutions thereof; and

5 (f) a light chain variable region CDR3 domain comprising the amino acid sequence of SEQ ID NO: 46, and conservative substitutions thereof.

F19. The foregoing kit of F18, wherein one or more of the capture antibody, first capture antibody and second capture antibody comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID
10 NO: 54, and conservative substitutions thereof; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 50, and conservative substitutions thereof.

F20. The foregoing kit of F19, wherein one or more of the capture antibody, first capture antibody and second capture antibody comprises:

15 (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 22, and conservative substitutions thereof; and

(b) a light chain comprising the amino acid sequence of SEQ ID NO: 18, and conservative substitutions thereof.

20 F21. The foregoing kit of F15, wherein one or more of the detector antibody and first detector antibody comprises:

(a) a heavy chain variable region CDR1 comprising the amino acid sequence of SEQ ID NO: 29, and conservative substitutions thereof;

(b) a heavy chain variable region CDR2 domain comprising the amino acid sequence of SEQ ID NO: 33, and conservative substitutions thereof;

25 (c) a heavy chain variable region CDR3 domain comprising the amino acid sequence of SEQ ID NO: 37, and conservative substitutions thereof;

(d) a light chain variable region CDR1 domain comprising the amino acid sequence of SEQ ID NO: 41, and conservative substitutions thereof;

30 (e) a light chain variable region CDR2 domain comprising the amino acid sequence of SEQ ID NO: 45, and conservative substitutions thereof; and

(f) a light chain variable region CDR3 domain comprising the amino acid sequence of SEQ ID NO: 49, and conservative substitutions thereof.

F22. The foregoing kit of F21, wherein one or more of the detector antibody and first detector antibody comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 57, and conservative substitutions thereof; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 53, and conservative substitutions thereof.

5 F23. The foregoing kit of F22, wherein one or more of the detector antibody and first detector antibody comprises:

(a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 25, and conservative substitutions thereof; and

(b) a light chain comprising the amino acid sequence of SEQ ID NO: 21, and
10 conservative substitutions thereof.

F24. The foregoing kit of any one of D-F11, wherein one or more of the capture antibody, first capture antibody and second capture antibody competitively binds with an antibody comprising:

(a) a heavy chain variable region CDR1 comprising an amino acid sequence
15 selected from the group consisting of SEQ ID NOs: 26 and 27, and conservative substitutions thereof;

(b) a heavy chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 30 and 31, and conservative substitutions thereof;

(c) a heavy chain variable region CDR3 domain comprising an amino acid
20 sequence selected from the group consisting of SEQ ID NOs: 34 and 35, and conservative substitutions thereof;

(d) a light chain variable region CDR1 domain comprising an amino acid
25 sequence selected from the group consisting of SEQ ID NOs: 38 and 39, and conservative substitutions thereof;

(e) a light chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 42 and 43, and conservative substitutions thereof; and

(f) a light chain variable region CDR3 domain comprising an amino acid
30 sequence selected from the group consisting of SEQ ID NOs: 46 and 47, and conservative substitutions thereof.

F25. The foregoing kit of any one of D and F-F11, wherein one or more of the detector antibody and first detector antibody competitively binds with an antibody comprising:

(a) a heavy chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 28 and 29, and conservative substitutions thereof;

5 (b) a heavy chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 32 and 33, and conservative substitutions thereof;

(c) a heavy chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 36 and 37, and conservative substitutions thereof;

10 (d) a light chain variable region CDR1 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 40 and 41, and conservative substitutions thereof;

(e) a light chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 44 and 45, and conservative substitutions thereof; and

15 (f) a light chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 48 and 49, and conservative substitutions thereof.

F26. The foregoing kit of any one of D-F25, wherein the sample is a blood sample.

F27. The foregoing kit of any one of D-F25, wherein the sample is a plasma sample.

F28. The foregoing kit of any one of D-F27, wherein the kit detects the amount of total or active FGF21 protein in the sample at an in-well sensitivity from about 0.2 pg/ml to about 0.5 pg/ml.

25 G. In certain non-limiting embodiments, the presently disclosed subject matter provides for an isolated anti-FGF21 antibody, or an antigen-binding portion thereof, comprising:

(a) a heavy chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 26-29, and conservative substitutions thereof;

(b) a heavy chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 30-33, and conservative substitutions thereof;

(c) a heavy chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 34-37, and conservative substitutions thereof;

5 (d) a light chain variable region CDR1 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 38-41, and conservative substitutions thereof;

(e) a light chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 42-45, and conservative substitutions thereof; and

10 (f) a light chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 46-49, and conservative substitutions thereof.

G1. The foregoing isolated antibody of G, wherein the antibody, or antigen-binding portion thereof, comprises:

15 (a) a heavy chain variable region CDR1 comprising the amino acid sequence of SEQ ID NO: 26, and conservative substitutions thereof;

(b) a heavy chain variable region CDR2 domain comprising the amino acid sequence of SEQ ID NO: 30, and conservative substitutions thereof;

20 (c) a heavy chain variable region CDR3 domain comprising the amino acid sequence of SEQ ID NO: 34, and conservative substitutions thereof;

(d) a light chain variable region CDR1 domain comprising the amino acid sequence of SEQ ID NO: 38, and conservative substitutions thereof;

(e) a light chain variable region CDR2 domain comprising the amino acid sequence of SEQ ID NO: 42, and conservative substitutions thereof; and

25 (f) a light chain variable region CDR3 domain comprising the amino acid sequence of SEQ ID NO: 46, and conservative substitutions thereof.

G2. The foregoing isolated antibody of G, wherein the antibody, or antigen-binding portion thereof, comprises:

30 (a) a heavy chain variable region CDR1 comprising the amino acid sequence of SEQ ID NO: 27, and conservative substitutions thereof;

(b) a heavy chain variable region CDR2 domain comprising the amino acid sequence of SEQ ID NO: 31, and conservative substitutions thereof;

(c) a heavy chain variable region CDR3 domain comprising the amino acid sequence of SEQ ID NO: 35, and conservative substitutions thereof;

(d) a light chain variable region CDR1 domain comprising the amino acid sequence of SEQ ID NO: 39, and conservative substitutions thereof;

(e) a light chain variable region CDR2 domain comprising the amino acid sequence of SEQ ID NO: 43, and conservative substitutions thereof; and

5 (f) a light chain variable region CDR3 domain comprising the amino acid sequence of SEQ ID NO: 47, and conservative substitutions thereof.

G3. The foregoing isolated antibody of G, wherein the antibody, or antigen-binding portion thereof, comprises:

(a) a heavy chain variable region CDR1 comprising the amino acid sequence of
10 SEQ ID NO: 28, and conservative substitutions thereof;

(b) a heavy chain variable region CDR2 domain comprising the amino acid sequence of SEQ ID NO: 32, and conservative substitutions thereof;

(c) a heavy chain variable region CDR3 domain comprising the amino acid sequence of SEQ ID NO: 36, and conservative substitutions thereof;

15 (d) a light chain variable region CDR1 domain comprising the amino acid sequence of SEQ ID NO: 40, and conservative substitutions thereof;

(e) a light chain variable region CDR2 domain comprising the amino acid sequence of SEQ ID NO: 44, and conservative substitutions thereof; and

(f) a light chain variable region CDR3 domain comprising the amino acid
20 sequence of SEQ ID NO: 48, and conservative substitutions thereof.

G4. The foregoing isolated antibody of G, wherein the antibody, or antigen-binding portion thereof, comprises:

(a) a heavy chain variable region CDR1 comprising the amino acid sequence of
SEQ ID NO: 29, and conservative substitutions thereof;

25 (b) a heavy chain variable region CDR2 domain comprising the amino acid sequence of SEQ ID NO: 33, and conservative substitutions thereof;

(c) a heavy chain variable region CDR3 domain comprising the amino acid sequence of SEQ ID NO: 37, and conservative substitutions thereof;

(d) a light chain variable region CDR1 domain comprising the amino acid
30 sequence of SEQ ID NO: 41, and conservative substitutions thereof;

(e) a light chain variable region CDR2 domain comprising the amino acid sequence of SEQ ID NO: 45, and conservative substitutions thereof; and

(f) a light chain variable region CDR3 domain comprising the amino acid sequence of SEQ ID NO: 49, and conservative substitutions thereof.

G5. The foregoing isolated antibody of G1, wherein the antibody, or antigen-binding portion thereof, comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 54, and conservative substitutions thereof; and

5 (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 50, and conservative substitutions thereof.

G6. The foregoing isolated antibody of G2, wherein the antibody, or antigen-binding portion thereof, comprises:

10 (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 55, and conservative substitutions thereof; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 51, and conservative substitutions thereof.

G7. The foregoing isolated antibody of G3, wherein the antibody, or antigen-binding portion thereof, comprises:

15 (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 56, and conservative substitutions thereof; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 52, and conservative substitutions thereof.

20 G8. The foregoing isolated antibody of G4, wherein the antibody, or antigen-binding portion thereof, comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 57, and conservative substitutions thereof; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 53, and conservative substitutions thereof.

25 G9. The foregoing isolated antibody of G1, wherein the antibody, or antigen-binding portion thereof, comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 75, and conservative substitutions thereof; and

30 (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 71, and conservative substitutions thereof.

G10. The foregoing isolated antibody of G2, wherein the antibody, or antigen-binding portion thereof, comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 74, and conservative substitutions thereof; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 70, and conservative substitutions thereof.

G11. The foregoing isolated antibody of G3, wherein the antibody, or antigen-binding portion thereof, comprises:

5 (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 73, and conservative substitutions thereof; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 69, and conservative substitutions thereof.

10 G12. The foregoing isolated antibody of G4, wherein the antibody, or antigen-binding portion thereof, comprises:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 72, and conservative substitutions thereof; and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 68, and conservative substitutions thereof.

15 G13. The foregoing isolated antibody of G5, wherein the antibody, or antigen-binding portion thereof, comprises:

(a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 22, and conservative substitutions thereof; and

20 (b) a light chain comprising the amino acid sequence of SEQ ID NO: 18, and conservative substitutions thereof.

G14. The foregoing isolated antibody of G6, wherein the antibody, or antigen-binding portion thereof, comprises:

(a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 23, and conservative substitutions thereof; and

25 (b) a light chain comprising the amino acid sequence of SEQ ID NO: 19, and conservative substitutions thereof.

G15. The foregoing isolated antibody of G7, wherein the antibody, or antigen-binding portion thereof, comprises:

30 (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 24, and conservative substitutions thereof; and

(b) a light chain comprising the amino acid sequence of SEQ ID NO: 20, and conservative substitutions thereof.

G16. The foregoing isolated antibody of G8, wherein the antibody, or antigen-binding portion thereof, comprises:

(a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 25, and conservative substitutions thereof; and

(b) a light chain comprising the amino acid sequence of SEQ ID NO: 21, and conservative substitutions thereof.

5 G17. The foregoing isolated antibody of G9, wherein the antibody, or antigen-binding portion thereof, comprises:

(a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 67, and conservative substitutions thereof; and

10 (b) a light chain comprising the amino acid sequence of SEQ ID NO: 63, and conservative substitutions thereof.

G18. The foregoing isolated antibody of G10, wherein the antibody, or antigen-binding portion thereof, comprises:

(a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 66, and conservative substitutions thereof; and

15 (b) a light chain comprising the amino acid sequence of SEQ ID NO: 62, and conservative substitutions thereof.

G19. The foregoing isolated antibody of G11, wherein the antibody, or antigen-binding portion thereof, comprises:

20 (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 65, and conservative substitutions thereof; and

(b) a light chain comprising the amino acid sequence of SEQ ID NO: 61, and conservative substitutions thereof.

G20. The foregoing isolated antibody of G12, wherein the antibody, or antigen-binding portion thereof, comprises:

25 (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 64, and conservative substitutions thereof; and

(b) a light chain comprising the amino acid sequence of SEQ ID NO: 60, and conservative substitutions thereof.

30 H. In certain non-limiting embodiments, the presently disclosed subject matter provides for an isolated nucleic acid encoding the antibody, or antigen-binding portion thereof, of any one of G-G20.

I. In certain non-limiting embodiments, the presently disclosed subject matter provides for a host cell comprising the nucleic acid of H.

J. In certain non-limiting embodiments, the presently disclosed subject matter provides for a method of producing an antibody comprising culturing the host cell of I so that the antibody is produced.

5 J1. The foregoing method of J, further comprising recovering the antibody from the host cell.

K. In certain non-limiting embodiments, the presently disclosed subject matter provides for a composition comprising one or more antibodies, or antigen-binding portions thereof, of any one of G-G20.

10

EXAMPLES

The following examples are merely illustrative of the presently disclosed subject matter and should not be considered as limitations in any way.

Example 1: Anti-FGF21 Antibody Generation

15

Monoclonal antibodies were generated by immunizing SJL and Balb/c mice with recombinant human FGF21. 80 hybridoma supernatants were screened by ELISA (Figure 1). 20 hybridomas were selected based on binding to intact human FGF21 (PUR 98271), intact cynomolgus FGF21 (PUR 98270) and cleaved human FGF21 (PUR 102247) generated by digesting intact human FGF21 by human FAP.

20

Example 2: Anti-FGF21 Antibody Characterization

IgG obtained from the selected 20 hybridomas identified in Example 1 were further characterized by ELISA. ELISA was performed as follows: 96 well MaxiSorp plate (439454, Nalge Nunc International; Rochester, NY) was coated with 1 µg/mL of anti-FGF21 mAbs or anti-FGF21 sheep pAbs (Cat. No. RD184108100, Biovendor, Asheville, NC) in coating buffer (50 mM sodium carbonate, pH 9.6) overnight at 4°C. On the next day, after blocking with PBS containing 0.5% BSA and 10 ppm ProClin pH 7.4, and washing with the Washing buffer (PBS, 0.05% Tween 20, pH7.2), plate was incubated with 0.00000186-2000 pg/mL of intact human FGF21 (full-length, uncleaved FGF21; Cat. No. 2539-FG, R&D Systems) or the FAP-cleaved human FGF21 in assay buffer (25 mM HEPES, pH 7.2, 150 mM NaCl, 0.2 mM CaCl₂, 0.1% bovine serum albumin (BSA), 0.05% Tween 20) for 1-2 h at room temperature. After washing with the Washing buffer, the plate was incubated with 0.5 µg/ml of the secondary antibody (R&D Systems, biotinylated goat anti-FGF21 pAb BAF2539) for 1-2 hr at room

temperature. After washing with Washing buffer, the plate was incubated with High Sensitivity Streptavidin-HRP (PIERCE Cat. No. 21130) diluted 1:1,000 in assay buffer. After washing with the Washing buffer, the binding of anti-FGF21 to recombinant FGF21 was assessed by adding substrate 3, 3', 5, 5' tetramethyl benzidine (TMBE 1000, Moss; Pasadena, MD). The mean absorbance values from duplicate wells were plotted as a function of antibody concentration and the data were fitted to a three parameter equation to calculate the half maximal effective concentration (EC₅₀) values for each antibody using Prism 6 (GraphPad Software, Inc., La Jolla, CA) (Table 2).

Table 2. EC₅₀ values for each FGF21 antibody.

Primary Ab	Secondary Ab	Intact FGF21 EC₅₀ (pg/ml)	Cleaved FGF21 EC₅₀ (pg/ml)	EC₅₀ ratio (Cleaved/Intact)
mAb1	Goat anti-FGF21 pAb	126	228	1.8
mAb2	Goat anti-FGF21 pAb	2108	3187	1.5
mAb3	Goat anti-FGF21 pAb	292	450	1.5
mAb4	Goat anti-FGF21 pAb	90	170	1.9
mAb5	Goat anti-FGF21 pAb	5506	19331	3.5
mAb6	Goat anti-FGF21 pAb	1993	4813	2.4
mAb7	Goat anti-FGF21 pAb	8797	25403	2.9
mAb8	Goat anti-FGF21 pAb	503	777	1.5
mAb9	Goat anti-FGF21 pAb	855	1385	1.6
mAb10	Goat anti-FGF21 pAb	136	249	1.8
mAb11	Goat anti-FGF21 pAb	149	318	2.1
mAb12	Goat anti-FGF21 pAb	5633	30386	5.4
mAb13	Goat anti-FGF21 pAb	169	300	1.8
Sheep pAb	Goat anti-FGF21 pAb	48	84	1.7

On the basis of differential detection of intact versus cleaved FGF21 and absolute EC₅₀ values, antibodies mAb5, mAb6, mAb7 and mAb12 were excluded from further analysis. Antibodies mAb1, mAb2, mAb3, mAb4, mAb8, mAb9, mAb10, mAb11, mAb13, mAb15 and mAb16 were biotinylated by using EZ-Link™ NHS-PEG

Solid-Phase Biotinylation Kit (PIERCE Cat. No. 21450) and sandwich ELISA was conducted in a pairwise combinatorial manner using intact FGF21 (Tables 3 and 4). Biotinylated goat anti-FGF21 pAb BAF2539 (R&D Systems) was used as a positive control.

5

Table 3. Compatibility of anti-FGF21 mAbs in sandwich ELISA.

	BIO-1	BIO-10	BIO-11	BIO-4	BIO-9	BIO-13	BIO-2	BIO-3	BIO-8	BAF2539
mAb1				XX	XX					XX
mAb10				XX	XX	X				XX
mAb11				XX	XX	X				XX
mAb4	XX	XX	XX							XX
mAb9	XX	XX	XX							XX
mAb13	X	X	X							XX
mAb8	XX	XX	XX	XX		X		X		XX
mAb2										XX
mAb3										XX
Sheep pAb										XX

XX: strong signal with OD>1, X: strong signal with OD<1

Table 4. Compatibility of anti-FGF21 mAbs in sandwich ELISA.

	BIO-4	BIO-11	BIO-15	BIO-16
mAb4		XX	XX	X
mAb8	-	-	-	
mAb9		XX	XX	X
mAb11	XX		XX	
mAb15	XX	X		
mAb16	-			

10 XX: strong signal with OD>1.5, X: strong signal with 0.5<OD<1.5, -: OD<0.5 when 653 pg/mL FGF21 was used. The average value with intact FGF21 and FAP-cleaved FGF21 was used to generate the table.

On the basis of the results provided in Table 3, antibodies mAb2, 3 and 13 were excluded from further analysis. The results from Table 3 placed antibodies mAb1, 4, 8, 9, 10 and 11 into three epitope bins (Table 5).

5

Table 5. Epitope binning.

Epitope bin	mAb
1	1, 10, 11
2	4, 9
3	8

Antibodies mAb1, 4, 8, 9, 10 and 11 were then tested in ELISA using intact human FGF21 (Cat. No. 2539-FG, R&D Systems) in a combinatorial manner. The absorbance values were plotted as a function of antibody concentration and the data were fitted to a three parameter equation to calculate the half maximal effective concentration (EC₅₀) values for each antibody using Prism 6 (GraphPad Software, Inc., La Jolla, CA) (Table 6). As shown in Table 6, better potency was observed when antibodies mAb4 or 9 were used as the capture antibody and antibodies mAb10 or 11 were used as the detector antibody for intact human FGF21.

15

Table 6. EC₅₀ values with various anti-FGF21 mAb combinations in sandwich ELISA.

Capture mAb	Detector mAb	EC ₅₀ (pg/ml)
4	10	108
4	11	133
9	11	156
9	10	161
11	4	161
10	4	172
8	10+4	182
10	9	191
4	1	193
11	9	195
8	11+4	198
8	1+4	202

1	4	222
8	4	228
8	10	237
9	1	249
8	11	303
8	1	308
1	9	323

Antibodies mAb4, 8, 9, 10, 11, 15 and 16 were then tested in ELISA using intact human FGF21 (Cat. No. 2539-FG, R&D systems) or the FAP-cleaved human FGF21 in a combinatorial manner. The absorbance values were plotted as a function of antibody concentration and the data were fitted to a three parameter equation for each antibody using Prism 6 (GraphPad Software, Inc., La Jolla, CA). The most consistent result was observed when antibodies mAb4 or 9 were used as the capture antibody and antibody mAb11 or mAb15 was used as the detector antibody (Figure 2 and Table 7). Therefore, mAb8 and 16 were removed from further analysis. Figure 2 shows that the antibodies bind equally to intact and FAP-cleaved FGF21 (cFGF21), which is important to detect the concentration of total FGF21 (*i.e.*, both intact and FAP-cleaved).

Table 7. EC₅₀ values with various anti-FGF21 mAb combinations in sandwich ELISA.

Capture mAb	Detection mAb	EC ₅₀ (pg/ml) with intact FGF21	EC ₅₀ (pg/ml) with FAP-cleaved FGF21
9	11	165.8	156.3
4	11	194.7	148
11	4	204.5	203.8
4	15	232.1	262.6
9	15	431.4	362.6
15	4	536.8	561.7
11	15	774.4	630.1
4	16	1246	7044
15	11	1388	1239
9	16	1451	6234
16	4	4893	15750

8	11	40272	106195
8	15	42549	78857
8	4	44411	121300000

Antibodies mAb4, 9, 11 and 15 were further analyzed by BIACORE® surface plasmon resonance to determine the K_d . As shown in Figure 3, mAb4 has a K_d of 3.689×10^{10} , mAb9 has a K_d of 8.895×10^{10} , mAb11 has a K_d of 2.704×10^{10} and mAb15 has a K_d of 3.955×10^{12} .

Example 3: Epitope Analysis

Epitope mapping was conducted by expressing FGF19, FGF21 or FGF19-FGF21 chimeric proteins as FLAG-tagged proteins in transiently transfected HEK293 culture supernatant and testing the binding of antibodies mAb4, 9, 11 and 15 by ELISA. For ELISA, 96 well MaxiSorp plate (439454, Nalge Nunc International; Rochester, NY) was coated with a mixture of 15 μ l culture supernatant containing secreted protein and 135 μ l of 1x coating buffer (50 mM sodium carbonate, pH 9.6) overnight at 4°C. Commercial antibodies R5 and R9, which bind to the C-terminus of FGF21, were used as positive controls.

Human FGF19:

RPLAFSDAGPHVHYGWGDPIRLRHLTYTSGPHGLSSCFLRIRADGVVDCARGQSA
HSLLEIKAVALRTVAIKGVHVSRYLCMGADGKMQLLQYSEEDCAFEIEIRPDG
YNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLE
SDMFSSPLETDSMDPFGLVTGLEAVRSPSFKEK (SEQ ID NO: 2)

Human FGF21:

HPIPDSSPLLQFGGQVRQRYLYTDDAQQTAEHLEIREDDGTGVGGAADQSPESLLQL
KALKPGVIQILGVKTSRFLCQRPDGLYGLSHFDPEACSFRELLLEDGYNVYQSE
AHGLPLHLPGNKSPhRDPAPRGPARFLPLPGLPPALPEPPGILAPQPPDVGSSDPL
SMVGPSQGRSPSYAS (SEQ ID NO: 1)

Human FGF21-19 chimera proteins (the FGF21 portion is italicized and the FGF19 portion is underlined):

HPIPDSSPHVHYGWGDPIRLRHLTYTSGPHGLSSCFLRIRADGVVDCARGQSAHSL
LEIKAVALRTVAIKGVHVSRYLCMGADGKMQLLQYSEEDCAFEIEIRPDGYN
VYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESD
MFSSPLETDSMDPFGLVTGLEAVRSPSFKEK (SEQ ID NO: 3)

*HPIPDSSPLLQFGGQVRQRYLYTDDAQQTAEHLEIR*ADGVVDCARGQSAHSLLEIK

- AVALRTVAIKGVHSVRYLCMGADGKMQGLLOQYSEEDCAFEIEIRPDGYNVYRS
EKHRLPVSLSAKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSP
LETDSMDPFGLVTGLEAVRSPSFEK (SEQ ID NO: 4)
- 5 HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGVVDCARGQSAHSLLEIK
AVALRTVAIKGVHSVRYLCMGADGKMQGLLOQYSEEDCAFEIEIRPDGYNVYRS
EKHRLPVSLSAKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSP
LETDSMDPFGLVTGLEAVRSPSFEK (SEQ ID NO: 5)
- 10 HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQSAHSLLEIKA
VALRTVAIKGVHSVRYLCMGADGKMQGLLOQYSEEDCAFEIEIRPDGYNVYRSE
KHRLPVSLSAKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPL
ETDSMDPFGLVTGLEAVRSPSFEK (SEQ ID NO: 6)
- 15 HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQSPESLLEIKA
VALRTVAIKGVHSVRYLCMGADGKMQGLLOQYSEEDCAFEIEIRPDGYNVYRSE
KHRLPVSLSAKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPL
ETDSMDPFGLVTGLEAVRSPSFEK (SEQ ID NO: 7)
- 20 HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQSPESLLQLKA
LKPGVIQILGVHSVRYLCMGADGKMQGLLOQYSEEDCAFEIEIRPDGYNVYRSEK
HRLPVSLSAKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPLE
TDSMDPFGLVTGLEAVRSPSFEK (SEQ ID NO: 8)
- 25 HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQSPESLLQLKA
LKPGVIQILGVKTSRFLCMGADGKMQGLLOQYSEEDCAFEIEIRPDGYNVYRSEKH
RLPVSLSAKQRQLYKNRGFLPLSHFLPMLPMVPEEPEDLRGHLESDMFSSPLET
DSMDPFGLVTGLEAVRSPSFEK (SEQ ID NO: 9)
- 30 HPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQSPESLLQLKA
LKPGVIQILGVKTSRFLCQRPDGAALYGLHFDPEACSFRELLLEDGYNVYQSEAHGLP
LHLPGNKSPHRDPAPRGPAPFLPMLPMVPEEPEDLRGHLESDMFSSPLETDSMDP
FGLVTGLEAVRSPSFEK (SEQ ID NO: 10)
- 35 RPLAFSDAGPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDGTVGGAADQSPESLLQ
LKALKPGVIQILGVKTSRFLCQRPDGAALYGLHFDPEACSFRELLLEDGYNVYQSEAH
GLPLHLPGNKSPHRDPAPRGPAPFLPGLPALPEPPGILAPQPPDVGSSDPLSMV
GPSQGRSPSYAS (SEQ ID NO: 11)
- 40 RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIREDGTVGGAADQSPE
SLLQLKALKPGVIQILGVKTSRFLCQRPDGAALYGLHFDPEACSFRELLLEDGYNVYQ
SEAHGLPLHLPGNKSPHRDPAPRGPAPFLPGLPALPEPPGILAPQPPDVGSSDPL
SMVGPSQGRSPSYAS (SEQ ID NO: 12)
- 45 RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGTVGGAADQSPE
SLLQLKALKPGVIQILGVKTSRFLCQRPDGAALYGLHFDPEACSFRELLLEDGYNVYQ
SEAHGLPLHLPGNKSPHRDPAPRGPAPFLPGLPALPEPPGILAPQPPDVGSSDPL
SMVGPSQGRSPSYAS (SEQ ID NO: 13)
- 50 RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQSP

*ESLLQLKALKPGVIQILGVKTSRFLCQRPDGALYGLHFDPEACSFRELLLEDGYNVY
QSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLPGLPPALPEPPGILAPQPPDVGSSD
PLSMVGPSQGRSPSYAS (SEQ ID NO: 14)*

5 RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQSA
HSLQLKALKPGVIQILGVKTSRFLCQRPDGALYGLHFDPEACSFRELLLEDGYNVY
QSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLPGLPPALPEPPGILAPQPPDVGSSD
PLSMVGPSQGRSPSYAS (SEQ ID NO: 15)

10 RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQSA
HSLLEIKAVALLRTVAIKGVHVSRYLCQRPDGALYGLHFDPEACSFRELLLEDGYN
VYQSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLPGLPPALPEPPGILAPQPPDVGSS
DPLSMVGPSQGRSPSYAS (SEQ ID NO: 16)

15 RPLAFSDAGPHVHYGWGDPIRLRHLYTSGPHGLSSCFLRIRADGVVDCARGQSA
HSLLEIKAVALLRTVAIKGVHVSRYLCMGADGKMQGLLOYSEEDCAFEIEIRPDG
YNVYRSEKHRLPVSLSSAKQRQLYKNRGFLPLSHFLPLPGLPPALPEPPGILAPQP
PDVGSSDPLSMVGPSQGRSPSYAS (SEQ ID NO: 17)

20 As shown in Figure 4, antibodies mAb4, 9, 11 and 15 bind to the core FGF
folds of human FGF21 and do not bind to the N-terminal or C-terminal flexible regions.

Example 4: FGF21 ELISA Assay

The utility of antibodies mAb4 and 11 as capture antibodies in the detection
25 of intact FGF21 was tested in combination with a C-terminal specific anti-FGF21 pAb
(Cat. No. 30661, Epitope Diagnostics, San Diego, CA; also referred to herein as “C-ter
pAb”)) biotinylated by using EZ-Link™ NHS-PEG Solid-Phase Biotinylation Kit
(PIERCE #21450). A schematic of the immunoassays to determine total FGF21 and
active FGF21 levels is shown in Figure 5.

30 The ELISA assay was performed as follows: 96 well MaxiSorp plate (Cat.
No. 439454, Nalge Nunc International; Rochester, NY) was coated with 0.5 µg/mL of
anti-FGF21 mAbs in coating buffer (50 mM sodium carbonate, pH 9.6) overnight at 4°C.
On the next day, after blocking with PBS containing 0.5% BSA and 10ppm Proclin pH
7.4, and washing with Washing buffer (PBS, 0.05% Tween 20, pH7.2), plate was
35 incubated with 0.0004-32000 pg/mL of intact human FGF21 (2539-FG, R&D systems)
in assay buffer (25 mM HEPES, pH 7.2, 150 mM NaCl, 0.2 mM CaCl₂, 0.1% bovine
serum albumin [BSA], 0.05% Tween-20) for 1-2 h at room temperature. After washing
with Washing buffer, the plate was incubated with 0.5 µg/ml of the secondary Ab
(biotinylated anti-FGF21 C-terminal pAb 30661 or anti-FGF21 mAb11 or 15) in Magic

buffer (1X PBS pH 7.4, 0.5% BSA, 0.05% Tween 20, 0.2% BgG, 0.25% CHAPS, 5mM EDTA, 0.35M NaCl, 10PPM Proclin) for 1-2 hr at room temperature. After washing with Washing buffer, plate was incubated with High Sensitivity Streptavidin-HRP (PIERCE #21130) diluted 1:1,000 in assay buffer. After washing with Washing buffer, the binding of anti-FGF21 to recombinant FGF21 was assessed by adding substrate 3, 3', 5, 5'-tetramethyl benzidine (TMBE-1000, Moss; Pasadena, MD). A more detailed protocol is provided in Figure 6. The mean absorbance values from duplicate wells were plotted as a function of antibody concentration and the data were fitted to a three-parameter equation using Prism 6 (GraphPad Software, Inc., La Jolla, CA) (Figure 7).

As shown in Figure 8, the total FGF21 ELISA assay had an in-well sensitivity of 5 pg/ml and the active FGF21 ELISA assay had an in-well sensitivity of 28 pg/ml. The active FGF21 ELISA assay did not detect the cleaved form of FGF21 that is missing the last 10 C-terminal amino acids.

Further experiments were performed to determine the effect of serum has on the total FGF21 ELISA assay. FGF21 ELISA assays using mAb4 as the capture antibody and mAb15 at the detector antibody were performed. As shown in Figure 9, there was minimal serum interference on the assay. The specificity of the assay for human FGF21 was also tested. As shown in Figure 9, the assay for total FGF21 detected human FGF21 that was expressed in human-FGF21 knock-in mice as compared to control mice. Figure 10 also shows that the assay using the disclosed antibodies was specific for human FGF21 and did not detect mouse FGF21.

On the basis of these data, 4 antibodies, mAb4, mAb9, mAb11 and mAb15, were chosen for cDNA cloning for recombinant expression. The amino acid sequences of these antibodies are provided in Tables 8-13 and Figures 41A and 41B. Recombinant mAbs were expressed in 100 mL CHO culture in the murine IgG2a background.

Table 8. Full-length light chain (LC) sequences for murine anti-FGF21 monoclonal antibodies.

Antibody	Full-length Light Chain Amino Acid Sequence
4	QIVLTQSPAIMSAPLGERVTMTCTASSSVSSSYLHWYQQKPGSSPKVWIYRT TNLASGVPTRFSGSGSGTSLTSSMEAEDAATYYCHQYHRSPPTWTFGGG

	TKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSE RQNGVLNSWTDQDSKDYSTYSMSSTLTTLTKDEYERHNSYTCEATHKTSTSPI VKSFNRNEC (SEQ ID NO: 18)
9	DIQMTQSPASLSASVGETVIITCRASENIYSYLAWYQQKQKSPQLLVYNIRT LAEGVPSRFSGSGSGTQFSLKINSIQPEDFGSYQCQHHYDSPWTFGGGKLEI KRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSE RQNGVLNSWTDQDSKDYSTYSMSSTLTTLTKDEYERHNSYTCEATHKTSTSPI VKSFNRNEC (SEQ ID NO: 19)
11	QIVLTQSPALMSASPGERVTMTCSAGSSVSIMYQYQQKPRSSPKPWIYLT SLASGVPARFSGSGSGTSLTISSEMEAEAAATYYCQQWSSNPRTFGGGTKL EIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSE RQNGVLNSWTDQDSKDYSTYSMSSTLTTLTKDEYERHNSYTCEATHKTSTSPI VKSFNRNEC (SEQ ID NO: 20)
15	DVLTMTQTPSLPVSIGDQASISCRSSQIVHNNGDTYLEWYLQKPGQSPKLLI YKISNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHVPTFGG GTKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSE RQNGVLNSWTDQDSKDYSTYSMSSTLTTLTKDEYERHNSYTCEATHKTSTSPI VKSFNRNEC (SEQ ID NO: 21)

Table 9. Full-length heavy chain (HC) sequences for murine anti-FGF21 monoclonal antibodies.

Antibody	Full-length Heavy Chain Amino Acid Sequence
4	EVKLVESGGDLVKPGGSLKLSCAASGFTFSSYGMSWVRQTPDKRLEWVATI STGGGYTYYPDSVKGRFTISRDNANKNTLYLQMSSLRSEDAMYYCARHDL VDWYFDVWGTGTTVTVSSAKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGY FPEPVTLTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNVA HPASSTKVDKKIEPRGPTIKPCPPCKCPAPNLLGGPSVFIKPKIKDVLMI SLSPIVTCVVVDVSEDDPDVQISWVFNNEVHTAQTQTHREDYNSTLRVVSALPI QHQQDWMSGKEFKCKVNNKDLPAPIERTISKPKGSRAPQVYVLPPEEEMT KKQVTLTCMVTDMPEDIVVEWTNNGKTELNYKNTEPVLDSGYSYFMYSK

	LRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGK (SEQ ID NO: 22)
9	EVQLQQSGPELVKPGASVKIPCKASGYTFTDYYMGWVKQSHGKSLEWIGDI NPNNGVTINNQNFKGKATLTVDKSSSTAYMELRSLASEDTAVYYCTRGYG GALDYWGQGTSTVTVSSAKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGYFPE PVTLTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNVAHPA SSTKVDKKIEPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMISSLPIVT CVVVDVSEDDPDVQISWVFNNEVHTAQTQTHREDYNSTLRVVSALPIQHQQ DWMSGKEFKCKVNNKDLPAPIERTISKPKGSVRAPQVYVLPPEEEMTKKQ VTLTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSGGSYFMYSKLRV EKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGK (SEQ ID NO: 23)
11	QVQLQQSGAELARPGASVKLSCKASGYTFTNYGISWVKQRTGQGLEWIGEI YPRSDNTYYNEKFKGKATLTADKSSSTAYMELRSLTSEDSAVYFCTRSDYG FFDYWGQGTTTLTVSSAKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGYFPEP VTLTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNVAHPAS STKVDKKIEPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMISSLPIVTC VVVDVSEDDPDVQISWVFNNEVHTAQTQTHREDYNSTLRVVSALPIQHQQD WMSGKEFKCKVNNKDLPAPIERTISKPKGSVRAPQVYVLPPEEEMTKKQV TLTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSGGSYFMYSKLRVE KKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGK (SEQ ID NO: 24)
15	QVQLIQSGPGLVQPSQSL SITCTVSGFSLTG YAIHWVRQSPGKGLEWLGMIV KSGNTDYNAAFMSRLSITKDNSKSQVFFKMNSLQADDTAIYYCARNGYDY EFVYWGQGTLVTVSAAKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGYFPEP VTLTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNVAHPAS STKVDKKIEPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMISSLPIVTC VVVDVSEDDPDVQISWVFNNEVHTAQTQTHREDYNSTLRVVSALPIQHQQD WMSGKEFKCKVNNKDLPAPIERTISKPKGSVRAPQVYVLPPEEEMTKKQV TLTCMVTDFMPEDIYVEWTNNGKTELNYKNTEPVLDSGGSYFMYSKLRVE KKNWVERNSYSCSVVHEGLHNHHTTKSFSRTPGK (SEQ ID NO: 25)

Table 10. Light Chain Variable Region (VL) sequences for murine anti-FGF21 monoclonal antibodies.

Antibody	Light Chain Variable Region Amino Acid Sequence
4	QIVLTQSPAISAPLGERVTMTCTASSSVSSSYLHWYQQKPGSSPKVWIYRT TNLASGVPTRFSGSGSGTSLTSSMEAEDAATYYCHQYHRSPPTWTFGGG TKLEIK (SEQ ID NO: 50)
9	DIQMTQSPASLSASVGETVIITCRASENIYSYLAWYQQKQKSPQLLVYNIRT LAEGVPSRFSGSGSGTQFSLKINSLQPEDFGSYQCQHHYDSPWTFGGGKLEI K (SEQ ID NO: 51)
11	QIVLTQSPALMSASPGERVTMTCSAGSSVS MYWYQQKPRSSPKPWIYLT NLASGVPARFSGSGSGTSLTSSMEAEDAATYYCQWSSNPRTFGGGTKL EIK (SEQ ID NO: 52)
15	DVLMTQTPLSLPVSLGDQASISCRSSQIVHNNGDTYLEWYLQKPGQSPKLLI YKISNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHVPYTFGG GTKLEIK (SEQ ID NO: 53)

Table 11. Heavy Chain Variable Region (VH) sequences for murine anti-FGF21 monoclonal antibodies.

Antibody	Heavy Chain Variable Region Amino Acid Sequence
4	EVKLVESGGDLVKPGGSLKLSCAASGFTFSSYGMSWVRQTPDKRLEWVATI STGGGYTYYPDSVKGRFTISRDNKNTLYLQMSSLRSEDAMYYCARHDL VDWYFDVWGTGTTVTVSS (SEQ ID NO: 54)
9	EVQLQQSGPELVKPGASVKIPCKASGYTFTDYYMGWVKQSHGKSLEWIGDI NPNNGVTINNQNFKGKATLTVDKSSSTAYMELRSLASEDTAVYYCTR GYG GALDYWGQGTSVTVSS (SEQ ID NO: 55)
11	QVQLQQSGAELARPGASVKLSCKASGYTFTNYGISWVKQRTGQGLEWIGE IYPRSDNTYYNEKFKGKATLTADKSSSTAYMELRSLTSEDSAVYFCTRSDY G FFDYWGQGTTTLTVSS (SEQ ID NO: 56)

15	QVQLIQSGPGLVQPSQSL SITCTVSGFSLTGYAIHWVRQSPGKGLEWLGMW KSGNTDYNAAFMSRLSITKDNSKSQVFFKMNSLQADDTAIYYCARNGYDY EFVYWGGQGLVTVSA (SEQ ID NO: 57)
----	---

Table 12. Heavy Chain CDR sequences for murine anti-FGF21 monoclonal antibodies.

Antibody	CDR H1	CDR H2	CDR H3
4	SYGMS (SEQ ID NO: 26)	TISTGGGYTYYPDSVKG (SEQ ID NO: 30)	HDLVDWYFDV (SEQ ID NO: 34)
9	DYYMG (SEQ ID NO: 27)	DINPNNGVTINNQNFKG (SEQ ID NO: 31)	GYGGALDY (SEQ ID NO: 35)
11	NYGIS (SEQ ID NO: 28)	EIYPRSDNTYYNEKFKG (SEQ ID NO: 32)	SDYGFFDY (SEQ ID NO: 36)
15	GYAIH (SEQ ID NO: 29)	MIWKSGNTDYNAAFMS (SEQ ID NO: 33)	NGYDYEFVY (SEQ ID NO: 37)

5 Table 13. Light Chain CDR sequences for murine anti-FGF21 monoclonal antibodies.

Antibody	CDR L1	CDR L2	CDR L3
4	TASSSVSSSYLH (SEQ ID NO: 38)	RTTNLAS (SEQ ID NO: 42)	HQYHRSPPTWT (SEQ ID NO: 46)
9	RASENIYSYLA (SEQ ID NO: 39)	NIRTLAE (SEQ ID NO: 43)	QHHYDSPWT (SEQ ID NO: 47)
11	SAGSSVSYMY (SEQ ID NO: 40)	LTSNLAS (SEQ ID NO: 44)	QQWSSNPRT (SEQ ID NO: 48)
15	RSSQIIVHNNGDTYPE (SEQ ID NO: 41)	KISNRFS (SEQ ID NO: 45)	FQGSHVPYT (SEQ ID NO: 49)

Example 5: Optimization of the FGF21 ELISA Assay

The FGF21 ELISA Assay described in Example 4 was further optimized to improve the sensitivity of the assay.

5 Different capture antibodies were compared to determine which capture antibody resulted in more superior detection. Antibodies mAb4 and mAb9 were both tested as the capture antibody. As shown in Figure 11, better assay sensitivity was obtained using mAb4 as the capture antibody compared to mAb9.

10 Different types of coating buffers and different concentrations of coating antibody at fix concentration of detecting antibody were analyzed for the total FGF21 assay and the active FGF21 assay. A bicarbonate coating buffer and a PBS coating buffer were analyzed at different coating antibody concentrations. As shown in Figure 12, for the total FGF21 assay, similar in-well sensitivities were observed for sodium bicarbonate and PBS coating buffer, even at different concentrations of coating antibody. For example, coating 2 µg/ml of mAb4 in PBS had an in-well sensitivity of 2 pg/ml and
15 coating 2 µg/ml of mAb4 had an in-well sensitivity of 3 pg/ml.

For the active FGF21 assay, similar in-well sensitivities were observed for sodium bicarbonate and PBS coating buffer (Figure 13).

Additional experiments were performed to determine the effects the concentration of the detector antibody (mAb15) and the concentration of the horseradish
20 peroxide (HRP) had on the sensitivity of the total FGF21 assay. Concentrations of 0.2, 1 and 2 µg/ml were tested for the detector antibody and dilution of 1/100 and 1/500 were tested for HRP. As shown in Figure 14, higher concentrations of the detector antibody and HRP did not significantly improve the sensitivity of the assays.

25 Example 6: FGF21 Detection Assays Using Quanterix Simoa

Based on optimizations from the ELISA format, as discussed in Example 5, an assay using the Quanterix Simoa HD-1 Analyzer™ was adapted to use mAb4 as the capture antibody, and either biotinylated mAb15 (to detect total FGF21) or biotinylated C-ter pAb (to detect active FGF21) as the detector antibodies. A schematic of the assay
30 is shown in Figure 15.

A summary of the immunoassay is provided. The Quanterix Simoa immunoassay starts with the capture and labeling of total FGF21 with an enzyme conjugate (streptavidin β-galactosidase (SBG)), using a 2-step assay protocol (Figure 16). Total FGF21 captured with magnetic beads conjugated to mAb4 and biotinylated

detection antibody (either mAb15-Biotin for total FGF21 or C-ter pAb-Biotin for active FGF21) are added together to form a captured analyte sandwich in the first step, then SBG is added for detection in the second step. Between each step, the beads are washed. During each wash cycle, the instrument uses a magnet to pellet beads before automated aspiration of the supernatant. After the final wash cycle, the capture beads are resuspended in resorufin β -D-galactopyranoside (RGP) substrate. The beads are then transferred to the entry port of a Simoa Disc in preparation for imaging and analyte quantification.

After capture and labeling of FGF21, the capture beads are loaded into an array containing 216,000 40-fL wells that have been sized to hold no more than one bead per well (4.25 μ m width, 3.25 μ m depth). The bead suspension is pulled through the entry channel and over the array. Beads are allowed to settle into the wells via gravity for approximately 90 seconds. An aliquot of oil is dispensed in the array entry channel and pulled over the array, trapping the beads and RGP substrate in the microwells as well as removing excess beads from the surface. If a FGF21 molecule has been captured and labeled, the SBG hydrolyzes RGP substrate into the fluorescent product resorufin. The fluorescent product builds up within the sealed microwells, enabling detection of single molecules.

The multiplex capture beads were prepared using a two-step EDAC coupling protocol (Simoa Homebrew 2.0 Multiplex Bead Coating Protocol USER-213-11). Beads are coupled with 0.5 mg/mL mAb4 and 0.25 mg/mL EDAC. The coupling reaction occurs between the antibody primary amino groups and the carboxyl groups on the beads.

Quanterix Simoa assay was performed in 96-well Nunc™ 96-Well Polypropylene MicroWell™ Plates (V-bottom, Thermo Scientific Nunc 249944, Rochester, NY). For the standard curve, recombinant intact human FGF21 (iFGF21) and cleaved human FGF21 (cFGF21) were serially diluted in Simoa buffer (PBS pH 7.4, 2% BSA (Fraction B, Protease-Free), 0.1% Tween, 5 mM EDTA) from 0.200-500 pg/mL (Figure 17) or Magic Buffer (BA010) (Figure 19-25, 28-32 and 33-37). To determine the unknown concentration of FGF21 (*e.g.*, in plasma or serum), the test samples were diluted at 1:5-1:20 in Simoa buffer or Magic Buffer. The assay plate, along with the required recommended reagents were loaded into the Simoa HD-1 Analyzer. In each well, for each reaction, 32 μ L of capture beads conjugated to mAb 4, 32 μ L of detector antibodies at 1 μ g/mL (mAb15-Biotin or C-ter pAb-Biotin) and 110 μ L of SBG were

used. For each well, the assay was performed in duplicate. The manufacturer's default Homebrew Assay was selected as the program for the automated procedures. Additional information regarding the assay protocol is provided in Figure 18.

As shown in Figure 19, the total (T) FGF21 Quanterix Simoa-based assay (QSA) detected intact (Wild Type (WT)) FGF21 with an in-well sensitivity (based on 2 x mean AEB of blank wells) of 0.3 pg/ml and the cleaved (CL) form of FGF21, which does not have the last 10 C-terminal amino acids, with an in-well sensitivity of 0.6 pg/ml. The active (A) FGF21 QSA detected intact FGF21 with an in-well sensitivity of 1.8 pg/ml. A significant improvement in assay sensitivity was observed in both total FGF21 and active FGF21 QSAs as compared to traditional ELISA. Figure 20 shows a representative of the standard curve performance for the total and active FGF21 assays. Good standard curve performance was observed.

Example 7: Optimization of the FGF21 Detection Assays Using Quanterix Simoa

The FGF21 QSAs described in Example 6 was further optimized to improve the sensitivity of the assay.

The effect of the type of assay diluent had on the sensitivity of the assays was analyzed. Two different diluents were tested, the BA010 diluent (PBS, 0.5% BSA, 0.25% CHAPS, 5mM EDTA, 0.35M NaCl, 0.05% Tween-20, 0.05% Proclin 300, pH 7.4) and the IL-12 diluent (PBS, 1.5% BSA, 0.15% Tween-20, 0.05% Proclin 300, pH 7.4). The BA010 diluent worked well for both the total and active FGF21 assays, and resulted in lower background and improved sensitivity (Figure 21).

The effect of the concentration of the paramagnetic beads had on the sensitivity of the assay was also analyzed. Two different concentrations were tested, a "high" bead concentration of 1.22×10^7 beads/ml and a "low" bead concentration of 0.59×10^7 beads/ml. As shown in Figure 22, similar assay sensitivity was observed between high bead concentrations and low bead concentrations for the total FGF21 assay. However, improved sensitivity was observed at the low bead concentration for the active FGF21 assay (Figure 22). In particular, the active FGF21 assay had an in-well sensitivity of 1.2 pg/ml when the high bead concentration was used as compared to the in-well sensitivity of 0.6 pg/ml, which was observed with the low bead concentration. Three different paramagnetic bead lots were also analyzed. As shown in Figure 23, similar binding curves and assay sensitivity were observed with the current and new lots of capture paramagnetic beads. Optimized assay parameters are shown in Table 14.

Table 14. Optimized Assay Parameters.

Reagent	Concentration
Assay Diluent (BA010)	1X
Bead	0.59×10^7 beads/ml
Detector Antibody (Total, Active)	0.8 μ g/mL, 2.2 μ g/mL
SBG	310pM

Different detector antibodies were tested for the total FGF21 assay.

Antibodies mAb11, mAb15 and C-ter pAb were tested. Similar sensitivities were

5 observed with the various detector antibodies in the total FGF21 assay (Figure 24).

However, the curve for mAb15 had the lowest background.

From the results shown in Figures 14, 19, and 22, the optimized concentrations of the detector antibodies and SBG for the total and active FGF21 assays were determined (Table 15). Assay sensitivity for both the total FGF21 and active

10 FGF21 assay were improved when the concentrations of the detector antibody and SBG were increased. The sensitivity of the total FGF21 assay improved with a detector antibody concentration of 0.8 μ g/mL and an SBG concentration of 310 pM, and the sensitivity of the activity FGF21 assay improved with a detector antibody concentration of 2.2 μ g/mL and an SBG concentration of 310 pM.

15

Table 15. Optimization of Detector Antibody Concentration and SBG.

Total FGF21 Assay Sensitivity (pg/ml)		
Detector Antibody (μ g/mL)	SBG (pM)	
	310	155
0.8	0.1	0.4
0.4	0.7	0.3
Active FGF21 Assay Sensitivity (pg/ml)		
Detector Antibody (μ g/mL)	SBG (pM)	
	310	155
2.2	0.5	1.1
1.1	2.2	1.4

The total FGF21 assay was further analyzed to determine if a hook effect is observed. A hook effect is typically observed when a high amount of analyte is present in a sample and the observed value is falsely lower. The assays were performed as follows: for the total assay, capture using performed by mAb4 conjugated paramagnetic beads at a concentration of 0.59×10^7 beads/ml and detection was performed using 0.8 $\mu\text{g/mL}$ of biotinylated mAb15; for the active assay, capture was performed using mAb4 conjugated paramagnetic beads at a concentration of 0.59×10^7 beads/ml and detection was performed using 2.2 $\mu\text{g/mL}$ biotinylated Sheep anti-FGF21 C-term pAb. As shown in Figure 25, no hook effect was observed with the total FGF21 assay. Further, the total FGF21 assay detected intact human FGF21 and FAP-cleaved human FGF21 (CL hFGF21) with similar sensitivities (Figure 25).

Example 8: Analysis of Plasma Samples Using FGF21 QSA

The total and active FGF21 QSAs were used to analyze samples obtained and freshly prepared from a healthy human donor. The assays were performed as described in Example 6. As shown in Figure 26, the assay was able to detect low levels of active FGF21 in the serum sample of the healthy donor. Additional experiments were performed in donors that were hypertensive or were not on any medications and were compared with the use of MS-SAFE, a protease inhibitor cocktail (Figure 27). Additional experiments were performed in type 2 diabetes patients. As shown in Figure 28A-B, out of 14 samples, FGF21 was detected in all samples (100%) using the total FGF21 assay. For the active FGF21 assay, FGF21 protein was detected in 12/14 samples (86%) (Figure 28A-B). The results obtained from the assays were reproducible (Figure 29). Reproducibility was acceptable within $\pm 30\%$ difference in both total FGF21 and active FGF21 assays.

The linearity of dilution was analyzed for the total and active FGF21 assays. A linearity of dilution was acceptable within $\pm 30\%$ change from minimum required dilution (MRD) (1:20 dilution) for the total FGF21 and at 1:40 dilution in the active FGF21 assay (Figure 30). A trend of a higher concentration at the initial MRD was observed. The LLOQ was determined for the total and active FGF21 assays. Preliminary LLOQ was determined to be 3.15 pg/ml and 10.94 pg/ml for the total FGF21 and active FGF21 assays, respectively, based on acceptable recovery within $\pm 30\%$ of the mean calculated concentration at the highest dilution factor (Figure 31).

The specificity of the assays was analyzed further. As shown in Figure 32, specificity was demonstrated by greater than 90% inhibition of AEB values of all six type 2 diabetic plasma sample in the presence of 10 µg/mL of mAb4 in the total FGF21 and active FGF21 assays.

5 The use of the P800 blood collection system, which includes a combination of protease, esterase and DPP-IV inhibitors and includes the anticoagulant K₂-EDTA, was compared with the use of K₂-EDTA alone (Figure 33). Comparable results within acceptable ±30% difference between P800 and K₂EDTA screen plasma samples were observed in the total FGF21 and active FGF21 assays (Figure 34). A good correlation
10 between P800 and K₂-EDTA screen plasma samples were observed in the total FGF21 and active FGF21 assays (Figures 35-36). The stability of the plasma samples after being stored at 2-8°C were analyzed. As shown in Figure 37, sample stability within acceptable ±30% recovery from 2-8°C stability sample was observed in total FGF21 and active FGF21 assays.

15 As shown in Figure 38 and 39, a higher than 100% active ratio was observed in K₂-EDTA screen plasma samples that were analyzed by the total and active FGF21 assays suggesting interference by heterophilic antibodies. In particular, a higher than 100% active ratio was observed from K₂-EDTA screen plasma samples 16 and 17 but not 9 and 10 from the GC29819 study when assay diluent was used alone. Samples 16
20 and 17, which had an active ratio higher than 100%, contained human anti-mouse antibodies (HAMA) and human anti-sheep antibodies (HASA), indicating that the presence of HAMA and HASA in patient plasma samples interfered with the accuracy of the total and active assays. As shown in Figures 38 and 39, HAMA affected both the total and active assay; whereas, HASA affected only the active assay. The addition of 10
25 µg/ml of mouse IgG to the diluent of the total assay and the addition of 10 µg/ml of sheep IgG in the diluent of the active assay effectively removed HAMA and HASA interference, respectively, and resolved the higher than 100% active ratio that was observed (Figure 38 and 39). As shown in Figure 40, the presence of 10 µg/ml of anti-mouse IgG or anti-sheep IgG in the assay diluent did not affect the standard curve of the
30 total and active assays, respectively.

Example 9: Chimeric Anti-FGF21 Antibodies

Antibodies mAb4, mAb9, mAb11 and mAb15 were grafted onto human IgG1 frameworks with a K149C mutation to generate mouse/human chimeric anti-FGF21

antibodies that have the mouse VH and VL regions and the human constant region with the K149C mutation. Amino acid sequences for the chimeric antibodies are provided below in Tables 16-19 and Figures 41A and 41B.

5 Table 16

Chimeric Ab4 (FGF21.GN36.4.hIgG1; PRO418189)
<u>Full-length Light Chain Amino Acid Sequence</u> QIVLTQSPAIMSAPLGERVTMTCTASSSVSSSYLHWYQQKPGSSPKVWIYRTTNL ASGVPTRFSGSGSGTSYSLTISSMEAEDAATYYCHQYHRSPPTWTFGGGTKVEIK RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWCVDNALQSGNSQE SVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 63)
<u>Full-length Heavy Chain Amino Acid Sequence</u> EVKLVESGGDLVKPGGSLKLSCAASGFTFSSYGMSWVRQTPDKRLEWVATISTG GGYTYYPDSVKGRFTISRDNKNTLYLQMSSLRSEDAMYYCARHDLVDWYF DVWGTGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSV HEALHNHYTQKSLSLSPGK (SEQ ID NO: 67)
<u>Light Chain Variable Region</u> QIVLTQSPAIMSAPLGERVTMTCTASSSVSSSYLHWYQQKPGSSPKVWIYRTTNL ASGVPTRFSGSGSGTSYSLTISSMEAEDAATYYCHQYHRSPPTWTFGGGTKVEIK (SEQ ID NO: 71)
<u>Heavy Chain Variable Region</u> EVKLVESGGDLVKPGGSLKLSCAASGFTFSSYGMSWVRQTPDKRLEWVATISTG GGYTYYPDSVKGRFTISRDNKNTLYLQMSSLRSEDAMYYCARHDLVDWYF DVWGTGTTVTVSS (SEQ ID NO: 75)

Table 17

Chimeric Ab9 (FGF21.GN36.9.hIgG1; PRO418190)
<u>Full-length Light Chain Amino Acid Sequence</u> DIQMTQSPASLSASVGETVIITCRASENIYSYLAWYQQKQGKSPQLLVYNIRTLA EGVPSRFSGSGSGTQFSLKINSLQPEDFGSYQCQHHYDSPWTFGGGKVEIKRTV AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWCVDNALQSGNSQESVT EQDSKDSTYSLSSLTTLTKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 62)
<u>Full-length Heavy Chain Amino Acid Sequence</u> EVQLQQSGPELVKPGASVKIPCKASGYTFTDYYMGWVKQSHGKSLEWIGDINP NNGVTINNQNFKGKATLTVDKSSSTAYMELRSLASEDTAVYYCTRGYGGALDY WGQGTSVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEHA LHNHYTQKSLSLSPGK (SEQ ID NO: 66)
<u>Light Chain Variable Region</u> DIQMTQSPASLSASVGETVIITCRASENIYSYLAWYQQKQGKSPQLLVYNIRTLA EGVPSRFSGSGSGTQFSLKINSLQPEDFGSYQCQHHYDSPWTFGGGKVEIK (SEQ ID NO: 70)
<u>Heavy Chain Variable Region</u> EVQLQQSGPELVKPGASVKIPCKASGYTFTDYYMGWVKQSHGKSLEWIGDINP NNGVTINNQNFKGKATLTVDKSSSTAYMELRSLASEDTAVYYCTRGYGGALDY WGQGTSVTVSS (SEQ ID NO: 74)

Table 18

Chimeric Ab11 (FGF21.GN36.11.hIgG1; PRO418191)
<u>Full-length Light Chain Amino Acid Sequence</u> QIVLTQSPALMSASPGERVMTCSAGSSVSVMYQKPRSSPKPWIYLTSLA SGVPARFSGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPRTFGGGKVEIKRTV AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWCVDNALQSGNSQESVT

EQDSKDSTYSLSSTLTLSKADYKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 61)
<u>Full-length Heavy Chain Amino Acid Sequence</u> QVQLQQSGAELARPGASVKLSCKASGYTFTNYGISWVKQRTGQGLEWIGEIYPR SDNTYYNEKFKGKATLTADKSSSTAYMELRSLTSEDSAVYFCTRSDYGFFDYW GQGTTTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP KSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV SNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMEALHNHYTQKSLSLSPGK (SEQ ID NO: 65)
<u>Light Chain Variable Region</u> ALMSASPGERVMTCSAGSSVS MYWYQQKPRSSPKWIYLTSLASGVPARFS GSGSGTSYSLTISSEAEADAATYYCQQWSSNPRTFGGGTKVEIK (SEQ ID NO: 69)
<u>Heavy Chain Variable Region</u> QVQLQQSGAELARPGASVKLSCKASGYTFTNYGISWVKQRTGQGLEWIGEIYPR SDNTYYNEKFKGKATLTADKSSSTAYMELRSLTSEDSAVYFCTRSDYGFFDYW GQGTTTLTVSS (SEQ ID NO: 73)

Table 19

Chimeric Ab15 (FGF21.GN36.15.hIgG1; PRO418192)
<u>Full-length Light Chain Amino Acid Sequence</u> DVLMTQTPLSLPVSLGDQASISCRSSQIIVHNNGDTYLEWYLQKPGQSPKLLIYKI SNRFSGVDPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHVPYTFGGGTKVEI KRTVAAPS VFIFPPSDEQLKSGTASVCLLN NFYPREAKVQWCVDNALQSGNSQ ESVTEQDSKDSTYSL SSTLTLSKADYKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 60)
<u>Full-length Heavy Chain Amino Acid Sequence</u> QVQLIQSGPGLVQPSQSL SITCTVSGFSLTG YAIHWVRQSPGKGLEWLGMWIKSG NTDYNAAFMSRLSITKD NSKSQVFFKMNSLQADDTAIYYCARNGYDYEFVYWG QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL

TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPK
 SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV
 KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV
 NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV
 EWESNGQPENNYKTTTPVLDSDGSFFLYSLKLTVDKSRWQQGNVFSQVMHEAL
 HNHYTQKSLSLSPGK (SEQ ID NO: 64)

Light Chain Variable Region

DVLMTQTPLSLPVSLGDQASISCRSSQIIVHNNGDTYLEWYLQKPGQSPKLLIYKI
 SNRFGSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHVPYTFGGGGTKVEI
 K (SEQ ID NO: 68)

Heavy Chain Variable Region

QVQLIQSGPGLVQPSQSLITCTVSGFSLTGYAIHWVRQSPGKGLEWLGMWIKSG
 NTDYNAAFMSRLSITKDNSKSQVFFKMNSLQADDTAIYYCARNGYDYEFVYWG
 QGTLVTVSS (SEQ ID NO: 72)

In addition to the various embodiments depicted and claimed, the disclosed subject matter is also directed to other embodiments having other combinations of the features disclosed and claimed herein. As such, the particular features presented herein

5 can be combined with each other in other manners within the scope of the disclosed subject matter such that the disclosed subject matter includes any suitable combination of the features disclosed herein. The foregoing description of specific embodiments of the disclosed subject matter has been presented for purposes of illustration and description. It is not intended to be exhaustive or to limit the disclosed subject matter to those

10 embodiments disclosed.

It will be apparent to those skilled in the art that various modifications and variations can be made in the compositions and methods of the disclosed subject matter without departing from the spirit or scope of the disclosed subject matter. Thus, it is intended that the disclosed subject matter include modifications and variations that are

15 within the scope of the appended claims and their equivalents.

Various publications, patents and patent applications are cited herein, the contents of which are hereby incorporated by reference in their entireties.

WHAT IS CLAIMED IS:

1. An immunoassay method for determining the amount of total FGF21 protein in a sample comprising:
 - (a) contacting a capture antibody that binds to an epitope present within amino acid residues 5-172 of FGF21 with the sample to generate a sample-capture antibody combination material;
 - (b) contacting the sample-capture antibody combination material with a detector antibody that binds to an epitope present within amino acid residues 5-172 of FGF21;
 - (c) detecting the detector antibody bound to the sample-capture antibody combination material; and
 - (d) calculating an amount of total FGF21 protein present in the sample based on the level of the detector antibody bound.
2. The immunoassay method of claim 1, wherein the capture antibody and the detector antibody bind to different epitopes within amino acid residues 5-172 of FGF21.
3. An immunoassay method for determining the amount of active FGF21 protein in a sample comprising:
 - (a) contacting a capture antibody that binds to an epitope present within amino acid residues 5-172 of FGF21 with the sample to generate a sample-capture antibody combination material;
 - (b) contacting the sample-capture antibody combination material with a detector antibody that binds to an epitope present within amino acid residues 173-182 of FGF21;
 - (c) detecting the detector antibody bound to the sample-capture antibody combination material; and
 - (d) calculating an amount of active FGF21 protein present in the sample based on the level of the detector antibody bound.
4. An immunoassay method for determining the ratio of active FGF21 protein to total FGF21 protein in a sample comprising:
 - (a) (i) contacting a first capture antibody that binds to an epitope present within amino acid residues 5-172 of FGF21 with the sample to generate a first sample-capture antibody combination material; (ii) contacting the first sample-capture antibody combination material with a first detector antibody that binds to an epitope present within amino acid residues 5-172 of FGF21; (iii) detecting the first detector antibody bound to the sample-capture antibody combination material; and (iv) calculating an amount of total FGF21 protein present in the sample based on the level of the first detector antibody bound;

- (b) (i) contacting a second capture antibody that binds to an epitope present within amino acid residues 5-172 of FGF21 with the sample to generate a second sample-capture antibody combination material; (ii) contacting the second sample-capture antibody combination material with a second detector antibody that binds to an epitope present within amino acid residues 173-182 of FGF21; (iii) detecting the second detector antibody bound to the sample-capture antibody combination material; and (iv) calculating an amount of active FGF21 protein present in the sample based on the level of the second detector antibody bound; and
 - (c) comparing the amount of total FGF21 protein as determined by step (a) with the amount of active FGF21 protein as determined by step (b) to determine the ratio of active FGF21 protein to total FGF21 protein in the sample.
- 5. The immunoassay method of claim 4, wherein the first capture antibody and the first detector antibody bind to different epitopes within amino acid residues 5-172 of FGF21.
- 6. The immunoassay method of claim 4, wherein the first capture antibody and second capture antibody are the same antibody.
- 7. The immunoassay method of any one of claims 1-6, wherein the immunoassay is an enzyme-linked immunosorbent assay (ELISA).
- 8. The immunoassay method of any one of claims 1-7, wherein one or more of the capture antibody, first capture antibody and second capture antibody is immobilized to a paramagnetic bead.
- 9. The immunoassay method of any one of claims 1-8, wherein one or more of the detector antibody, first detector antibody and second detector antibody is conjugated to biotin.
- 10. The immunoassay method of any one of claims 1-9, wherein one or more of the capture antibody, first capture antibody and second capture antibody binds to FGF21 with a K_d from about 10^{-10} M to 10^{-13} M.
- 11. The immunoassay method of any one of claims 1 and 4-9, wherein one or more of the detector antibody and first detector antibody binds to FGF21 with a K_d from about 10^{-10} M to 10^{-13} M.
- 12. The immunoassay method of any one of claims 1-11, wherein the sample is a blood sample.
- 13. The immunoassay method of any one of claims 1-11, wherein the sample is a plasma sample.
- 14. The immunoassay method of any one of claims 1-13, wherein the method detects the amount of total or active FGF21 protein in the sample at an in-well sensitivity from about 2 pg/ml to about 20 pg/ml.

15. The immunoassay method of any one of claims 1-13, wherein the immunoassay method is performed using a single molecule detection instrument.
16. The immunoassay method of claim 15, wherein the single molecule detection instrument is the Quanterix Simoa HD-1 Analyzer™.
17. The immunoassay method of claim 15 or 16, wherein the method detects the amount of total or active FGF21 protein in the sample at an in-well sensitivity from about 0.2 pg/ml to about 0.5 pg/ml.
18. The immunoassay method of any one of claims 1-17, wherein one or more of the capture antibody, first capture antibody and second capture antibody comprises:
 - (a) a heavy chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 26 and 27, and conservative substitutions thereof;
 - (b) a heavy chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 30 and 31, and conservative substitutions thereof;
 - (c) a heavy chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 34 and 35, and conservative substitutions thereof;
 - (d) a light chain variable region CDR1 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 38 and 39, and conservative substitutions thereof;
 - (e) a light chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 42 and 43, and conservative substitutions thereof; and
 - (f) a light chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 46 and 47, and conservative substitutions thereof.
19. The immunoassay of any one of claims 1-17, wherein one or more of the capture antibody, first capture antibody and second capture antibody comprises:
 - (a) a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 54, 55, 74 and 75, and conservative substitutions thereof; and
 - (b) a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 50, 51, 70 and 71, and conservative substitutions thereof.
20. The immunoassay of any one of claims 1-17, wherein one or more of the capture antibody, first capture antibody and second capture antibody comprises:

- (a) a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 22, 23, 66 and 67, and conservative substitutions thereof; and
 - (b) a light chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 18, 19, 62 and 63, and conservative substitutions thereof.
21. The immunoassay method of any one of claims 1 and 4-17, wherein one or more of the detector antibody and first detector antibody comprises:
- (a) a heavy chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 28 and 29, and conservative substitutions thereof;
 - (b) a heavy chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 32 and 33, and conservative substitutions thereof;
 - (c) a heavy chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 36 and 37, and conservative substitutions thereof;
 - (d) a light chain variable region CDR1 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 40 and 41, and conservative substitutions thereof;
 - (e) a light chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 44 and 45, and conservative substitutions thereof; and
 - (f) a light chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 48 and 49, and conservative substitutions thereof.
22. The immunoassay of any one of claims 1 and 4-17, wherein one or more of the detector antibody and first detector antibody comprises:
- (a) a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 56, 57, 72 and 73, and conservative substitutions thereof; and
 - (b) a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 52, 53, 68 and 69, and conservative substitutions thereof.
23. The immunoassay of any one of claims 1 and 4-17, wherein one or more of the detector antibody and first detector antibody comprises:
- (a) a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 24, 25, 64 and 65, and conservative substitutions thereof; and

- (b) a light chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 20, 21, 60 and 61, and conservative substitutions thereof.
24. The immunoassay method of claim 18, wherein one or more of the capture antibody, first capture antibody and second capture antibody comprises:
- (a) a heavy chain variable region CDR1 comprising the amino acid sequence of SEQ ID NO: 26, and conservative substitutions thereof;
 - (b) a heavy chain variable region CDR2 domain comprising the amino acid sequence of SEQ ID NO: 30, and conservative substitutions thereof;
 - (c) a heavy chain variable region CDR3 domain comprising the amino acid sequence of SEQ ID NO: 34, and conservative substitutions thereof;
 - (d) a light chain variable region CDR1 domain comprising the amino acid sequence of SEQ ID NO: 38, and conservative substitutions thereof;
 - (e) a light chain variable region CDR2 domain comprising the amino acid sequence of SEQ ID NO: 42, and conservative substitutions thereof; and
 - (f) a light chain variable region CDR3 domain comprising the amino acid sequence of SEQ ID NO: 46, and conservative substitutions thereof.
25. The immunoassay of claim 24, wherein one or more of the capture antibody, first capture antibody and second capture antibody comprises:
- (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 54, and conservative substitutions thereof; and
 - (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 50, and conservative substitutions thereof.
26. The immunoassay of claim 25, wherein one or more of the capture antibody, first capture antibody and second capture antibody comprises:
- (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 22, and conservative substitutions thereof; and
 - (b) a light chain comprising the amino acid sequence of SEQ ID NO: 18, and conservative substitutions thereof.
27. The immunoassay method of claim 21, wherein one or more of the detector antibody and first detector antibody comprises:
- (a) a heavy chain variable region CDR1 comprising the amino acid sequence of SEQ ID NO: 29, and conservative substitutions thereof;
 - (b) a heavy chain variable region CDR2 domain comprising the amino acid sequence of SEQ ID NO: 33, and conservative substitutions thereof;

- (c) a heavy chain variable region CDR3 domain comprising the amino acid sequence of SEQ ID NO: 37, and conservative substitutions thereof;
 - (d) a light chain variable region CDR1 domain comprising the amino acid sequence of SEQ ID NO: 41, and conservative substitutions thereof;
 - (e) a light chain variable region CDR2 domain comprising the amino acid sequence of SEQ ID NO: 45, and conservative substitutions thereof; and
 - (f) a light chain variable region CDR3 domain comprising the amino acid sequence of SEQ ID NO: 49, and conservative substitutions thereof.
28. The immunoassay of claim 27, wherein one or more of the detector antibody and first detector antibody comprises:
- (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 57, and conservative substitutions thereof; and
 - (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 53, and conservative substitutions thereof.
29. The immunoassay of claim 28, wherein one or more of the detector antibody and first detector antibody comprises:
- (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 25, and conservative substitutions thereof; and
 - (b) a light chain comprising the amino acid sequence of SEQ ID NO: 21, and conservative substitutions thereof.
30. The immunoassay method of any one of claims 1-17, wherein one or more of the capture antibody, first capture antibody and second capture antibody competitively binds with an antibody comprising:
- (a) a heavy chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 26 and 27, and conservative substitutions thereof;
 - (b) a heavy chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 30 and 31, and conservative substitutions thereof;
 - (c) a heavy chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 34 and 35, and conservative substitutions thereof;
 - (d) a light chain variable region CDR1 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 38 and 39, and conservative substitutions thereof;

- (e) a light chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 42 and 43, and conservative substitutions thereof; and
 - (f) a light chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 46 and 47, and conservative substitutions thereof.
31. The immunoassay method of any one of claims 1 and 4-17, wherein one or more of the detector antibody and first detector antibody competitively binds with an antibody comprising:
- (a) a heavy chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 28 and 29, and conservative substitutions thereof;
 - (b) a heavy chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 32 and 33, and conservative substitutions thereof;
 - (c) a heavy chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 36 and 37, and conservative substitutions thereof;
 - (d) a light chain variable region CDR1 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 40 and 41, and conservative substitutions thereof;
 - (e) a light chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 44 and 45, and conservative substitutions thereof; and
 - (f) a light chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 48 and 49, and conservative substitutions thereof.
32. A kit for detecting total FGF21 protein in a sample comprising:
- (a) a capture antibody, or an antigen-binding portion thereof, that binds to an epitope present within amino acid residues 5-172 of FGF21;
 - (b) a detector antibody, or an antigen-binding portion thereof, that binds to an epitope present within amino acid residues 5-172 of FGF21; and
 - (c) a detection agent.
33. The kit of claim 32, wherein the capture antibody and the detector antibody bind to different epitopes within amino acid residues 5-172 of FGF21.
34. A kit for detecting active FGF21 protein in a sample comprising:

- (a) a capture antibody, or an antigen-binding portion thereof, that binds to an epitope present within amino acid residues 5-172 of FGF21;
 - (b) a detector antibody, or an antigen-binding portion thereof, that binds to an epitope present within amino acid residues 173-182 of FGF21; and
 - (c) a detection agent.
35. A kit for determining the ratio of active FGF21 protein to total FGF21 protein in a sample comprising:
- (a) (i) a first capture antibody or an antigen-binding portion thereof, that binds to an epitope present within amino acid residues 5-172 of FGF21 and (ii) a first detector antibody, or an antigen-binding portion thereof, that binds to an epitope present within amino acid residues 5-172 of FGF21;
 - (b) (i) a second capture antibody, or an antigen-binding portion thereof, that binds to an epitope present within amino acid residues 5-172 of FGF21 and (ii) a second detector antibody, or an antigen-binding portion thereof, that binds to an epitope present within amino acid residues 173-182 of FGF21; and
 - (c) one or more detection agents.
36. The kit of claim 35, wherein the first capture antibody and second capture antibody are the same antibody.
37. The kit of claim 35, wherein the first capture antibody and the first detector antibody bind to different epitopes within amino acid residues 5-172 of FGF21.
38. The kit of any one of claims 32-37, wherein one or more of the capture antibody, first capture antibody and second capture antibody is immobilized to a paramagnetic bead.
39. The kit of any one of claims 32-38, wherein one or more of the detector antibody, first detector antibody and second detector antibody is conjugated to biotin.
40. The kit of any one of claims 32-39, wherein the detection agent is selected from the group consisting of a streptavidin- β -D-galactopyranose conjugate, a streptavidin-horseradish peroxidase conjugate and a combination thereof.
41. The kit of claim 40 further comprising resorufin β -D-galactopyranoside, tetramethylbenzidine, hydrogen peroxide or combinations thereof.
42. The kit of any one of claims 32-41, wherein one or more of the capture antibody, first capture antibody and second capture antibody binds to FGF21 with a K_d from about 10^{-10} M to 10^{-13} M.
43. The kit of any one of claims 32 and 35-42, wherein one or more of the detector antibody and first detector antibody binds to FGF21 with a K_d from about 10^{-10} M to 10^{-13} M.

44. The kit of any one of claims 32 and 35-43, wherein the detector antibody or first detector antibody has a concentration from about 0.1 $\mu\text{g/ml}$ to about 1 $\mu\text{g/ml}$.
45. The kit of any one of claims 33-42, wherein one or more of the detector antibody or second detector antibody has a concentration from about 1 $\mu\text{g/ml}$ to about 3 $\mu\text{g/ml}$.
46. The kit of claim 40, wherein the streptavidin- β -D-galactopyranose conjugate has a concentration from about 100 pM to about 400 pM.
47. The kit of any one of claims 32-46, wherein one or more of the capture antibody, first capture antibody and second capture antibody comprises:
- (a) a heavy chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 26 and 27, and conservative substitutions thereof;
 - (b) a heavy chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 30 and 31, and conservative substitutions thereof;
 - (c) a heavy chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 34 and 35, and conservative substitutions thereof;
 - (d) a light chain variable region CDR1 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 38 and 39, and conservative substitutions thereof;
 - (e) a light chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 42 and 43, and conservative substitutions thereof; and
 - (f) a light chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 46 and 47, and conservative substitutions thereof.
48. The kit of any one of claims 32-46, wherein one or more of the capture antibody, first capture antibody and second capture antibody comprises:
- (a) a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 54, 55, 74 and 75, and conservative substitutions thereof; and
 - (b) a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 50, 51, 70 and 71, and conservative substitutions thereof.
49. The kit of any one of claims 32-46, wherein one or more of the capture antibody, first capture antibody and second capture antibody comprises:

- (a) a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 22, 23, 66 and 67, and conservative substitutions thereof; and
 - (b) a light chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 18, 19, 62 and 63, and conservative substitutions thereof.
50. The kit of any one of claims 32 and 35-46, wherein one or more of the detector antibody and first detector antibody comprises:
- (a) a heavy chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 28 and 29, and conservative substitutions thereof;
 - (b) a heavy chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 32 and 33, and conservative substitutions thereof;
 - (c) a heavy chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 36 and 37, and conservative substitutions thereof;
 - (d) a light chain variable region CDR1 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 40 and 41, and conservative substitutions thereof;
 - (e) a light chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 44 and 45, and conservative substitutions thereof; and
 - (f) a light chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 48 and 49, and conservative substitutions thereof.
51. The kit of any one of claims 32 and 35-46, wherein one or more of the detector antibody and first detector antibody comprises:
- (a) a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 56, 57, 72 and 73, and conservative substitutions thereof; and
 - (b) a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 52, 53, 68 and 69, and conservative substitutions thereof.
52. The kit of any one of claims 32 and 35-46, wherein one or more of the detector antibody and first detector antibody comprises:
- (a) a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 24, 25, 64 and 65, and conservative substitutions thereof; and

- (b) a light chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 20, 21, 60 and 61, and conservative substitutions thereof.
53. The kit of claim 47, wherein one or more of the capture antibody, first capture antibody and second capture antibody comprises:
- (a) a heavy chain variable region CDR1 comprising the amino acid sequence of SEQ ID NO: 26, and conservative substitutions thereof;
 - (b) a heavy chain variable region CDR2 domain comprising the amino acid sequence of SEQ ID NO: 30, and conservative substitutions thereof;
 - (c) a heavy chain variable region CDR3 domain comprising the amino acid sequence of SEQ ID NO: 34, and conservative substitutions thereof;
 - (d) a light chain variable region CDR1 domain comprising the amino acid sequence of SEQ ID NO: 38, and conservative substitutions thereof;
 - (e) a light chain variable region CDR2 domain comprising the amino acid sequence of SEQ ID NO: 42, and conservative substitutions thereof; and
 - (f) a light chain variable region CDR3 domain comprising the amino acid sequence of SEQ ID NO: 46, and conservative substitutions thereof.
54. The kit of claim 53, wherein one or more of the capture antibody, first capture antibody and second capture antibody comprises:
- (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 54, and conservative substitutions thereof; and
 - (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 50, and conservative substitutions thereof.
55. The kit of claim 54, wherein one or more of the capture antibody, first capture antibody and second capture antibody comprises:
- (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 22, and conservative substitutions thereof; and
 - (b) a light chain comprising the amino acid sequence of SEQ ID NO: 18, and conservative substitutions thereof.
56. The kit of claim 50, wherein one or more of the detector antibody and first detector antibody comprises:
- (a) a heavy chain variable region CDR1 comprising the amino acid sequence of SEQ ID NO: 29, and conservative substitutions thereof;
 - (b) a heavy chain variable region CDR2 domain comprising the amino acid sequence of SEQ ID NO: 33, and conservative substitutions thereof;

- (c) a heavy chain variable region CDR3 domain comprising the amino acid sequence of SEQ ID NO: 37, and conservative substitutions thereof;
 - (d) a light chain variable region CDR1 domain comprising the amino acid sequence of SEQ ID NO: 41, and conservative substitutions thereof;
 - (e) a light chain variable region CDR2 domain comprising the amino acid sequence of SEQ ID NO: 45, and conservative substitutions thereof; and
 - (f) a light chain variable region CDR3 domain comprising the amino acid sequence of SEQ ID NO: 49, and conservative substitutions thereof.
57. The kit of claim 56, wherein one or more of the detector antibody and first detector antibody comprises:
- (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 57, and conservative substitutions thereof; and
 - (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 53, and conservative substitutions thereof.
58. The kit of claim 57, wherein one or more of the detector antibody and first detector antibody comprises:
- (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 25, and conservative substitutions thereof; and
 - (b) a light chain comprising the amino acid sequence of SEQ ID NO: 21, and conservative substitutions thereof.
59. The kit of any one of claims 32-46, wherein one or more of the capture antibody, first capture antibody and second capture antibody competitively binds with an antibody comprising:
- (a) a heavy chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 26 and 27, and conservative substitutions thereof;
 - (b) a heavy chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 30 and 31, and conservative substitutions thereof;
 - (c) a heavy chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 34 and 35, and conservative substitutions thereof;
 - (d) a light chain variable region CDR1 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 38 and 39, and conservative substitutions thereof;

- (e) a light chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 42 and 43, and conservative substitutions thereof; and
 - (f) a light chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 46 and 47, and conservative substitutions thereof.
60. The kit of any one of claims 32 and 35-46, wherein one or more of the detector antibody and first detector antibody competitively binds with an antibody comprising:
- (a) a heavy chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 28 and 29, and conservative substitutions thereof;
 - (b) a heavy chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 32 and 33, and conservative substitutions thereof;
 - (c) a heavy chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 36 and 37, and conservative substitutions thereof;
 - (d) a light chain variable region CDR1 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 40 and 41, and conservative substitutions thereof;
 - (e) a light chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 44 and 45, and conservative substitutions thereof; and
 - (f) a light chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 48 and 49, and conservative substitutions thereof.
61. The kit of any one of claims 32-60, wherein the sample is a blood sample.
62. The kit of any one of claims 32-60, wherein the sample is a plasma sample.
63. The kit of any one of claims 32-62, wherein the kit detects the amount of total or active FGF21 protein in the sample at an in-well sensitivity from about 0.2 pg/ml to about 0.5 pg/ml.
64. An isolated anti-FGF21 antibody, or an antigen-binding portion thereof, comprising:
- (a) a heavy chain variable region CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 26-29, and conservative substitutions thereof;

- (b) a heavy chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 30-33, and conservative substitutions thereof;
 - (c) a heavy chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 34-37, and conservative substitutions thereof;
 - (d) a light chain variable region CDR1 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 38-41, and conservative substitutions thereof;
 - (e) a light chain variable region CDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 42-45, and conservative substitutions thereof; and
 - (f) a light chain variable region CDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 46-49, and conservative substitutions thereof.
65. The isolated antibody of claim 64, wherein the antibody, or antigen-binding portion thereof, comprises:
- (a) a heavy chain variable region CDR1 comprising the amino acid sequence of SEQ ID NO: 26, and conservative substitutions thereof;
 - (b) a heavy chain variable region CDR2 domain comprising the amino acid sequence of SEQ ID NO: 30, and conservative substitutions thereof;
 - (c) a heavy chain variable region CDR3 domain comprising the amino acid sequence of SEQ ID NO: 34, and conservative substitutions thereof;
 - (d) a light chain variable region CDR1 domain comprising the amino acid sequence of SEQ ID NO: 38, and conservative substitutions thereof;
 - (e) a light chain variable region CDR2 domain comprising the amino acid sequence of SEQ ID NO: 42, and conservative substitutions thereof; and
 - (f) a light chain variable region CDR3 domain comprising the amino acid sequence of SEQ ID NO: 46, and conservative substitutions thereof.
66. The isolated antibody of claim 64, wherein the antibody, or antigen-binding portion thereof, comprises:
- (a) a heavy chain variable region CDR1 comprising the amino acid sequence of SEQ ID NO: 27, and conservative substitutions thereof;
 - (b) a heavy chain variable region CDR2 domain comprising the amino acid sequence of SEQ ID NO: 31, and conservative substitutions thereof;
 - (c) a heavy chain variable region CDR3 domain comprising the amino acid sequence of SEQ ID NO: 35, and conservative substitutions thereof;

- (d) a light chain variable region CDR1 domain comprising the amino acid sequence of SEQ ID NO: 39, and conservative substitutions thereof;
 - (e) a light chain variable region CDR2 domain comprising the amino acid sequence of SEQ ID NO: 43, and conservative substitutions thereof; and
 - (f) a light chain variable region CDR3 domain comprising the amino acid sequence of SEQ ID NO: 47, and conservative substitutions thereof.
67. The isolated antibody of claim 64, wherein the antibody, or antigen-binding portion thereof, comprises:
- (a) a heavy chain variable region CDR1 comprising the amino acid sequence of SEQ ID NO: 28, and conservative substitutions thereof;
 - (b) a heavy chain variable region CDR2 domain comprising the amino acid sequence of SEQ ID NO: 32, and conservative substitutions thereof;
 - (c) a heavy chain variable region CDR3 domain comprising the amino acid sequence of SEQ ID NO: 36, and conservative substitutions thereof;
 - (d) a light chain variable region CDR1 domain comprising the amino acid sequence of SEQ ID NO: 40, and conservative substitutions thereof;
 - (e) a light chain variable region CDR2 domain comprising the amino acid sequence of SEQ ID NO: 44, and conservative substitutions thereof; and
 - (f) a light chain variable region CDR3 domain comprising the amino acid sequence of SEQ ID NO: 48, and conservative substitutions thereof.
68. The isolated antibody of claim 64, wherein the antibody, or antigen-binding portion thereof, comprises:
- (a) a heavy chain variable region CDR1 comprising the amino acid sequence of SEQ ID NO: 29, and conservative substitutions thereof;
 - (b) a heavy chain variable region CDR2 domain comprising the amino acid sequence of SEQ ID NO: 33, and conservative substitutions thereof;
 - (c) a heavy chain variable region CDR3 domain comprising the amino acid sequence of SEQ ID NO: 37, and conservative substitutions thereof;
 - (d) a light chain variable region CDR1 domain comprising the amino acid sequence of SEQ ID NO: 41, and conservative substitutions thereof;
 - (e) a light chain variable region CDR2 domain comprising the amino acid sequence of SEQ ID NO: 45, and conservative substitutions thereof; and
 - (f) a light chain variable region CDR3 domain comprising the amino acid sequence of SEQ ID NO: 49, and conservative substitutions thereof.

69. The isolated antibody of claim 65, wherein the antibody, or antigen-binding portion thereof, comprises:
- (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 54, and conservative substitutions thereof; and
 - (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 50, and conservative substitutions thereof.
70. The isolated antibody of claim 66, wherein the antibody, or antigen-binding portion thereof, comprises:
- (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 55, and conservative substitutions thereof; and
 - (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 51, and conservative substitutions thereof.
71. The isolated antibody of claim 67, wherein the antibody, or antigen-binding portion thereof, comprises:
- (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 56, and conservative substitutions thereof; and
 - (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 52, and conservative substitutions thereof.
72. The isolated antibody of claim 68, wherein the antibody, or antigen-binding portion thereof, comprises:
- (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 57, and conservative substitutions thereof; and
 - (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 53, and conservative substitutions thereof.
73. The isolated antibody of claim 65, wherein the antibody, or antigen-binding portion thereof, comprises:
- (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 75, and conservative substitutions thereof; and
 - (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 71, and conservative substitutions thereof.
74. The isolated antibody of claim 66, wherein the antibody, or antigen-binding portion thereof, comprises:
- (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 74, and conservative substitutions thereof; and

- (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 70, and conservative substitutions thereof.
- 75. The isolated antibody of claim 67, wherein the antibody, or antigen-binding portion thereof, comprises:
 - (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 73, and conservative substitutions thereof; and
 - (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 69, and conservative substitutions thereof.
- 76. The isolated antibody of claim 68, wherein the antibody, or antigen-binding portion thereof, comprises:
 - (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 72, and conservative substitutions thereof; and
 - (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 68, and conservative substitutions thereof.
- 77. The isolated antibody of claim 69, wherein the antibody, or antigen-binding portion thereof, comprises:
 - (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 22, and conservative substitutions thereof; and
 - (b) a light chain comprising the amino acid sequence of SEQ ID NO: 18, and conservative substitutions thereof.
- 78. The isolated antibody of claim 70, wherein the antibody, or antigen-binding portion thereof, comprises:
 - (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 23, and conservative substitutions thereof; and
 - (b) a light chain comprising the amino acid sequence of SEQ ID NO: 19, and conservative substitutions thereof.
- 79. The isolated antibody of claim 71, wherein the antibody, or antigen-binding portion thereof, comprises:
 - (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 24, and conservative substitutions thereof; and
 - (b) a light chain comprising the amino acid sequence of SEQ ID NO: 20, and conservative substitutions thereof.
- 80. The isolated antibody of claim 72, wherein the antibody, or antigen-binding portion thereof, comprises:

- (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 25, and conservative substitutions thereof; and
 - (b) a light chain comprising the amino acid sequence of SEQ ID NO: 21, and conservative substitutions thereof.
81. The isolated antibody of claim 73, wherein the antibody, or antigen-binding portion thereof, comprises:
- (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 67, and conservative substitutions thereof; and
 - (b) a light chain comprising the amino acid sequence of SEQ ID NO: 63, and conservative substitutions thereof.
82. The isolated antibody of claim 74, wherein the antibody, or antigen-binding portion thereof, comprises:
- (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 66, and conservative substitutions thereof; and
 - (b) a light chain comprising the amino acid sequence of SEQ ID NO: 62, and conservative substitutions thereof.
83. The isolated antibody of claim 75, wherein the antibody, or antigen-binding portion thereof, comprises:
- (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 65, and conservative substitutions thereof; and
 - (b) a light chain comprising the amino acid sequence of SEQ ID NO: 61, and conservative substitutions thereof.
84. The isolated antibody of claim 76, wherein the antibody, or antigen-binding portion thereof, comprises:
- (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 64, and conservative substitutions thereof; and
 - (b) a light chain comprising the amino acid sequence of SEQ ID NO: 60, and conservative substitutions thereof.
85. An isolated nucleic acid encoding the antibody, or antigen-binding portion thereof, of any one of claims 64-84.
86. A host cell comprising the nucleic acid of claim 85.
87. A method of producing an antibody comprising culturing the host cell of claim 86 so that the antibody is produced.
88. The method of claim 87, further comprising recovering the antibody from the host cell.

89. A composition comprising one or more antibodies, or antigen-binding portions thereof, of any one of claims 64-84.

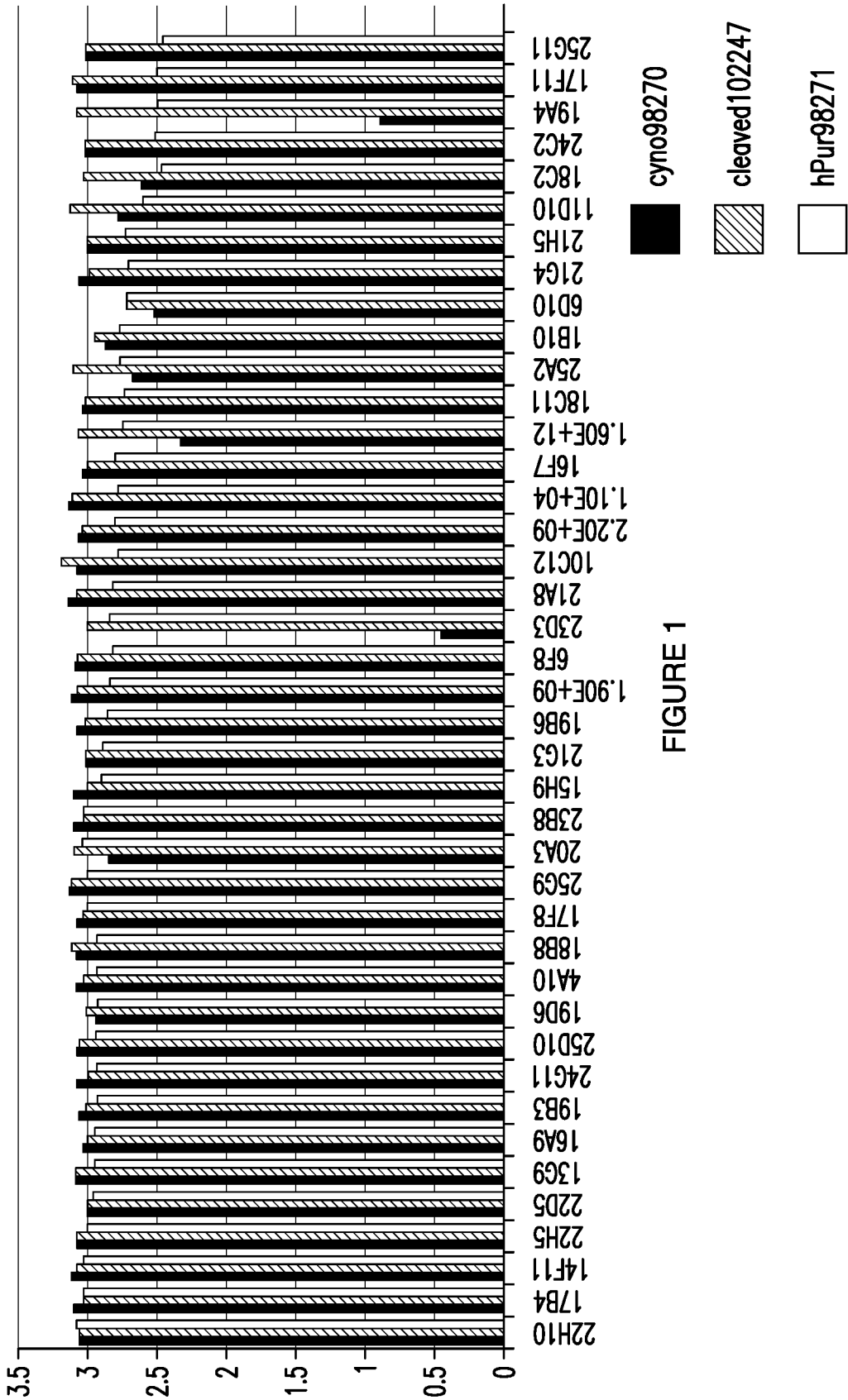


FIGURE 1

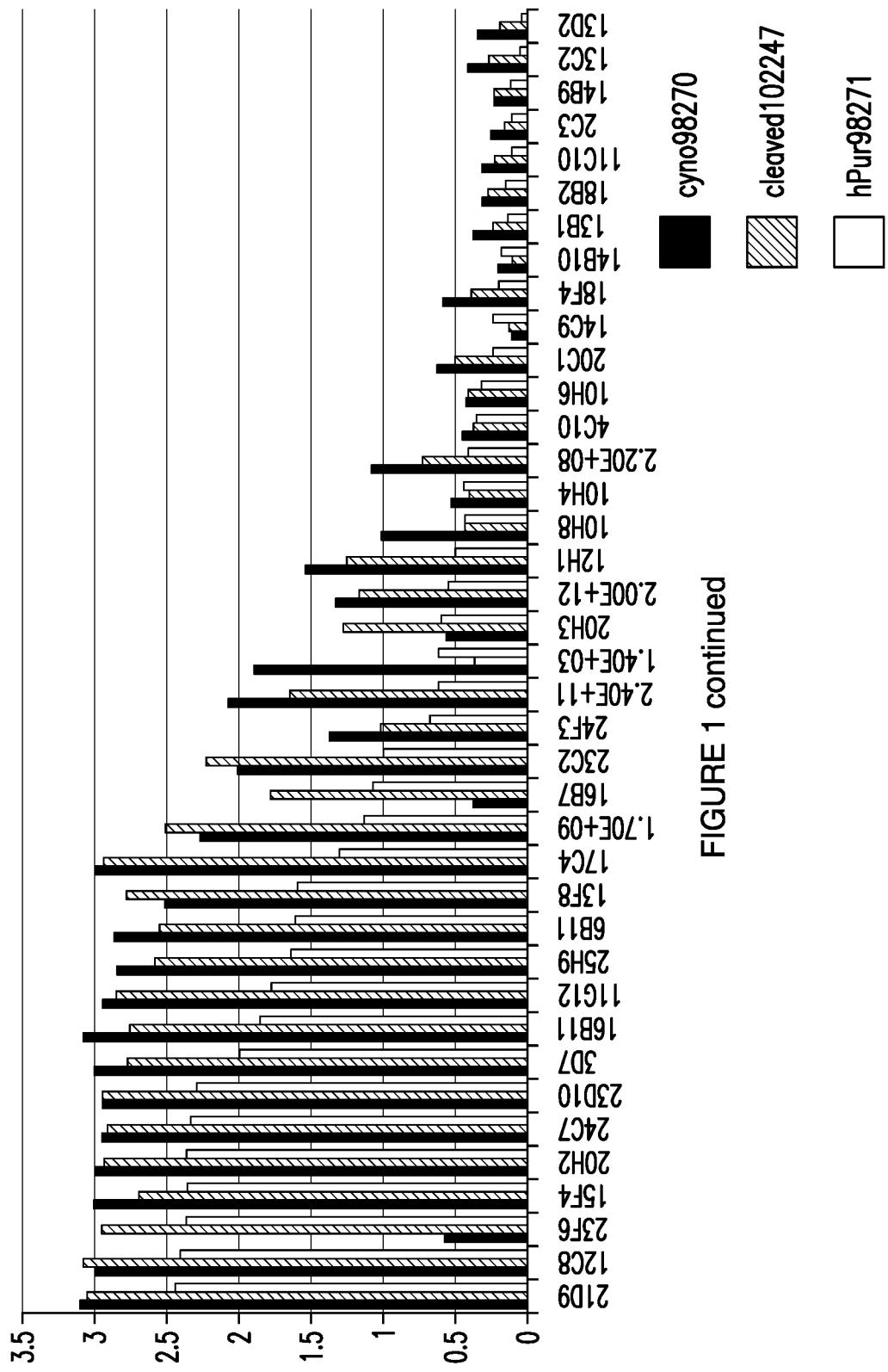
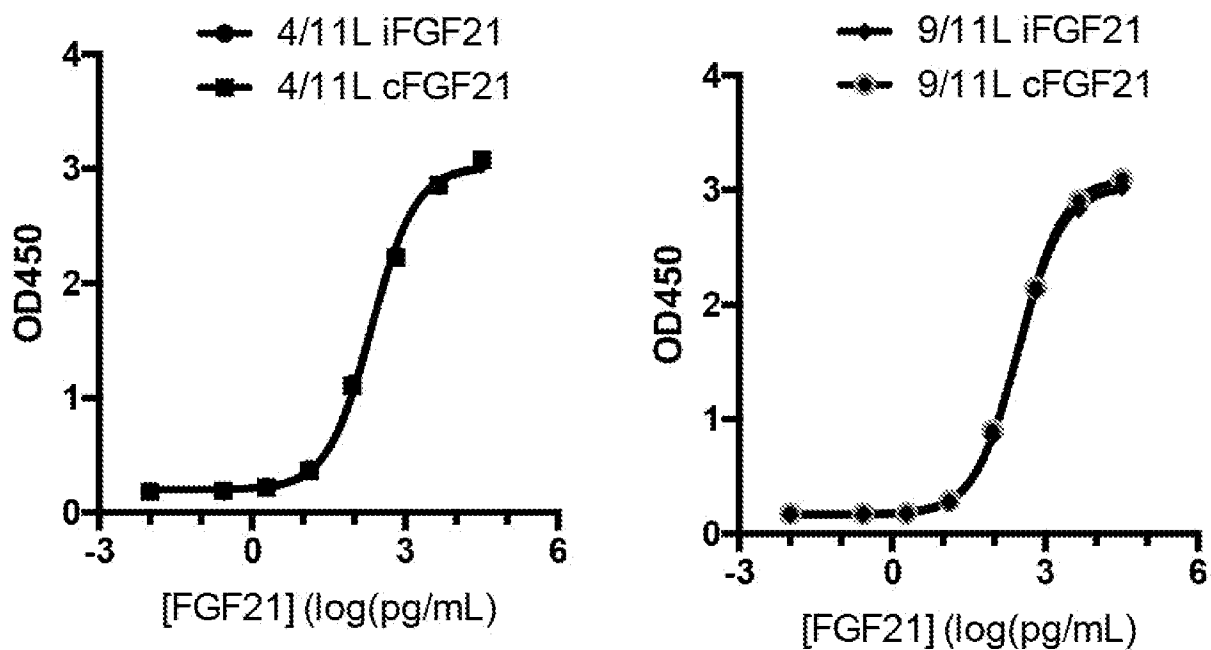


FIGURE 1 continued

3/46



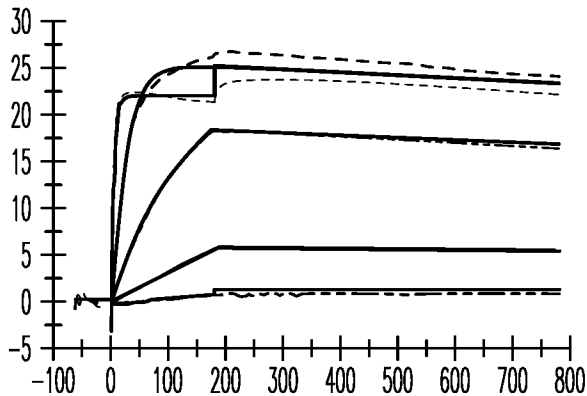
iFGF21: intact FGF21

cFGF21: cleaved FGF21

FIGURE 2

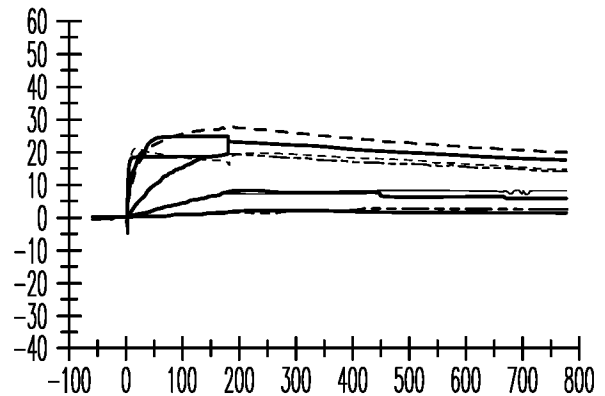
4/46

File: Anti-FGF21 Hyb Actual Run
 Item: FGF214
 Ligand: Hyb 4
 Sample: FGF21
 Curve: Fc=2-1 Temperature: 25°C



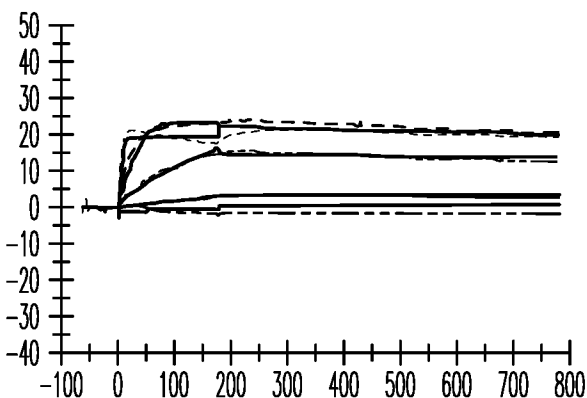
Model: 1:1 Binding
 k_a (1/Ms): $3.816E+5$ K_D (M): $3.689E-10$
 k_d (1/s): $1.408E-4$

File: Anti-FGF21 Hyb Actual Run
 Item: FGF216
 Ligand: Hyb 9
 Sample: FGF21
 Curve: Fc=4-1 Temperature: 25°C



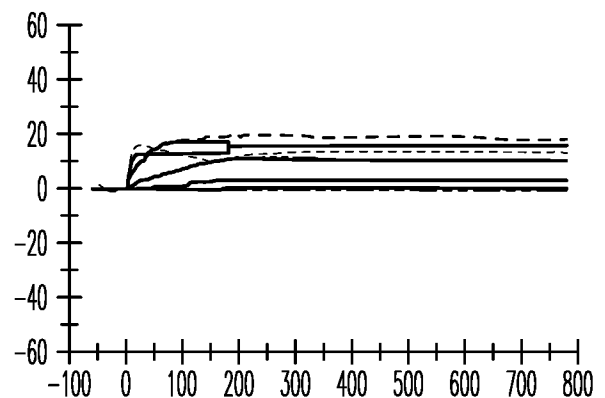
Model: 1:1 Binding
 k_a (1/Ms): $5.813E+5$ K_D (M): $8.895E-10$
 k_d (1/s): $5.171E-4$

File: Anti-FGF21 Hyb Actual Run
 Item: FGF213
 Ligand: Hyb 11
 Sample: FGF21
 Curve: Fc=4-1 Temperature: 25°C



Model: 1:1 Binding
 k_a (1/Ms): $7.075E+5$ K_D (M): $2.704E-10$
 k_d (1/s): $1.913E-4$

File: Anti-FGF21 Hyb Actual Run
 Item: FGF219
 Ligand: Hyb 15
 Sample: FGF21
 Curve: Fc=4-1 Temperature: 25°C



Model: 1:1 Binding
 k_a (1/Ms): $6.216E+5$ K_D (M): $3.955E-12$
 k_d (1/s): $2.459E-6$

FIGURE 3

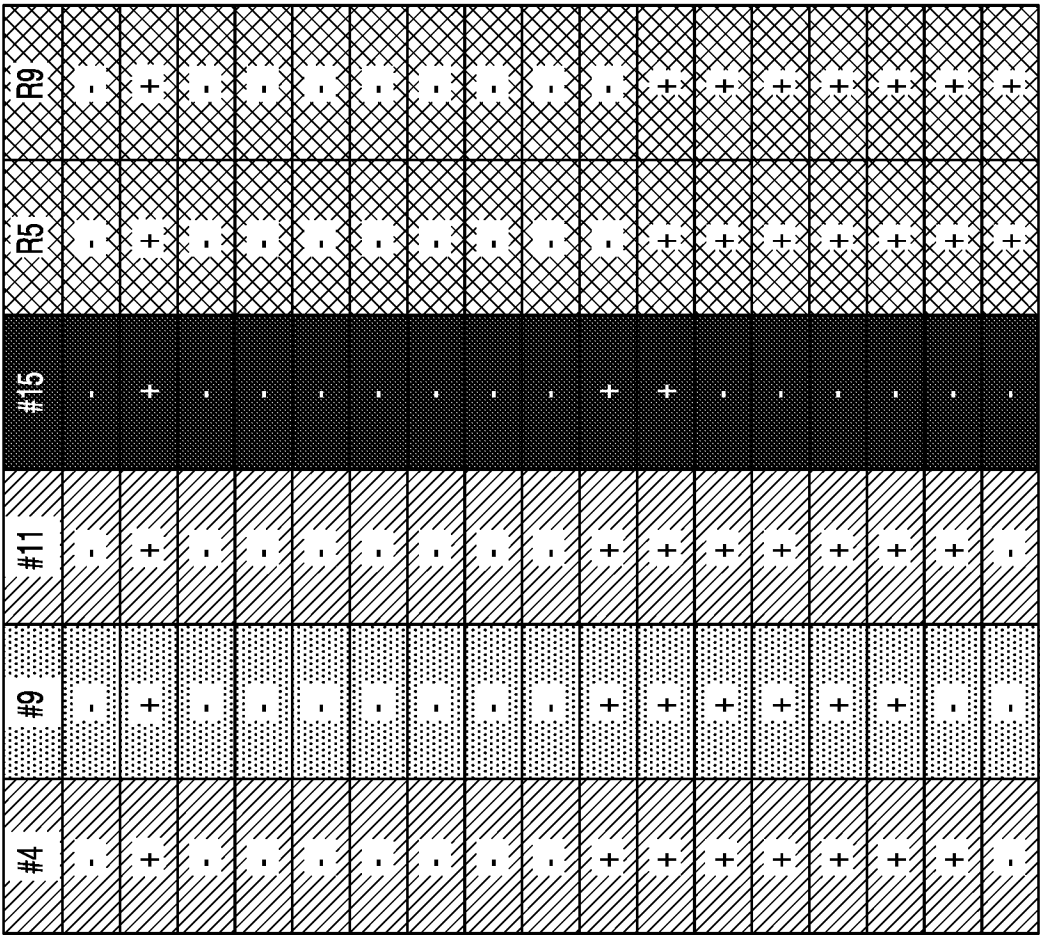
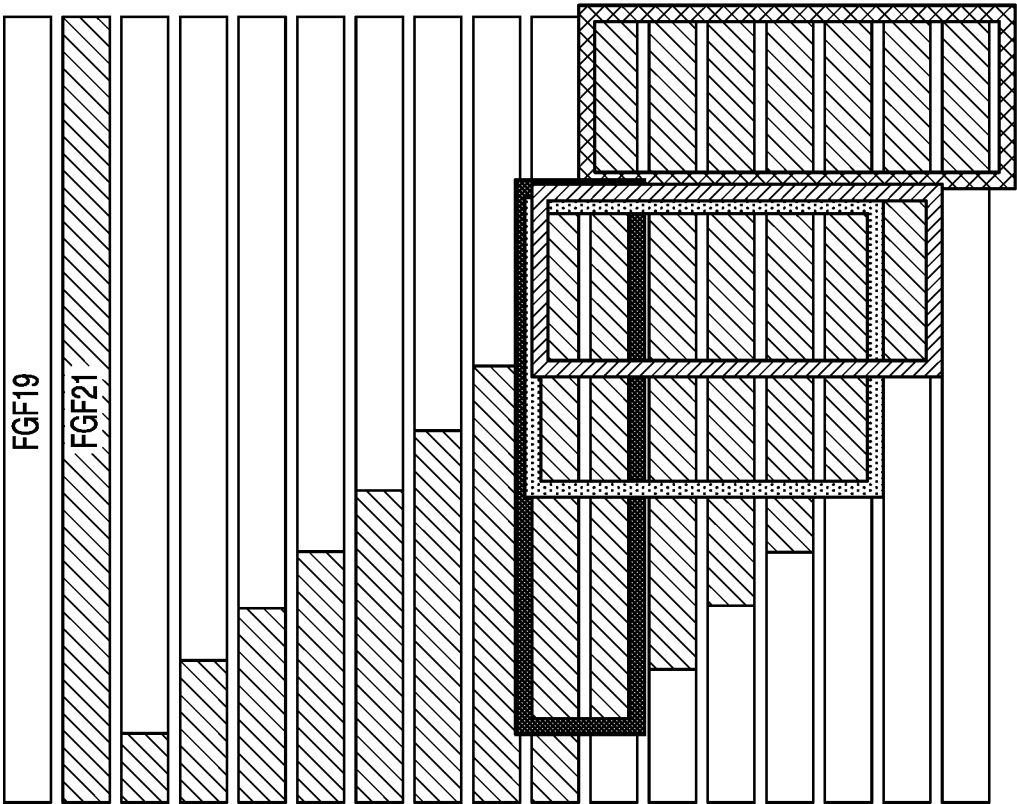


FIGURE 4



+: binding signal, -no binding signal

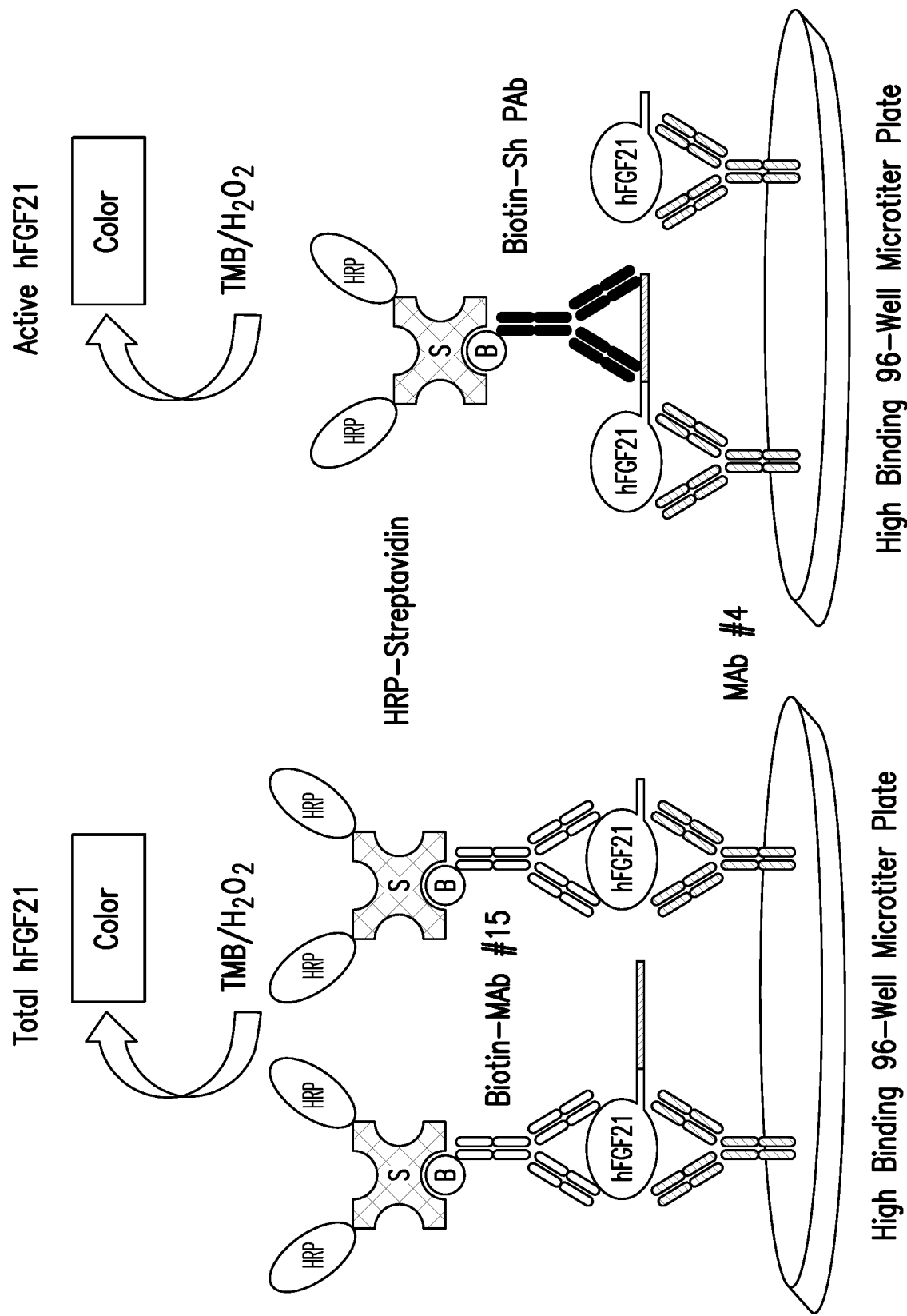


FIGURE 5

7/46

- 1 Coat microtiter plates overnight at 2–8°C with 100 μL per well of 0.5 $\mu\text{g/mL}$ of Coat Antibody in Coating Buffer.
- 2 Wash plate three times with 400 μL per well per cycle of Wash Buffer.
- 3 Add 200 μL per well of Blocking Buffer to plate and incubate plate at room temperature with shaking for 1 to 3 hours.
(Note: If coated microtiter plates were frozen, then incubate plates at room temperature for 1 to 3 hours in the hood with air blower turned on)
- 4 Prepare standard (Std) curve as following example:
WT hFGF21 std source at concentration of 1590 $\mu\text{g/mL}$
Prepare intermediate solution 2 as described in the table below as an example:

Stock	Conc. (pg/mL)	Source Conc. (pg/mL)	Fold dilution	Volume of Source (μL)	Volume of Std Diluent (μL)	Total Volume (μL)
Intermediate 1	10000000	1590000000	159	3.1	496.9	500
Intermediate 2	100000	10000000	100	5.0	495.0	500

	Std conc. (pg/mL)	Procedure
Std1	4000	40 μL of intermediate 2 at 100000 pg/mL + 960 μL Standard Diluent
Std2	1000	250 μL std1 + 750 μL Standard Diluent
Std3	250	250 μL std2 + 750 μL Standard Diluent
Std4	625	250 μL std3 + 750 μL Standard Diluent
Std5	15.625	250 μL std4 + 750 μL Standard Diluent
Std6	3.906	250 μL std5 + 750 μL Standard Diluent
Std7	0.977	250 μL std6 + 750 μL Standard Diluent
Std8	NA	NA
Negative Control (NC)	0.000	750 μL Standard Diluent

- 5 Dilute controls (QC) and samples at X (TBD) dilution in Assay Diluent
- 6 Wash plate three times with 400 μL per well per cycle of Wash Buffer.
- 7 Add diluted standards/controls/samples to plate, 100 μL per well in duplicate.
- 8 Incubate plate at room temperature with shaking for 2 hours.
- 9 Dilute Biotinylated Detection Antibody to 0.5 $\mu\text{g/mL}$ in Assay Diluent
- 10 Wash plate three times with 400 μL per well per cycle of Wash Buffer.
- 11 Add 100 μL per well of diluted Biotinylated Detection Antibody to plate.
- 12 Incubate plate at room temperature with shaking for 1 hour.
- 13 Dilute HRP Conjugate Stock Source to 1:500 dilution (TBD) in HRP-Conjugate Diluent.
- 14 Wash plate three times with 400 μL per well cycle of Wash Buffer.
- 15 Add 100 μL per well of diluted HRP-conjugate to plate.
- 16 Incubate plate at room temperature with shaking for 1 hour.
- 17 Wash plate four times with 400 μL per well per cycle of Wash Buffer.
- 18 Add 100 μL per well of TMB substrate to plate.
- 19 Incubate plate at room temperature with shaking for approximately 15 to 20 minutes.
- 20 Add 100 μL per well of 1 M phosphoric acid to plate.
- 21 Read plate using 450 nm read wavelength and 630 nm or 650 nm reference wavelength

FIGURE 6

8/46

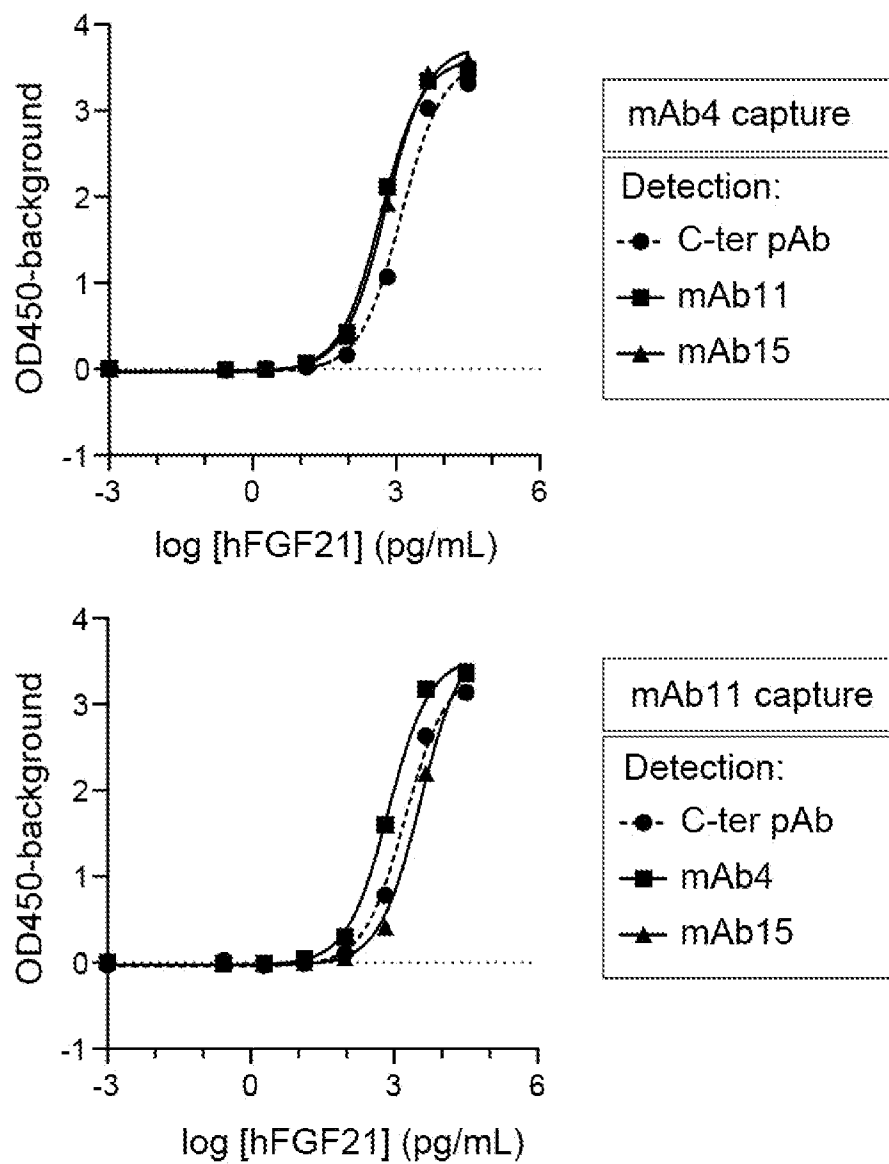
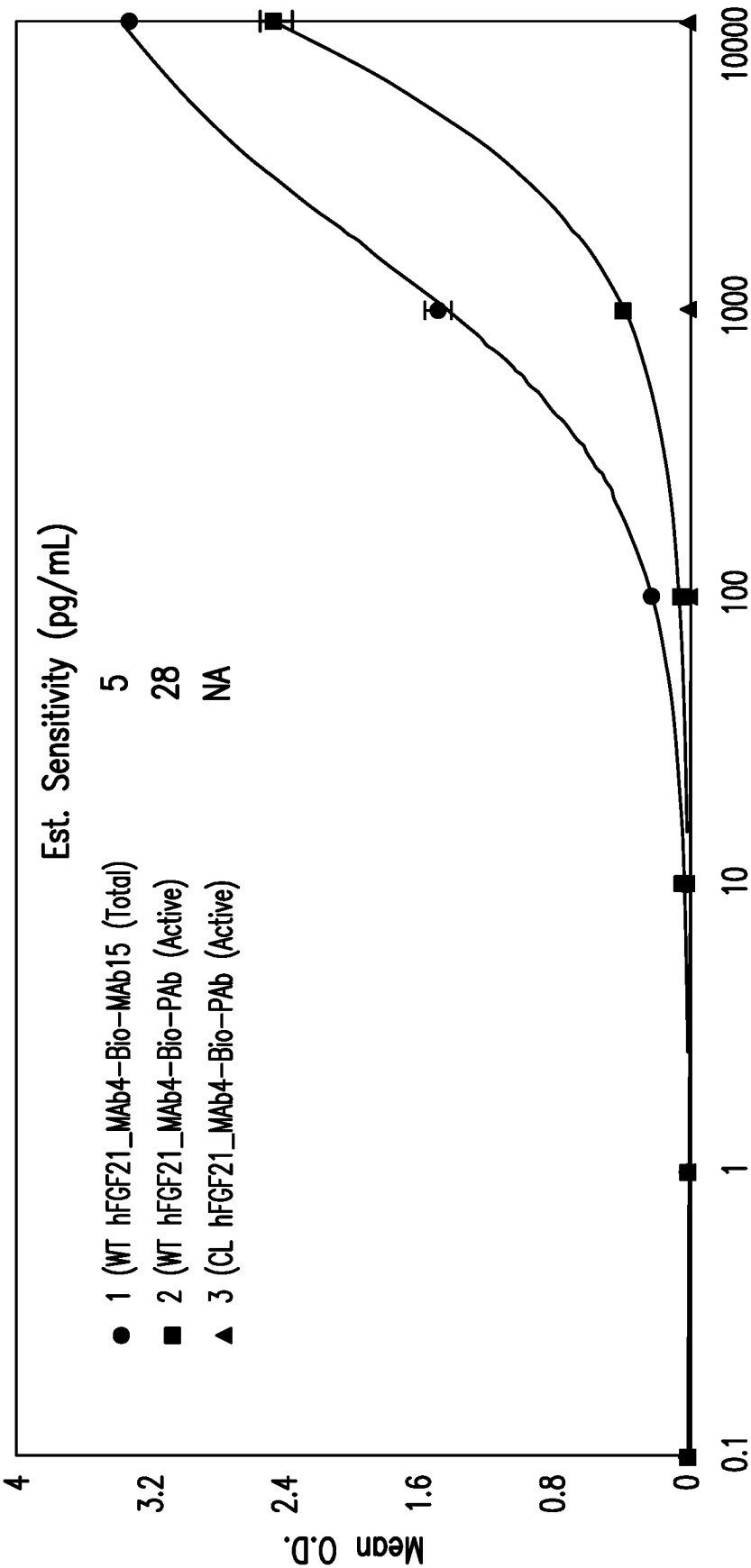


FIGURE 7



WT or Cleaved hFGF21 In-well Concentration in BA010 (pg/mL)

FIGURE 8

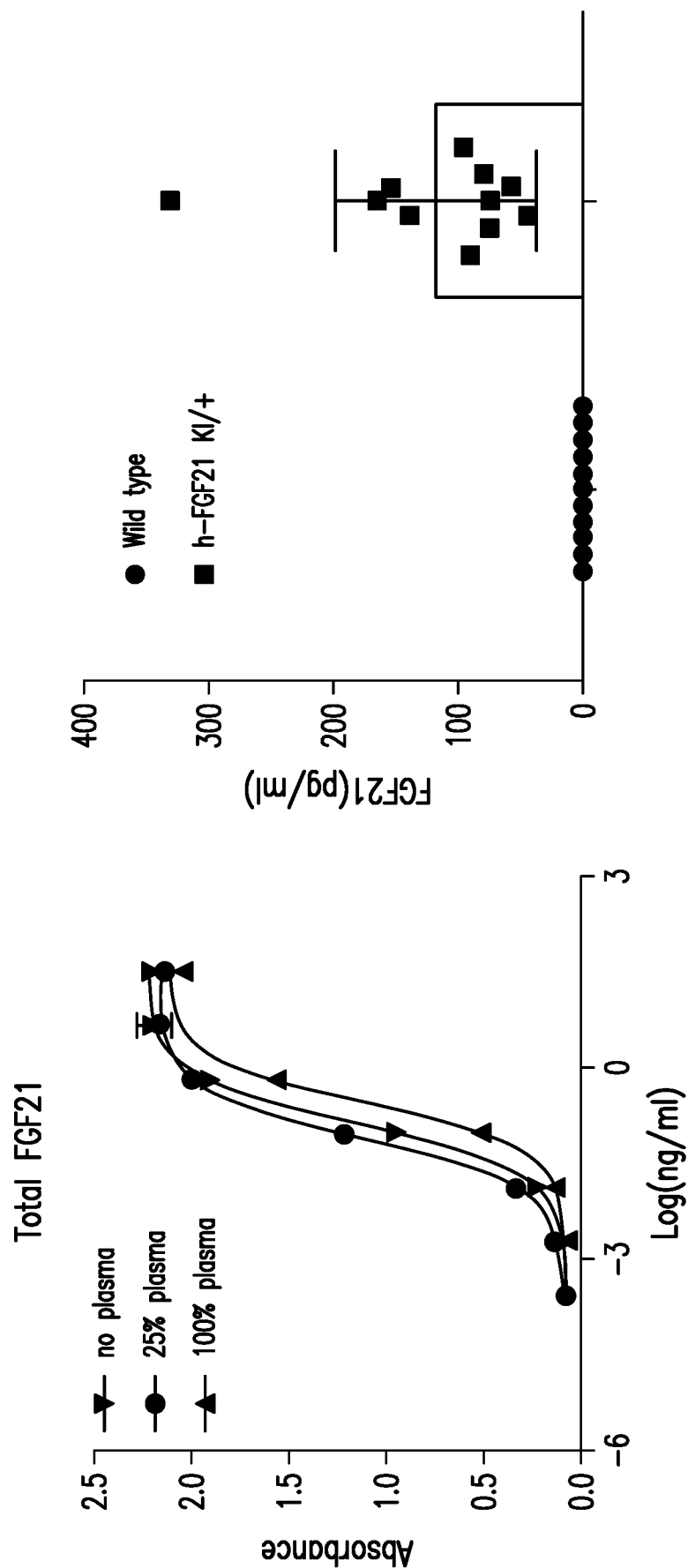


FIGURE 9

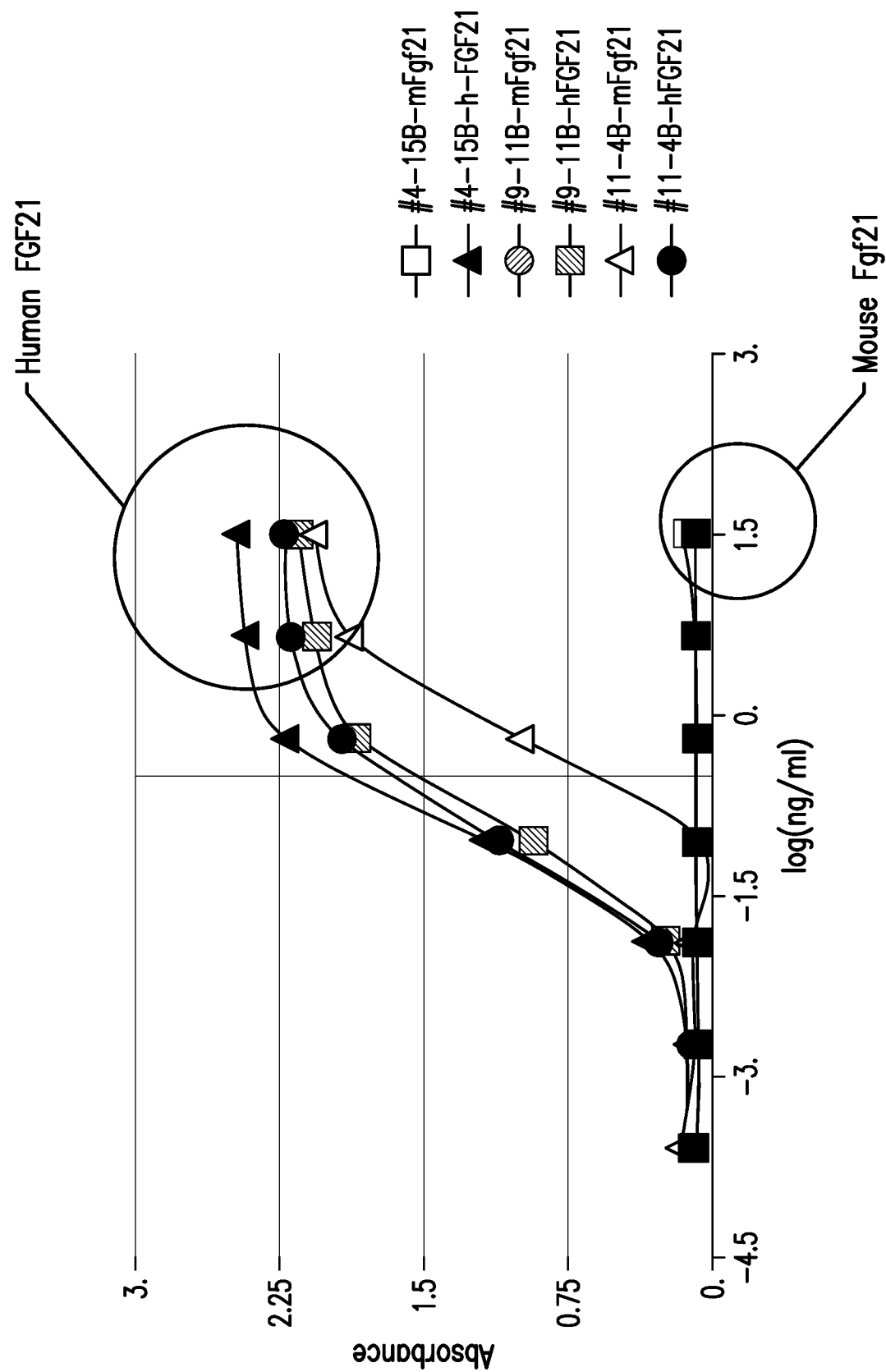


FIGURE 10

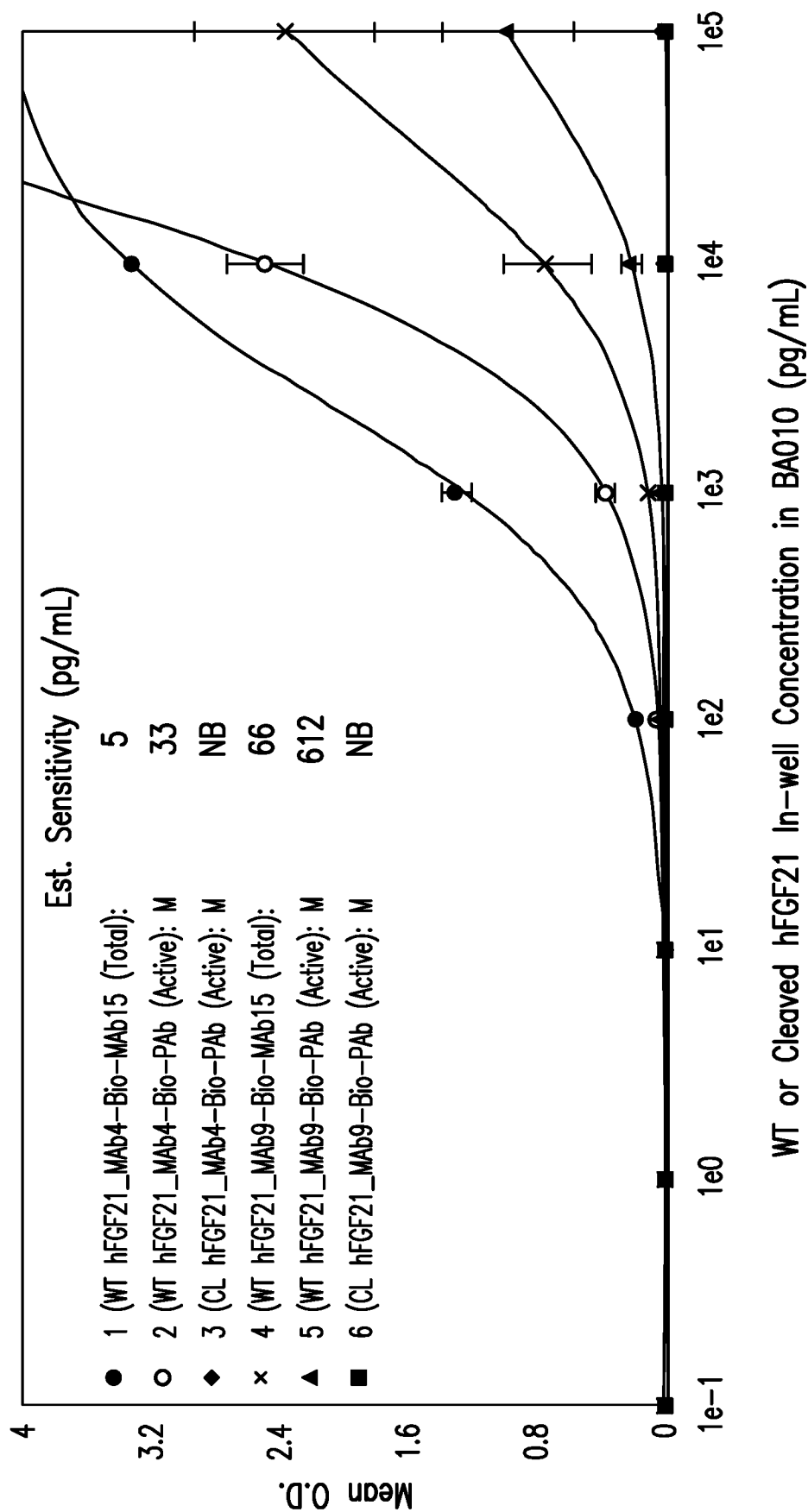


FIGURE 11

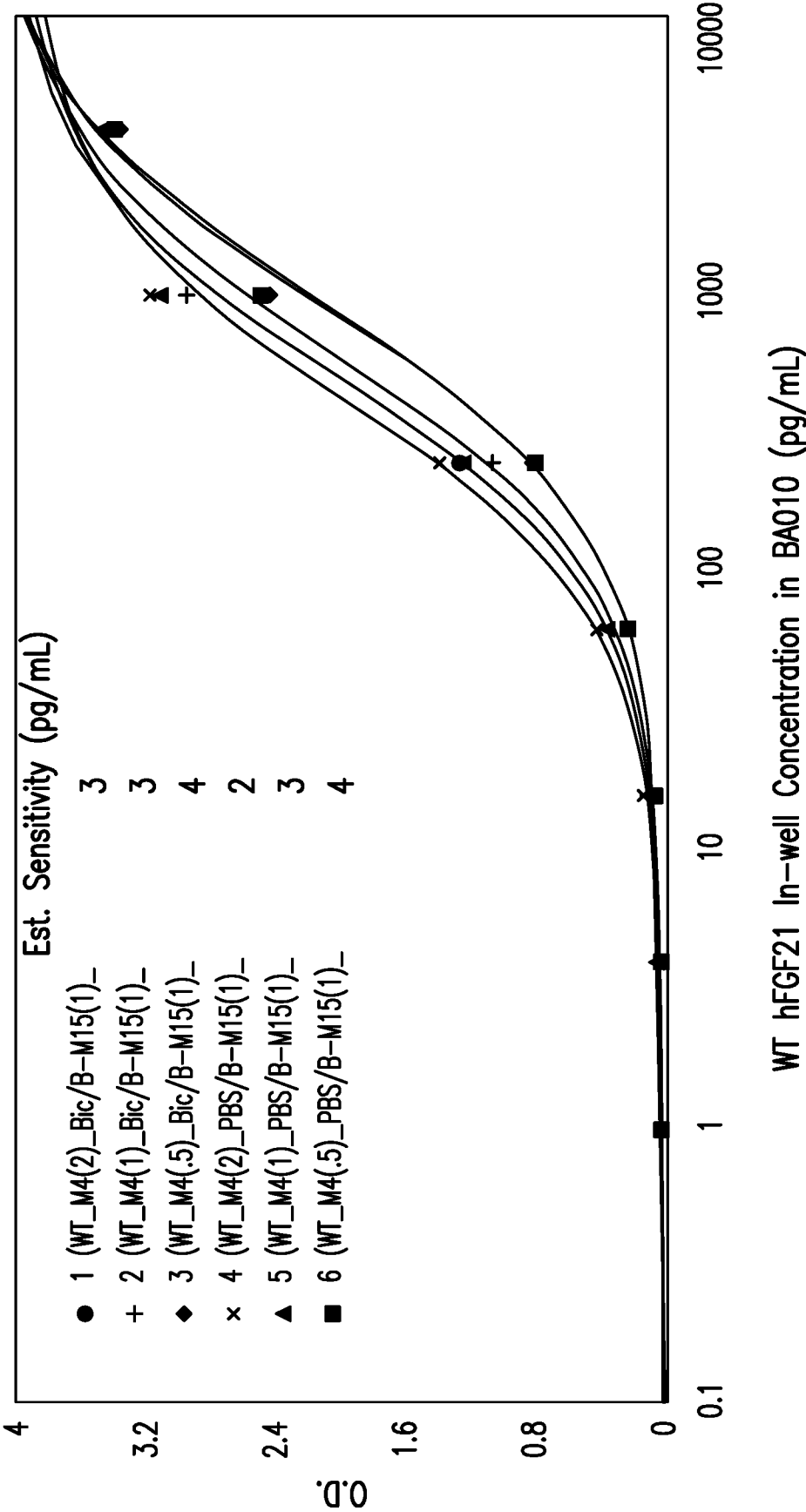
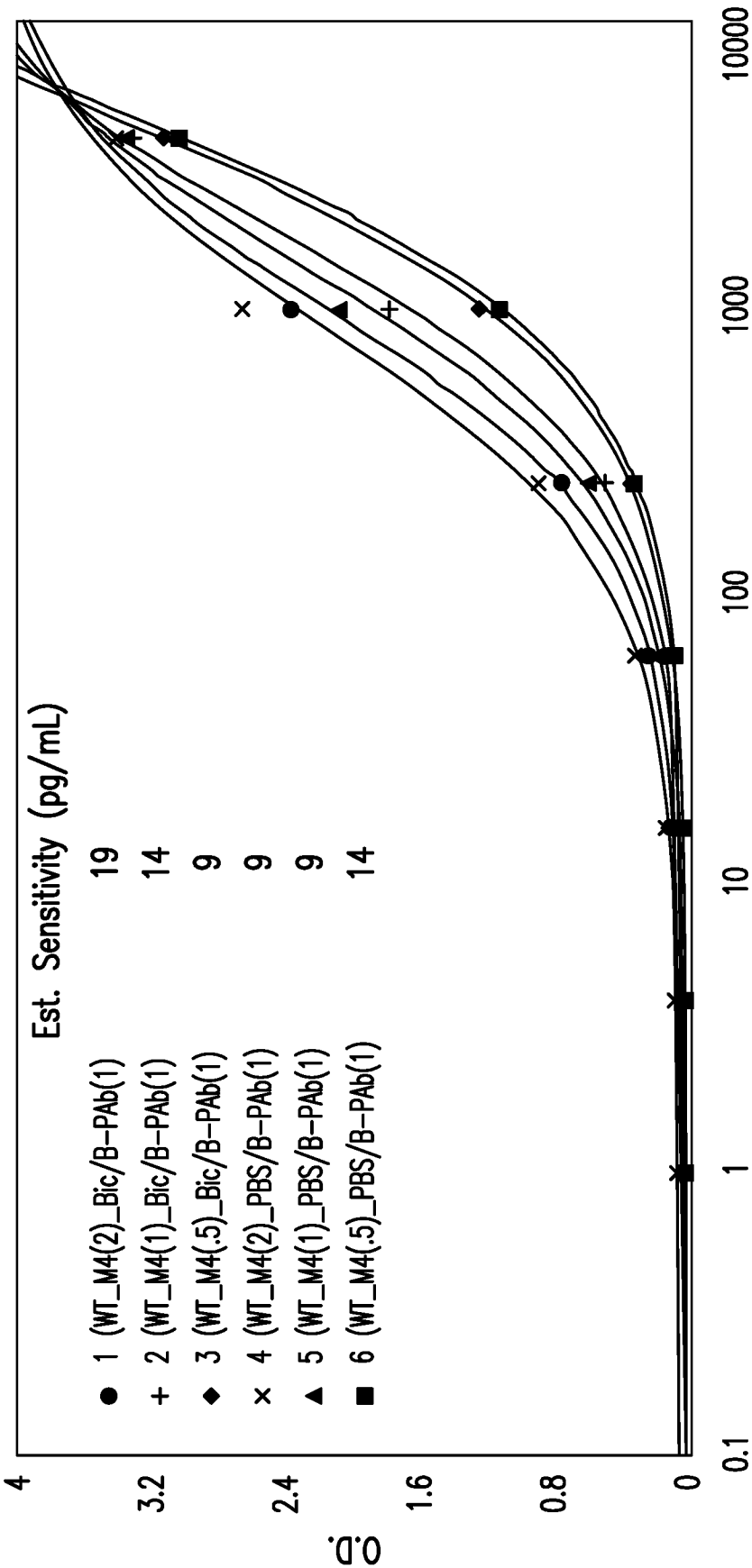


FIGURE 12



WT hFGF21 In-well Concentration in BA010 (pg/mL)

FIGURE 13

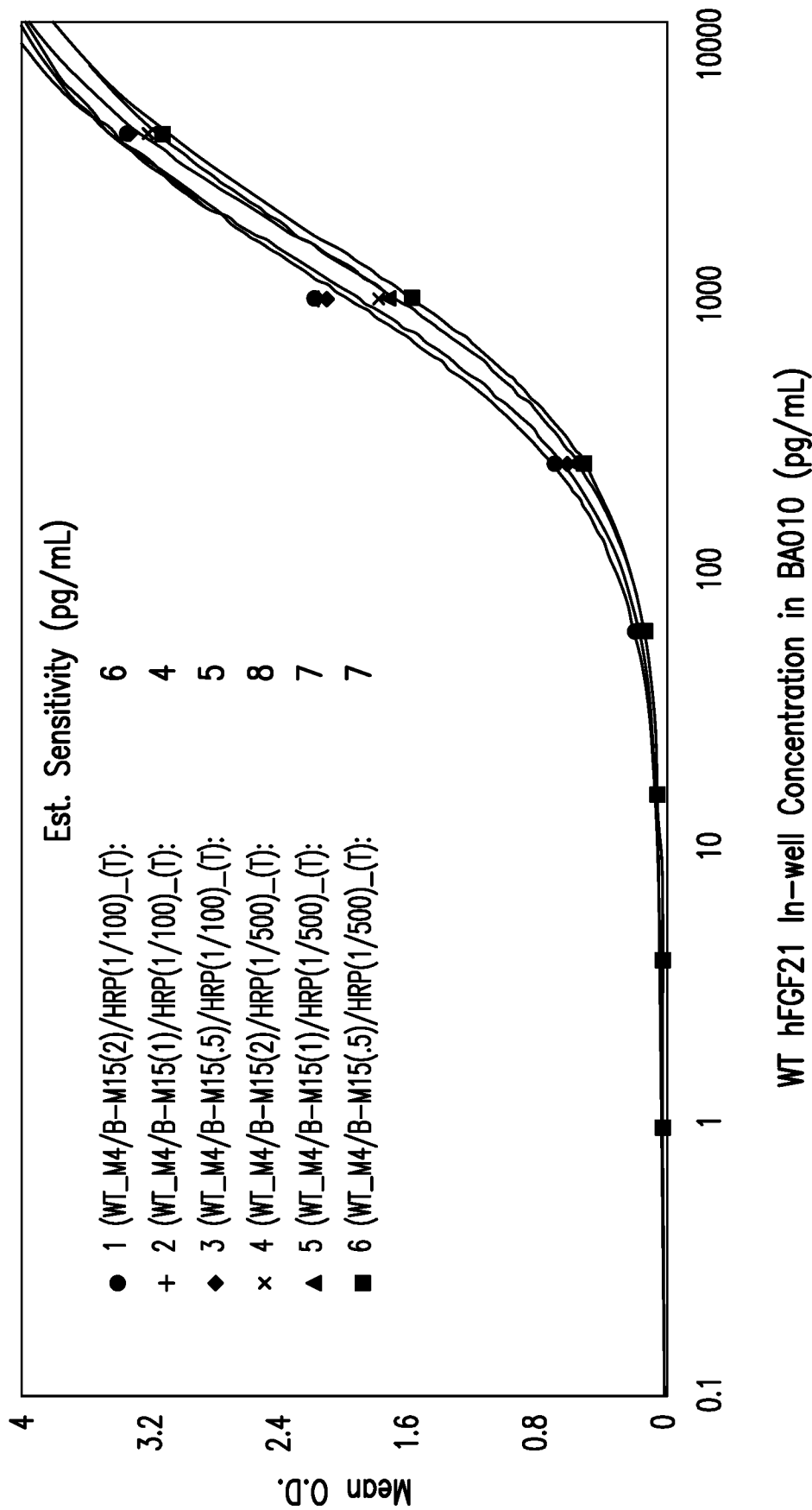


FIGURE 14

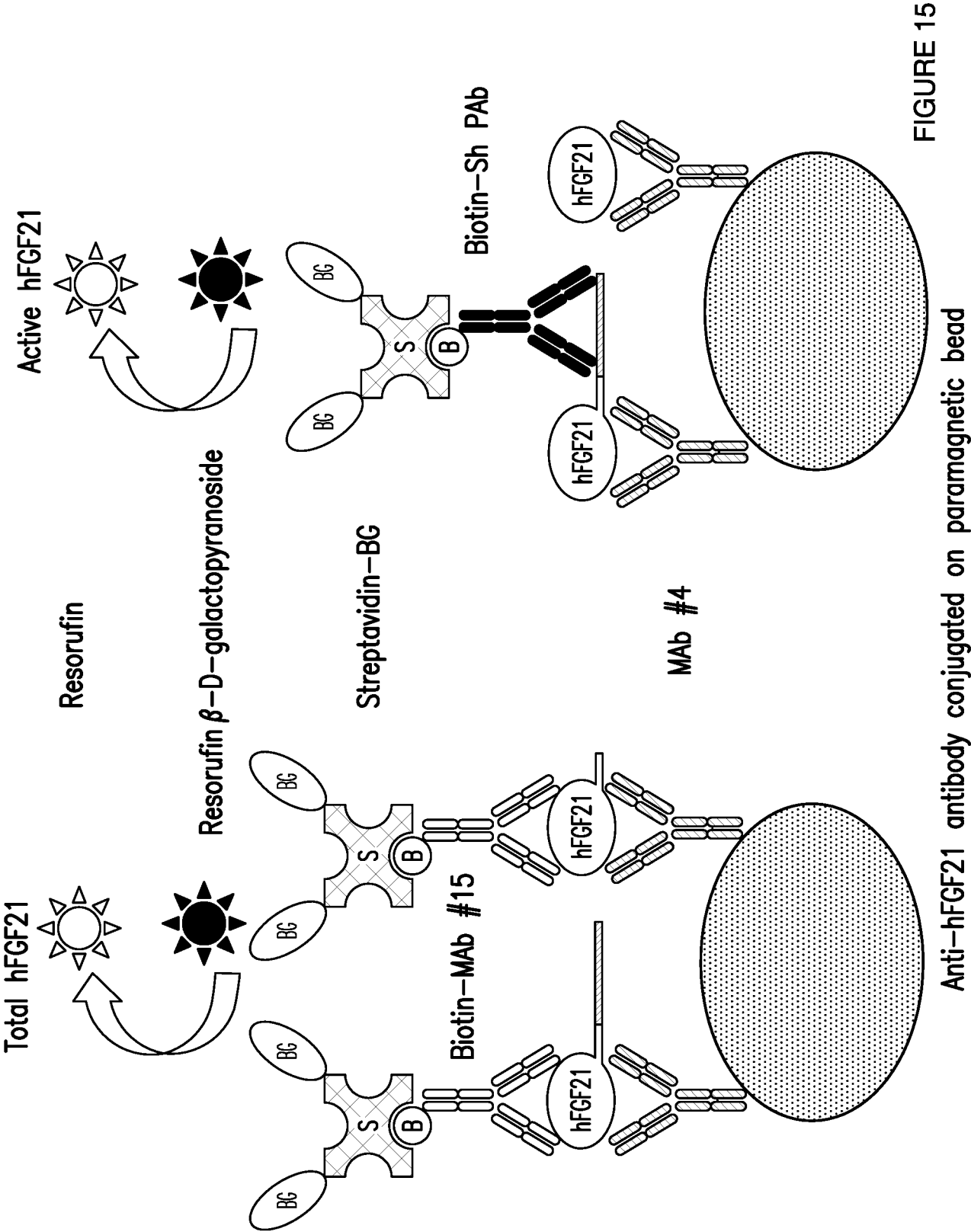


FIGURE 15

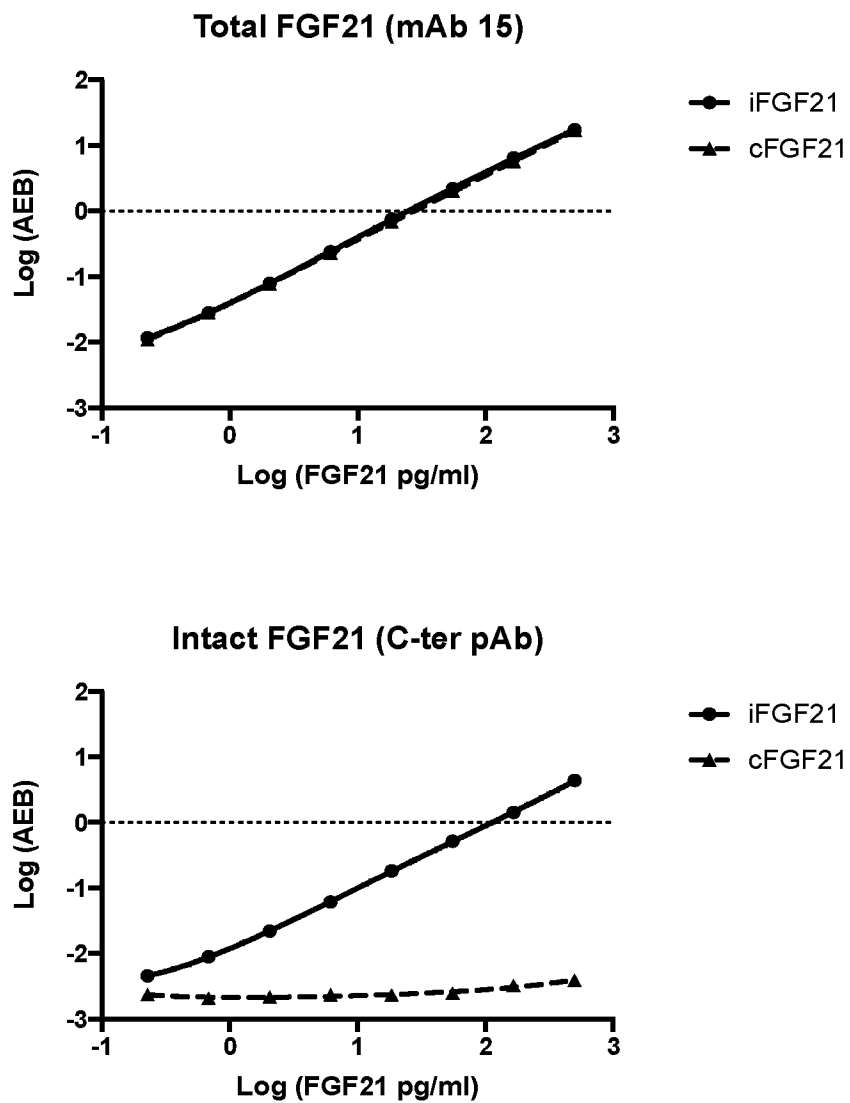
Anti-hFGF21 antibody conjugated on paramagnetic bead

17/46

Steps	Value	Unit	Min	Max	Note
~ Step 1					
Beads	Homebrew 1 Beads				
Liquid Volume	25	μL	10	185	
Sample/Calibrator					
Liquid Volume	100	μL	10	172	
Detector	Homebrew 1 Detect				
Liquid Volume	20	μL	10	185	
Incubation Time	80	cadences (45s)	1	1120	60:00 min
~ Step 2					
SBG	Homebrew 1 SBG				
Liquid Volume	100	μL	10	185	
Incubation Time	7	cadences (45s)	1	1120	5:15 min
~ Measuring					

FIGURE 16

18/46



iFGF21: intact FGF21

cFGF21: cleaved FGF21

FIGURE 17

19/46

- 1 Prepare working solution of Anti-hFGF21 MAb #4 coated paramagnetic beads, Biotin-Conjugate, and SBC in either 15 mL or 50 mL polypropylene centrifuge tubes.
- 2 Transfer each working solution to respective Quanterix assay reagent bottle.
- 3 Prepare standard (Std) curve as following example:
WT hFGF21 Std Stock 1 at concentration of 10 $\mu\text{g/mL}$.
Prepare intermediate solution 2 as described in the table below as an example:

Stock	Conc. (pg/mL)	Source Conc. (pg/mL)	Fold dilution	Volume of Source (μL)	Volume of Std Diluent (μL)	Total Volume (μL)
Intermediate 1	100000	10000000	100	20.0	1980.0	2000
Intermediate 2	1000	100000	100	20.0	1980.0	2000

	Std conc. (pg/mL)	Procedure
Std1	400	400 μL of intermediate 2 at 1000 pg/mL + 600 μL Standard Diluent
Std2	100	250 μL std1 + 750 μL Standard Diluent
Std3	25	250 μL std2 + 750 μL Standard Diluent
Std4	6.3	250 μL std3 + 750 μL Standard Diluent
Std5	1.5625	250 μL std4 + 750 μL Standard Diluent
Std6	0.391	250 μL std5 + 750 μL Standard Diluent
Std7	0.098	250 μL std6 + 750 μL Standard Diluent
Std8	0.024	250 μL std6 + 750 μL Standard Diluent
Negative Control (NC)	0.000	750 μL Standard Diluent

- 4 Use neat High and Low controls (QC) and dilute Endogenous control (EC) or samples at 1:20 minimum required dilution (M RD) and subsequent dilutions in Assay Diluent.
- 5 Add diluted standards/controls/samples to plate, 300 μL per well enough for duplicate.
- 6 Set up fully automated Quanterix Simoa HD-1 instrument and perform experiment.

FIGURE 18

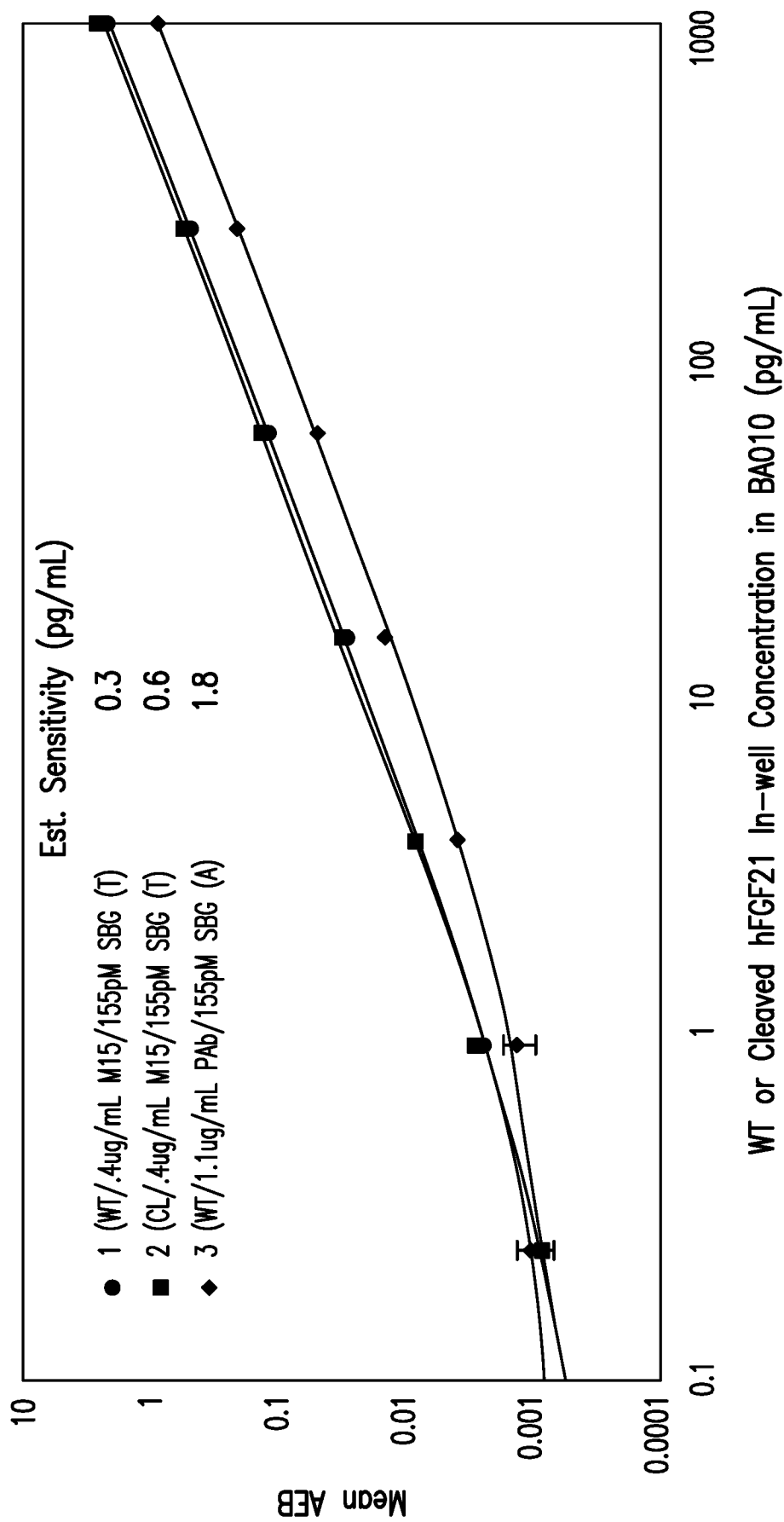


FIGURE 19

21/46

WT hFGF21	Conc. (pg/mL)	Mean AEB	Std.Dev.	CV%	S/N Ratio	Mean Results	% Recovery
1000		2.1119	0.0924	4.4	4036	1055	105
250		0.4766	0.0029	0.6	911	245	98
62.50		0.1130	0.0028	2.5	216	59	95
15.63		0.0289	0.0004	1.4	55	15	98
3.91		0.0080	0.0008	9.7	15	4	105
0.98		0.0024	0.0003	12	4	1.0	102
0.24		0.0010	0.0003	26	2	0.2	97
Blank		0.0005	0.0001	11	1	Range?	

Total Assay

CL hFGF21	Conc. (pg/mL)	Mean AEB	Std.Dev.	CV%	S/N Ratio	Mean Results	% Recovery
1000		2.5205	0.0523	2.1	3183	1126	113
250		0.5253	0.0014	0.3	663	240	96
62.50		0.1266	0.0005	0.4	160	59	95
15.63		0.0309	0.0007	2.2	39	15	93
3.91		0.0082	0.0003	3.1	10	4	98
0.98		0.0029	0.0001	3.5	4	1.3	129
0.24		0.0008	0.0002	20	1	0.2	91
Blank		0.0008	0.0001	15	1	0.2	

Total Assay

WT hFGF21	Conc. (pg/mL)	Mean AEB	Std.Dev.	CV%	S/N Ratio	Mean Results	% Recovery
1000		0.8592	0.0113	1.3	1085	1008	101
250		0.2030	0.0011	0.5	256	244	98
62.50		0.0492	0.0019	3.9	62	60	96
15.63		0.0142	0.0003	2.4	18	17	110
3.91		0.0039	0.0004	11	5	4	106
0.98		0.0013	0.0004	28	2	0.8	78
0.24		0.0010	0.0003	27	1	0.3	142
Blank		0.0010	0.0002	16	1	0.4	

Active Assay

FIGURE 20

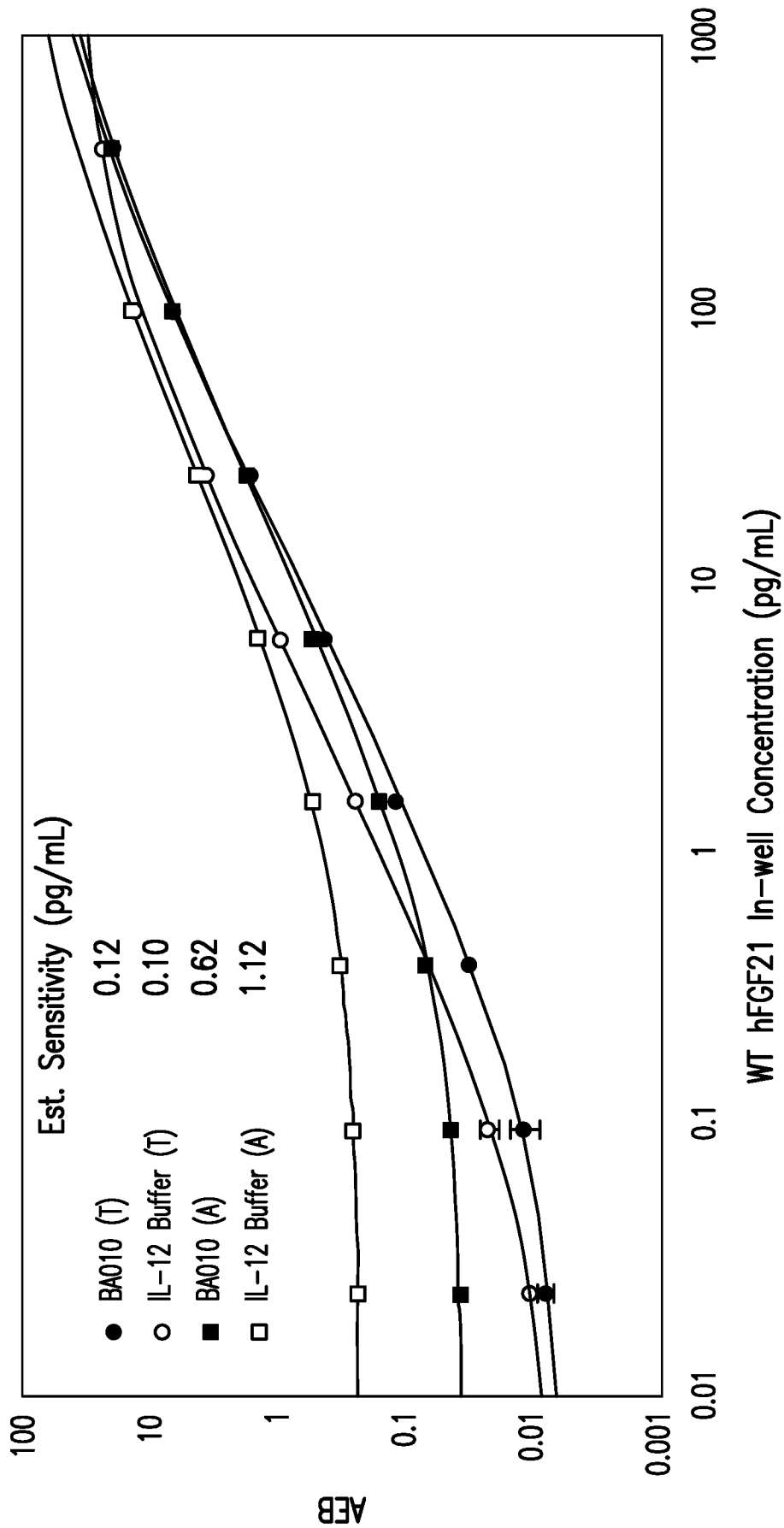


FIGURE 21

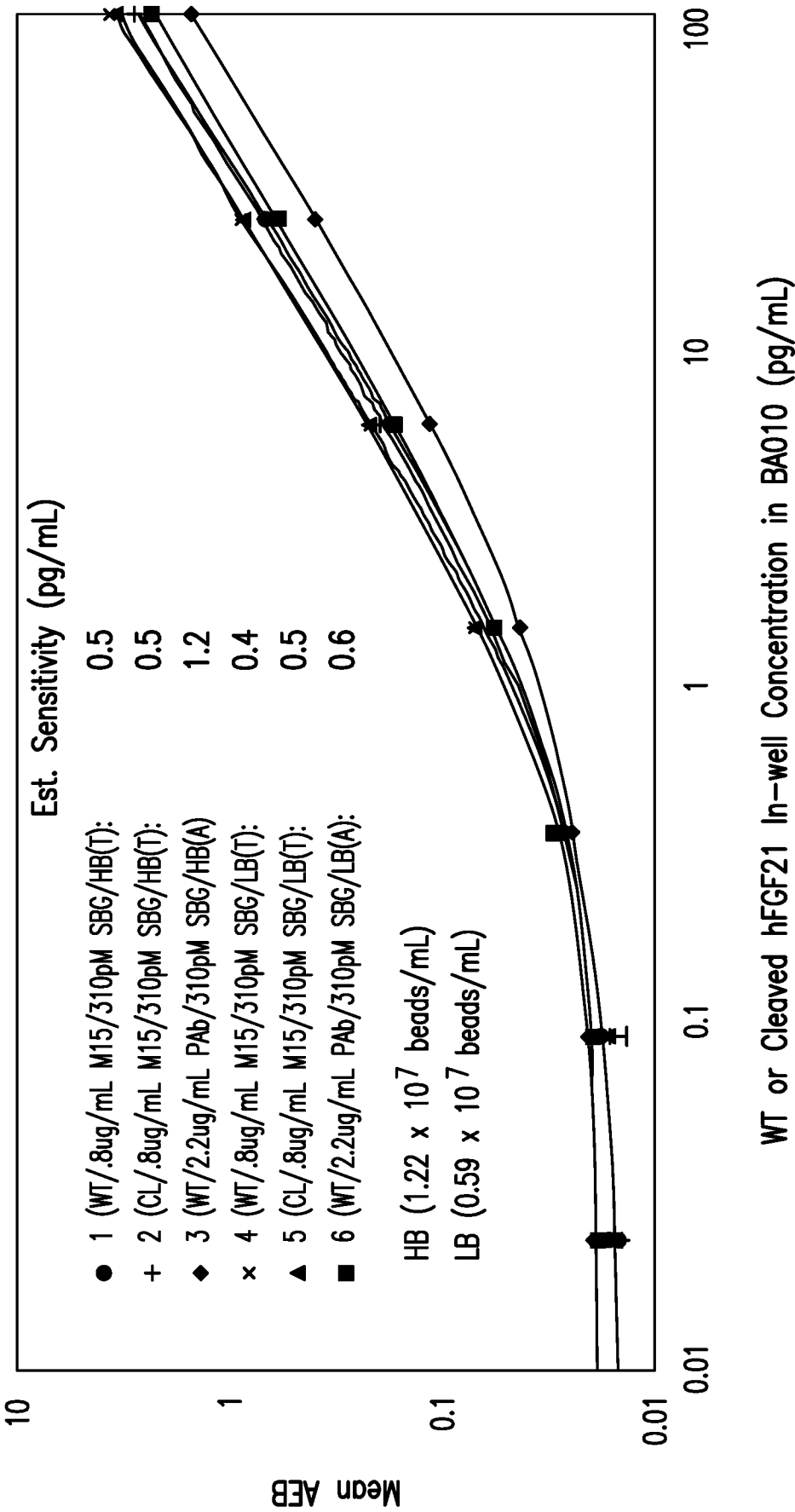


FIGURE 22

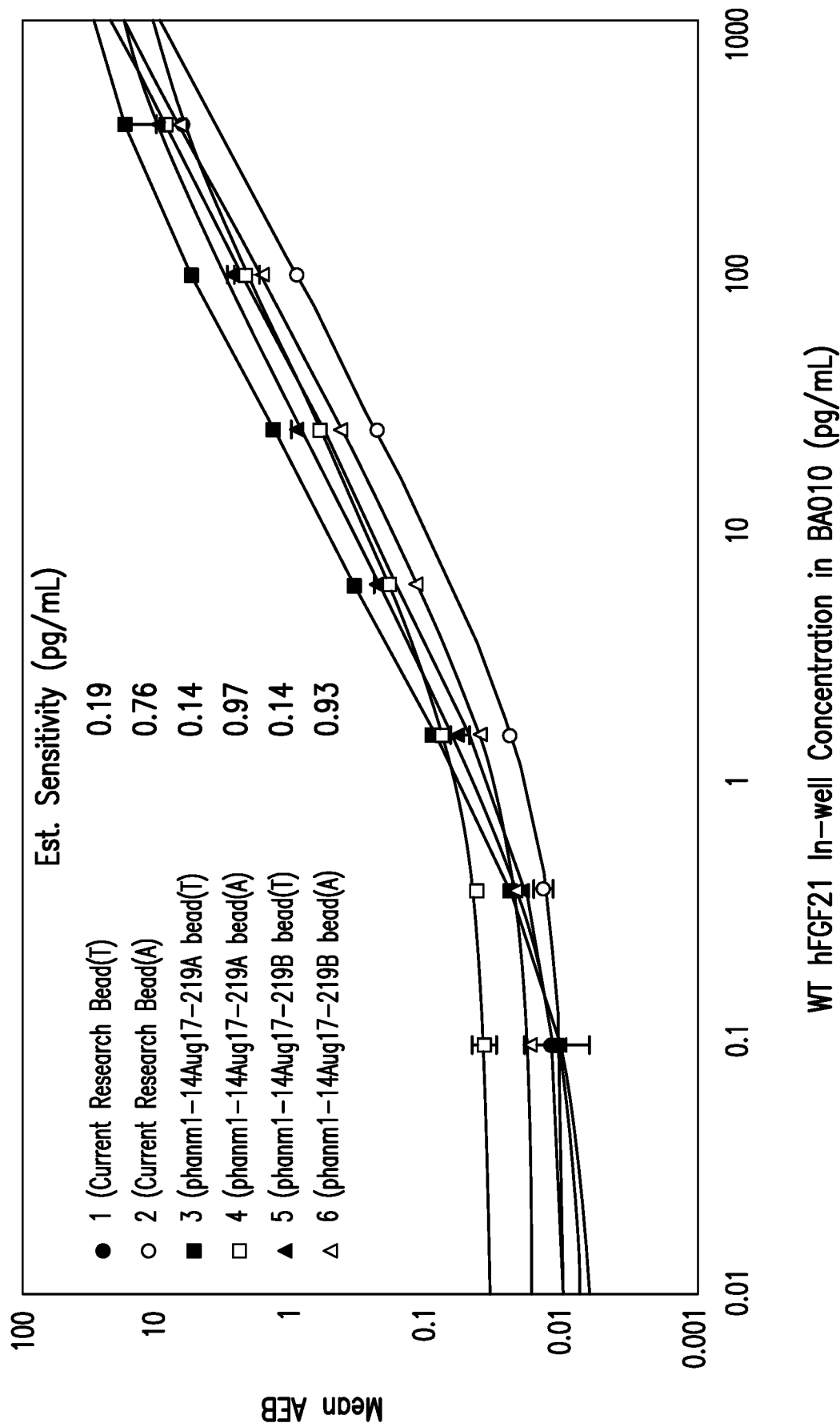


FIGURE 23

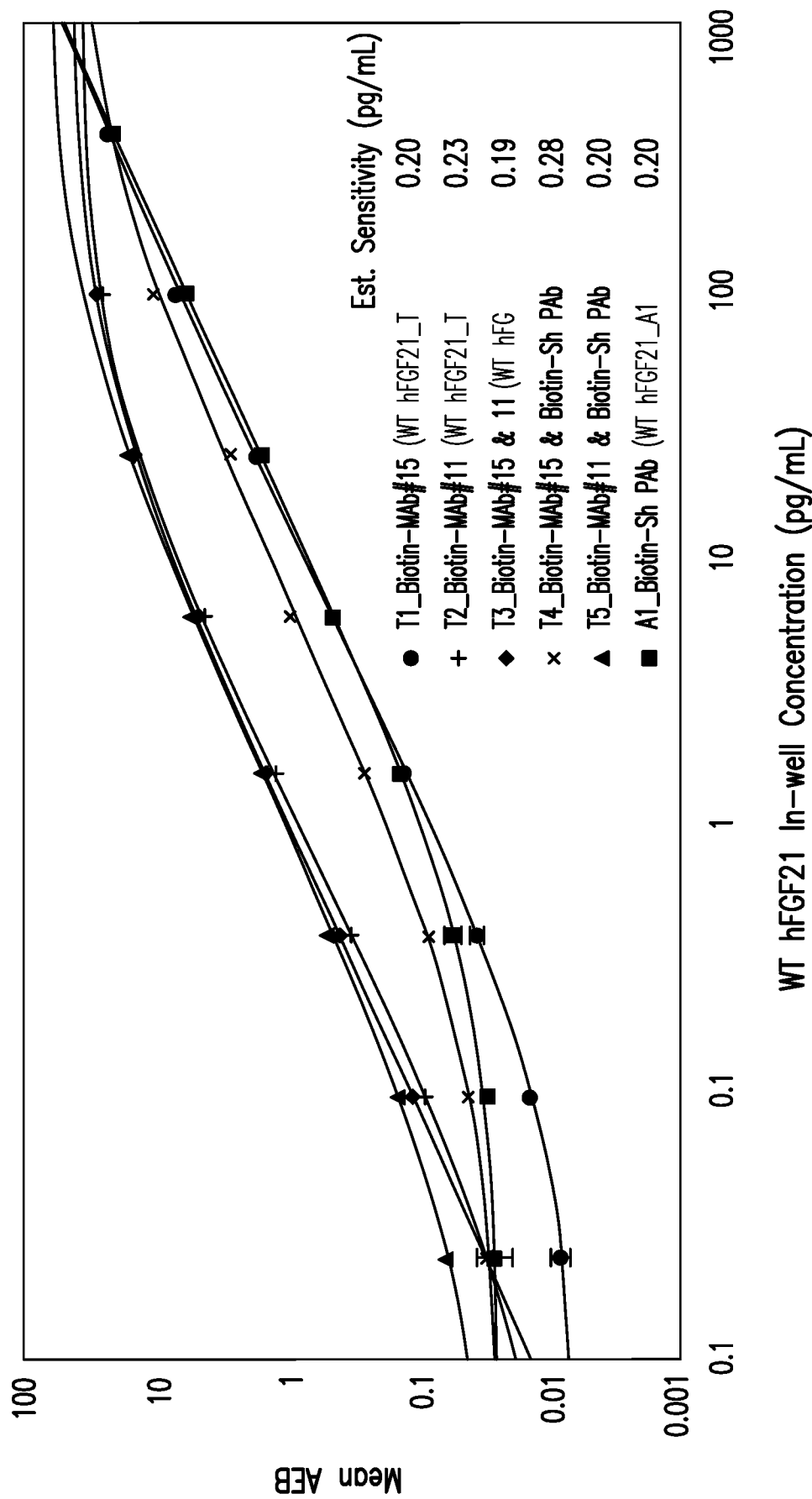


FIGURE 24

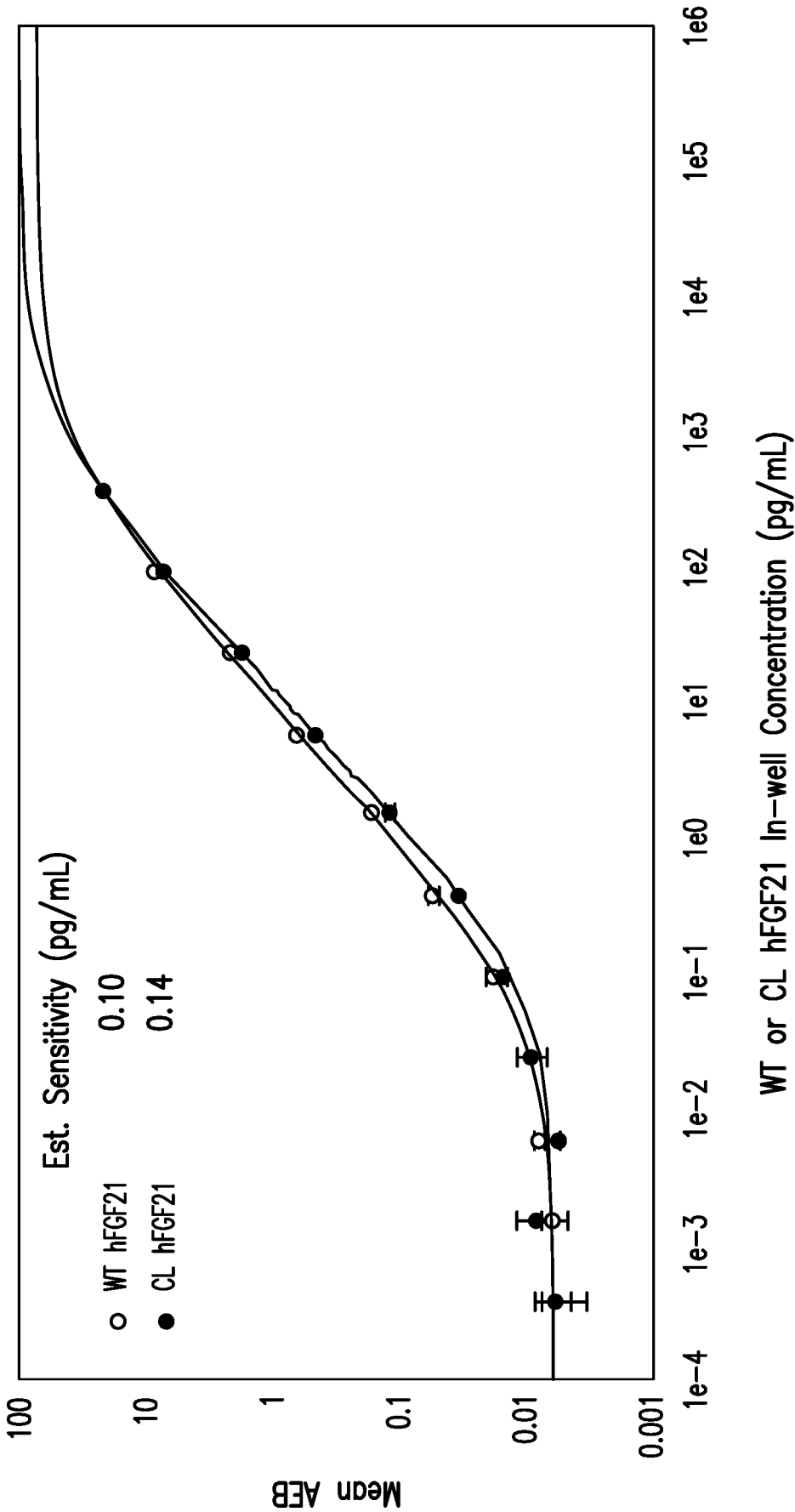


FIGURE 25

27/46

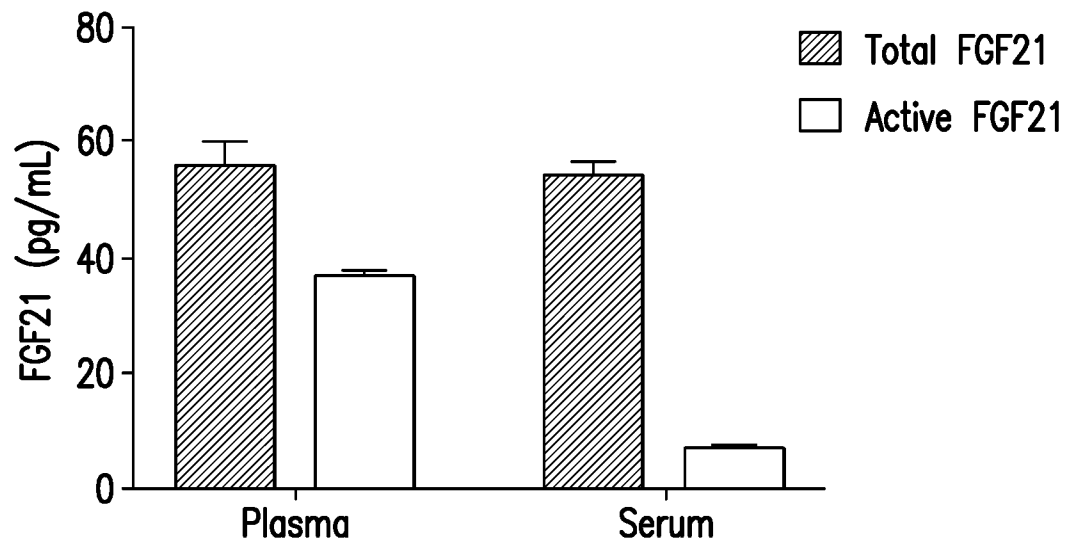


FIGURE 26

28/46

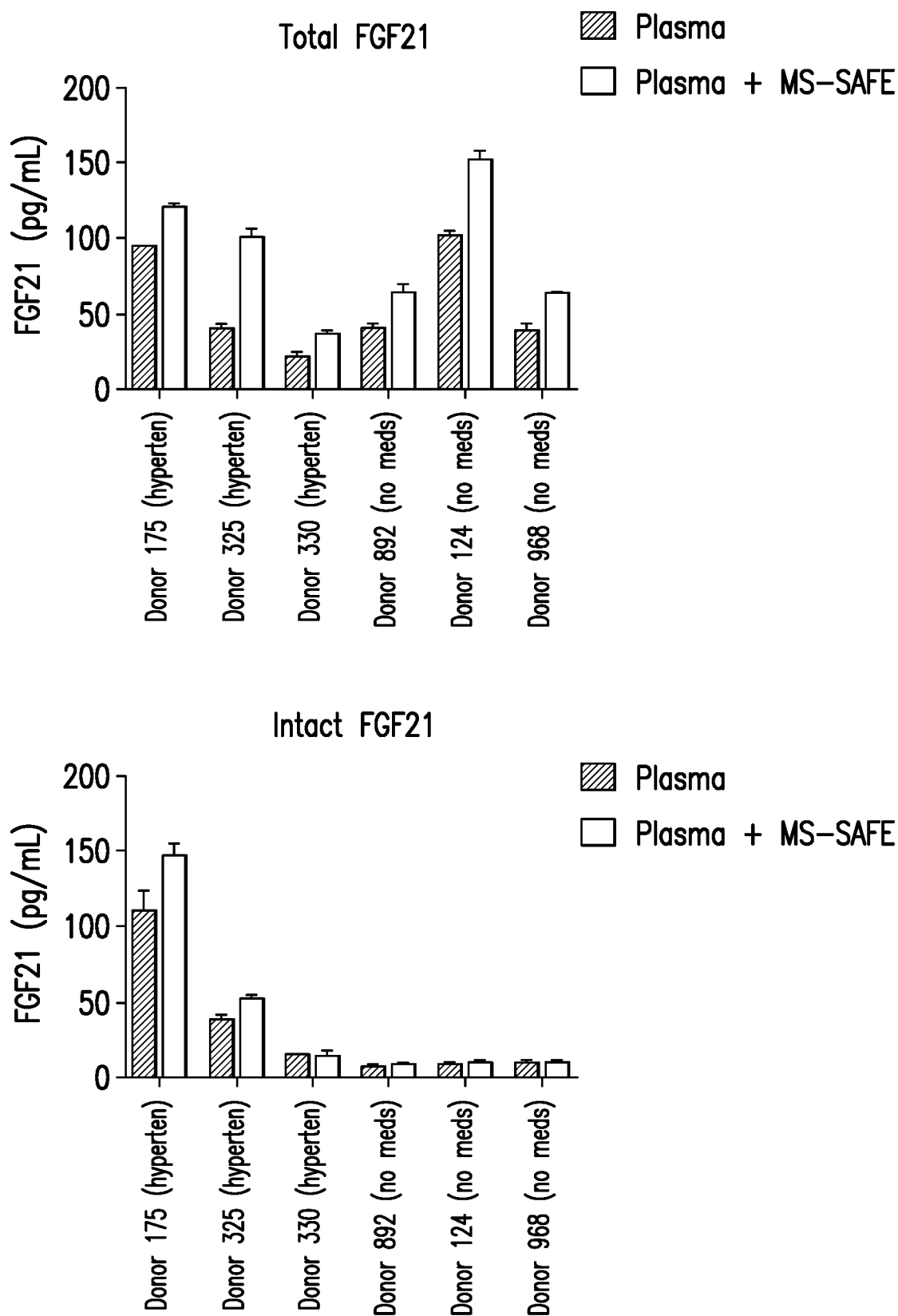
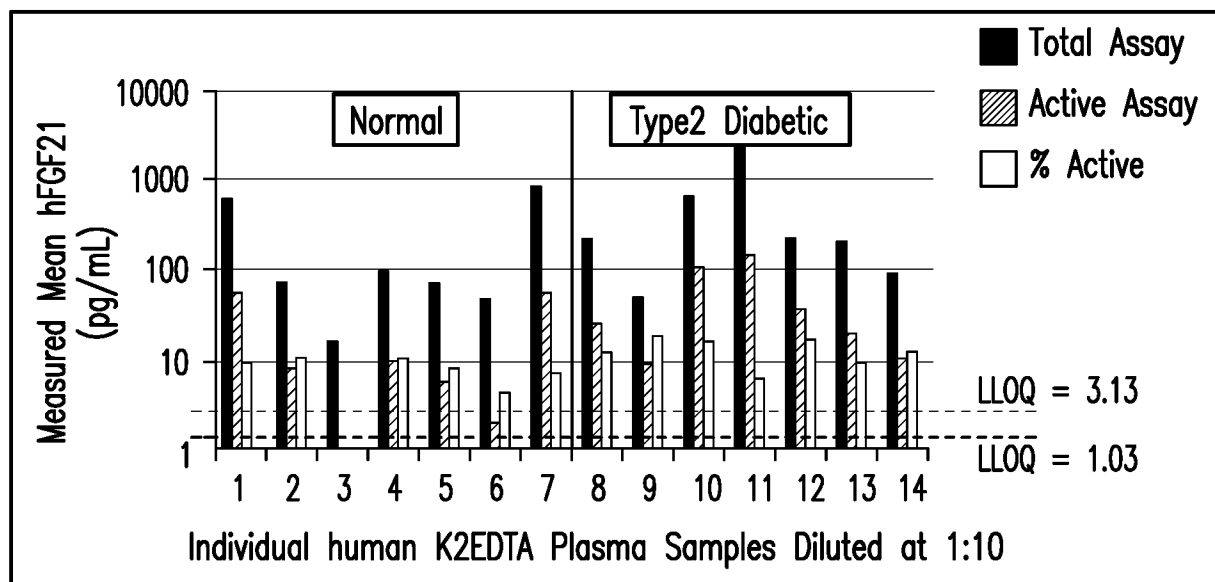


FIGURE 27

29/46

Detectability in Total and Active hFGF21 Assay (Day 1)

	K ₂ EDTA Plasma Sample	Total Assay	Active Assay	% Active
Normal	1	596	56	9
	2	75	8	11
	3	17	0	0
	4	96	10	10
	5	73	6	8
	6	47	2	4
	7	841	60	7
Type2 Diabetic	8	210	25	12
	9	49	9	18
	10	635	102	16
	11	2389	144	6
	12	216	36	17
	13	207	19	9
	14	85	10	12



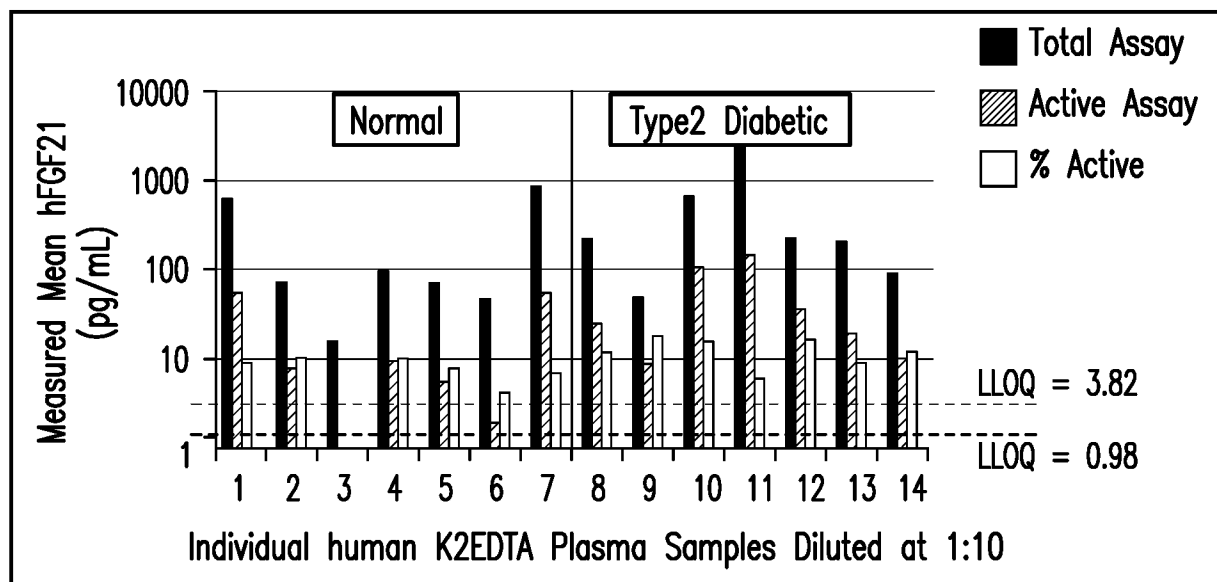
- Detectability is 14/14 (100%) in Total assay and 12/14 (86%) in Active assay.

FIGURE 28A

30/46

Detectability in Total and Active hFGF21 Assay (Day 2)

	K ₂ EDTA Plasma Sample	Total Assay	Active Assay	% Active
Normal	1	596	56	9
	2	75	8	11
	3	17	0	0
	4	96	10	10
	5	73	6	8
	6	47	2	4
	7	841	60	7
Type2 Diabetic	8	210	25	12
	9	49	9	18
	10	635	102	16
	11	2389	144	6
	12	216	36	17
	13	207	19	9
	14	85	10	12



- Detectability is 14/14 (100%) in Total assay and 12/14 (86%) in Active assay.

FIGURE 28B

31/46

		Total Assay (pg/ml)			Active Assay (pg/ml)		
	K ₂ EDTA Plasma Sample	Day 1	Day 2	%Diff	Day 1	Day 2	%Diff
Normal	1	596	345	-42	56	51	-9
	2	75	82	9	8	10	25
	3	17	19	12	0	0	NA
	4	96	93	-3	10	10	0
	5	73	75	3	6	6	0
	6	47	52	11	2	2	0
	7	841	787	-6	60	73	22
Type2 Diabetic	8	210	255	21	25	30	20
	9	49	57	16	9	11	22
	10	635	620	-2	102	124	22
	11	2389	2163	-9	144	171	19
	12	216	251	16	36	45	25
	13	207	202	-2	19	24	26
	14	85	89	5	10	13	30

FIGURE 29

32/46

			Total Assay			Active Assay		
			hFGF21 (pg/mL)	%Rec from MRD	%Change from MRD	hFGF21 (pg/mL)	%Rec from MRD	%Change from MRD
Type2 Diabetic	1	20	112	100	0	27	100	0
		40	110	98	-2	23	86	-14
		80	102	91	-9	16	58	-42
		160	96	86	-14	LTR	NA	NA
		320	98	88	-12	LTR	NA	NA
		640	108	97	-3	LTR	NA	NA
		1280	LTR	NA	NA	LTR	NA	NA
		2560	LTR	NA	NA	LTR	NA	NA
	2	20	185	100	0	37	100	0
		40	171	92	-8	34	92	-8
		80	163	88	-12	24	65	-35
		160	157	85	-15	23	63	-37
		320	158	85	-15	LTR	NA	NA
		640	171	92	-8	LTR	NA	NA
		1280	152	82	-18	LTR	NA	NA
		2560	LTR	NA	NA	LTR	NA	NA
	3	20	215	100	0	49	100	0
		40	194	90	-10	42	86	-14
		80	193	90	-10	35	70	-30
		160	186	86	-14	22	45	-55
		320	197	92	-8	LTR	NA	NA
		640	183	85	-15	LTR	NA	NA
		1280	217	101	1	LTR	NA	NA
		2560	LTR	NA	NA	LTR	NA	NA
	4	20	133	100	0	13	100	0
		40	143	108	8	13	96	-4
		80	132	100	0	LTR	NA	NA
		160	125	94	-6	LTR	NA	NA
		320	106	80	-20	LTR	NA	NA
		640	111	84	-16	LTR	NA	NA
		1280	LTR	NA	NA	LTR	NA	NA
		2560	LTR	NA	NA	LTR	NA	NA

FIGURE 30

33/46

			Total Assay			Active Assay		
	K ₂ EDTA Plasma Sample	Dilution	hFGF21 (pg/mL)	%Rec from MRD	LLOQ (pg/mL)	hFGF21 (pg/mL)	%Rec from MRD	LLOQ (pg/mL)
Type2 Diabetic	1	20	112	100		27	100	
		40	110	98		23	86	0.583
		80	102	91		16	58	
		160	96	86		LTR	NA	
		320	98	88		LTR	NA	
		640	108	97	0.169	LTR	NA	
		1280	LTR	NA		LTR	NA	
		2560	LTR	NA		LTR	NA	
	2	20	185	100		37	100	
		40	171	92		34	92	0.851
		80	163	88		24	65	
		160	157	85		23	63	
		320	158	85		LTR	NA	
		640	171	92		LTR	NA	
		1280	152	89	0.119	LTR	NA	
		2560	LTR	NA		LTR	NA	
	3	20	215	100		49	100	
		40	194	90		42	86	
		80	193	90		35	70	0.432
		160	186	86		22	45	
		320	197	92		LTR	NA	
		640	183	85		LTR	NA	
		1280	217	112	1.069	LTR	NA	
		2560	LTR	NA		LTR	NA	
	4	20	133	100		13	100	
		40	143	108		13	96	0.321
		80	132	100		LTR	NA	
		160	125	94		LTR	NA	
		320	106	80		LTR	NA	
		640	111	84	0.173	LTR	NA	
		1280	LTR	NA		LTR	NA	
		2560	LTR	NA		LTR	NA	
				Mean	0.158		Mean	0.547
				SD	0.026		SD	0.230
				%CV	16.5		%CV	42.0

Final LLOQ corrected for 1:20 dilution:

3.15

10.94

FIGURE 31

		Total Assay				Active Assay					
Type2 Diabetic K ₂ EDTA Plasma Sample	Dilution	Sample alone		Sample + 10 µg/mL Mab #4		% Inhibition (AEB)	Sample alone		Sample + 10 µg/mL Mab #4		% Inhibition (AEB)
		Mean AEB	hFGF21 (pg/mL)	Mean AEB	hFGF21 (pg/mL)		Mean AEB	hFGF21 (pg/mL)			
1	20	1.90	565	0.051	9	97	0.32	71	0.020	LTR	94
2	20	4.02	1321	0.015	LTR	100	1.07	284	0.015	LTR	99
3	20	12.03	4730	0.034	5	100	1.44	394	0.023	LTR	98
4	20	1.84	546	0.022	3	99	0.47	110	0.022	LTR	95
5	20	1.52	439	0.011	LTR	99	0.29	62	0.020	LTR	93
6	20	0.82	220	0.004	LTR	99	0.17	31	0.016	LTR	91

FIGURE 32

35/46

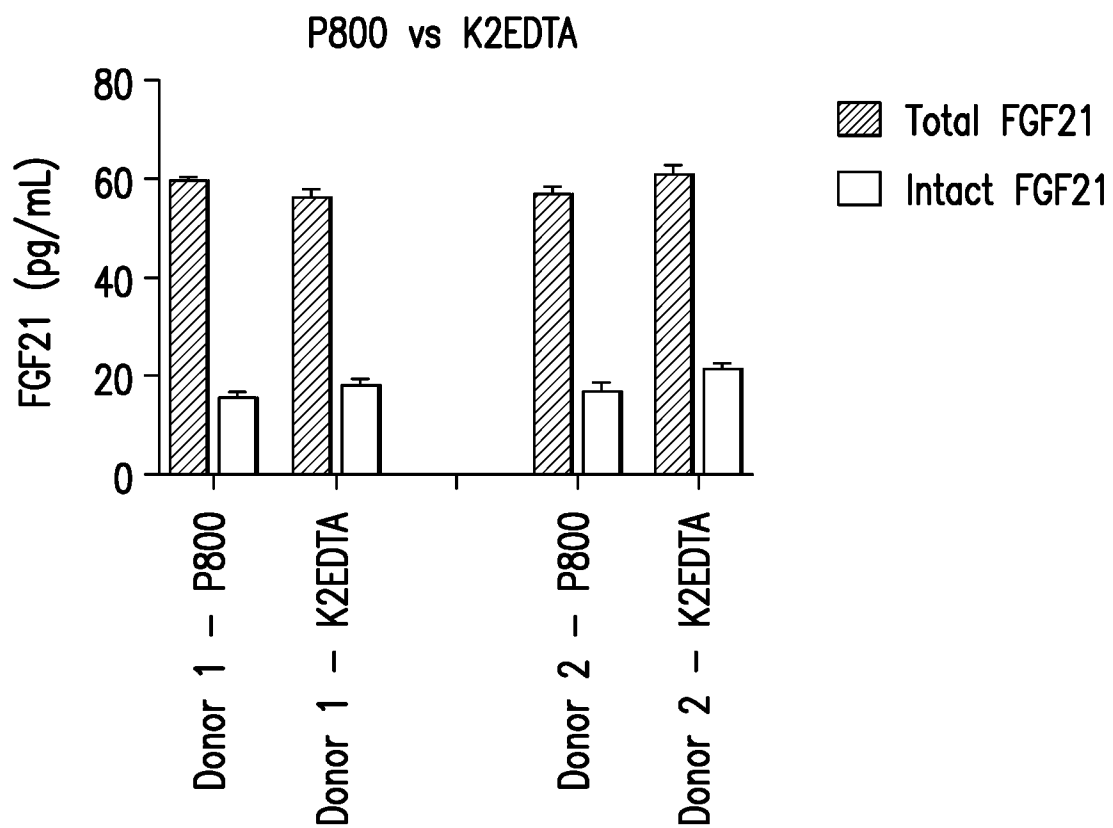


FIGURE 33

36/46

	Plasma Sample	Total Assay (pg/ml)			Active Assay (pg/ml)		
		P800	K ₂ EDTA	%Diff	P800	K ₂ EDTA	%Diff
GC29819 Study Patient Screen Samples	1	91	117	29	11	12	4
	2	607	735	21	145	138	-5
	3	169	184	9	35	34	-4
	4	49	57	16	LTR	LTR	NA
	5	LTR	99	NA	LTR	13	NA
	6	185	212	15	39	43	11
	7	699	840	20	158	166	5
	8	179	167	-7	39	49	27
	9	1137	959	-16	209	255	22
	10	628	557	-11	162	170	5
	11	334	298	-11	164	183	11
	12	122	108	-12	20	23	16
	13	147	128	-13	22	25	15
	14	181	179	-1.2	30	35	16
	15	279	248	-11	61	64	5
	16	132	109	-17	391	418	7
	17	259	249	-4	622	502	-19
	18	442	444	0.3	82	93	13
	19	579	536	-7	127	138	9
	20	148	125	-15	20	24	17
	21	49	47	-5	5	7	51
	22	247	209	-15	53	62	17
	23	211	177	-16	35	41	16
	24	524	557	6	93	110	18
	25	95	94	-2	15	16	8
	Mean	312	297		110	109	
	SD	263	257		143	127	
	%CV	84	86		129	117	

FIGURE 34

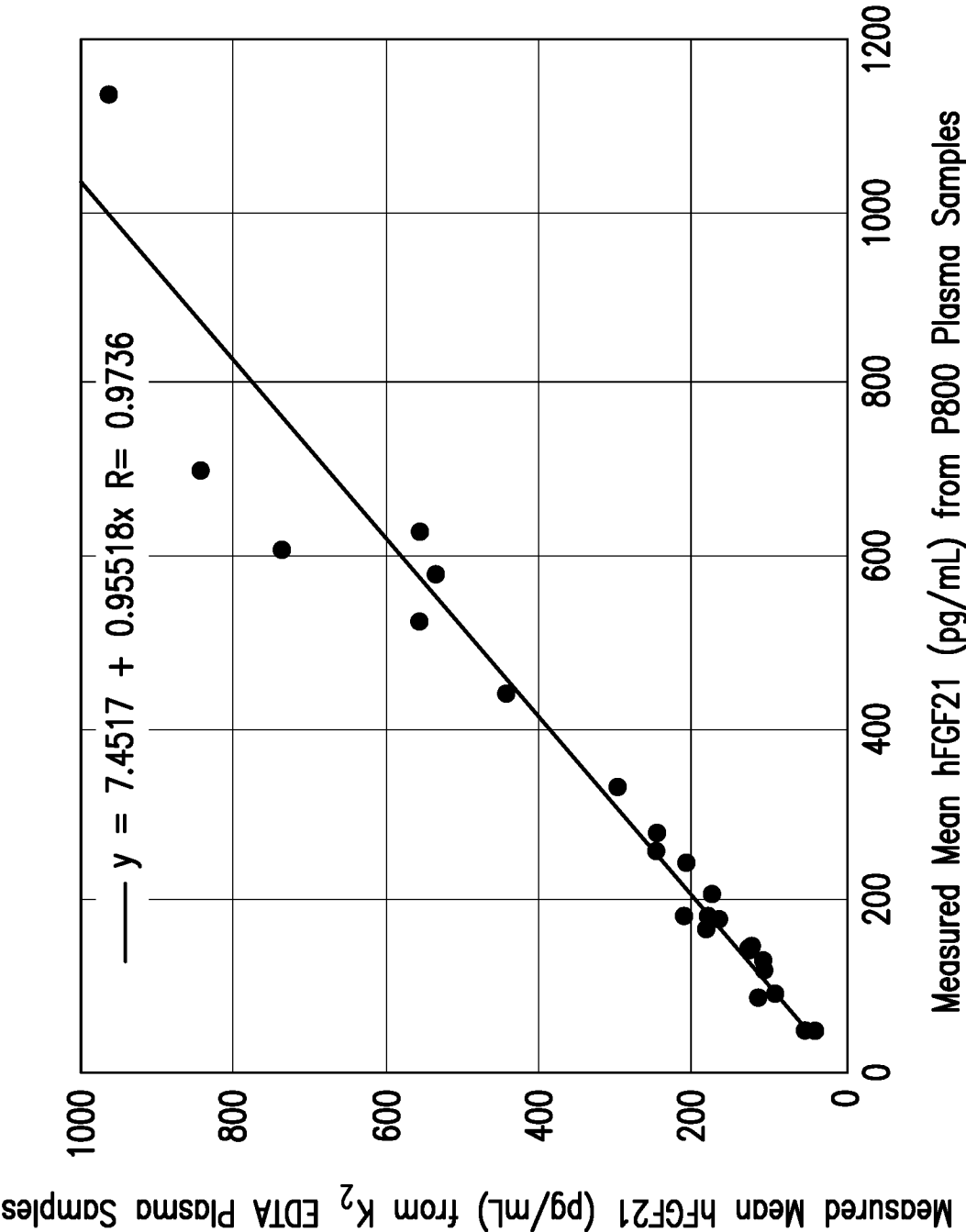


FIGURE 35

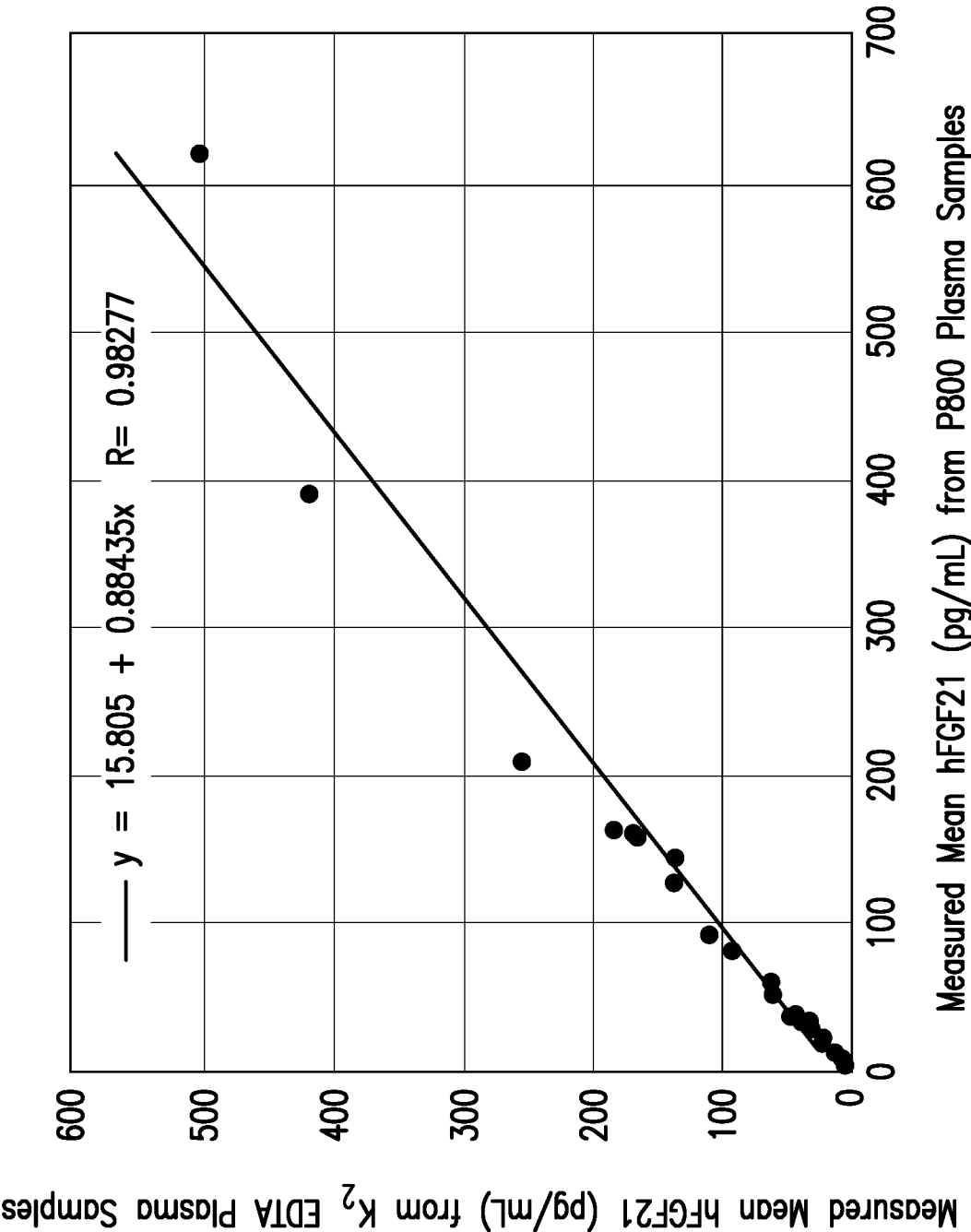


FIGURE 36

39/46

	Subject #	P800 Plasma Stability Sample	Total Assay	% Recovery from 2-8°C	Active Assay	% Recovery from 2-8°C	% Active
GC29819 Study Patient Screen Samples	1 ng/mL WT hFGF21 in AD	2-8°C	1064	100	885	100	83
		1X F/T	1000	94	853	96	85
		2X F/T	1016	95	837	95	82
		3X F/T	1009	95	843	95	84
	20008	2-8°C	425	100	80	100	19
		1X F/T	418	98	96	120	23
		2X F/T	422	99	94	118	22
		3X F/T	427	101	97	122	23
	20027	2-8°C	135	100	29	100	22
		1X F/T	121	90	24	82	20
		2X F/T	118	87	24	82	20
		3X F/T	120	89	23	79	19
	20024	2-8°C	480	100	107	100	22
		1X F/T	452	94	103	96	23
		2X F/T	425	89	96	90	23
		3X F/T	451	94	102	95	23
	20029	2-8°C	122	100	28	100	23
		1X F/T	106	87	24	86	22
		2X F/T	113	92	23	83	20
		3X F/T	104	85	22	81	21
	20036	2-8°C	218	100	86	100	39
		1X F/T	199	91	66	77	33
		2X F/T	191	88	63	73	33
		3X F/T	193	88	66	77	34

FIGURE 37

Sample Diluent	GC29819 K ₂ EDTA samples	Total Assay (pg/mL)	Active Assay (pg/mL)	% Active
Assay Diluent	S09	764	268	35
	S10	446	182	41
	S16	130	145	112
	S17	238	290	122
Assay Diluent/10 μ g/mL MslgG	S09	629	254	40
	S10	378	162	43
	S16	88	121	138
	S17	151	75	49
Assay Diluent/10 μ g/mL ShlgG	S09	755	277	37
	S10	420	167	40
	S16	170	15	9
	S17	254	54	21

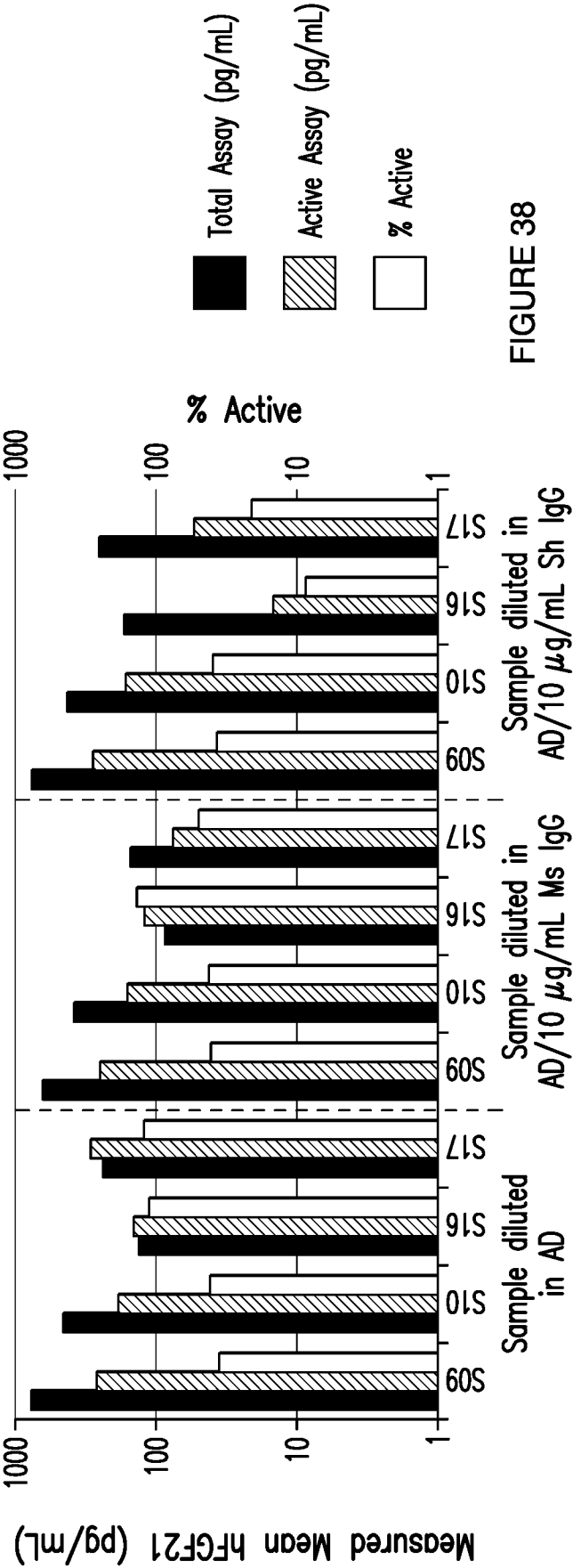


FIGURE 38

Sample Diluent	GC29819 K ₂ EDTA samples	Total Assay (pg/mL)	Active Assay (pg/mL)	% Active
Assay Diluent	S09	738	190	26
	S10	461	122	27
	S16	118	173	146
	S17	191	347	182
Assay Diluent/10 μg/mL Ms & Sh IgG	S09	848	209	25
	S10	501	131	26
	S16	119	18	15
	S17	190	44	23

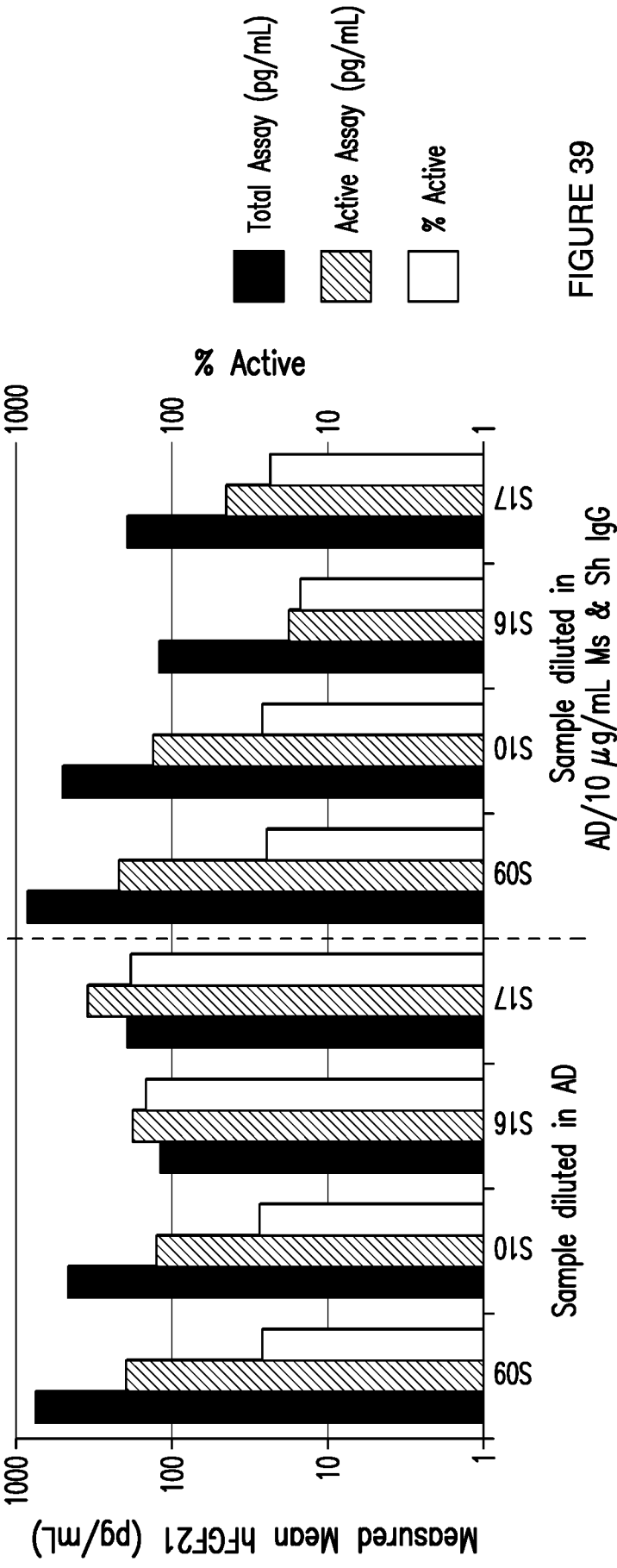


FIGURE 39

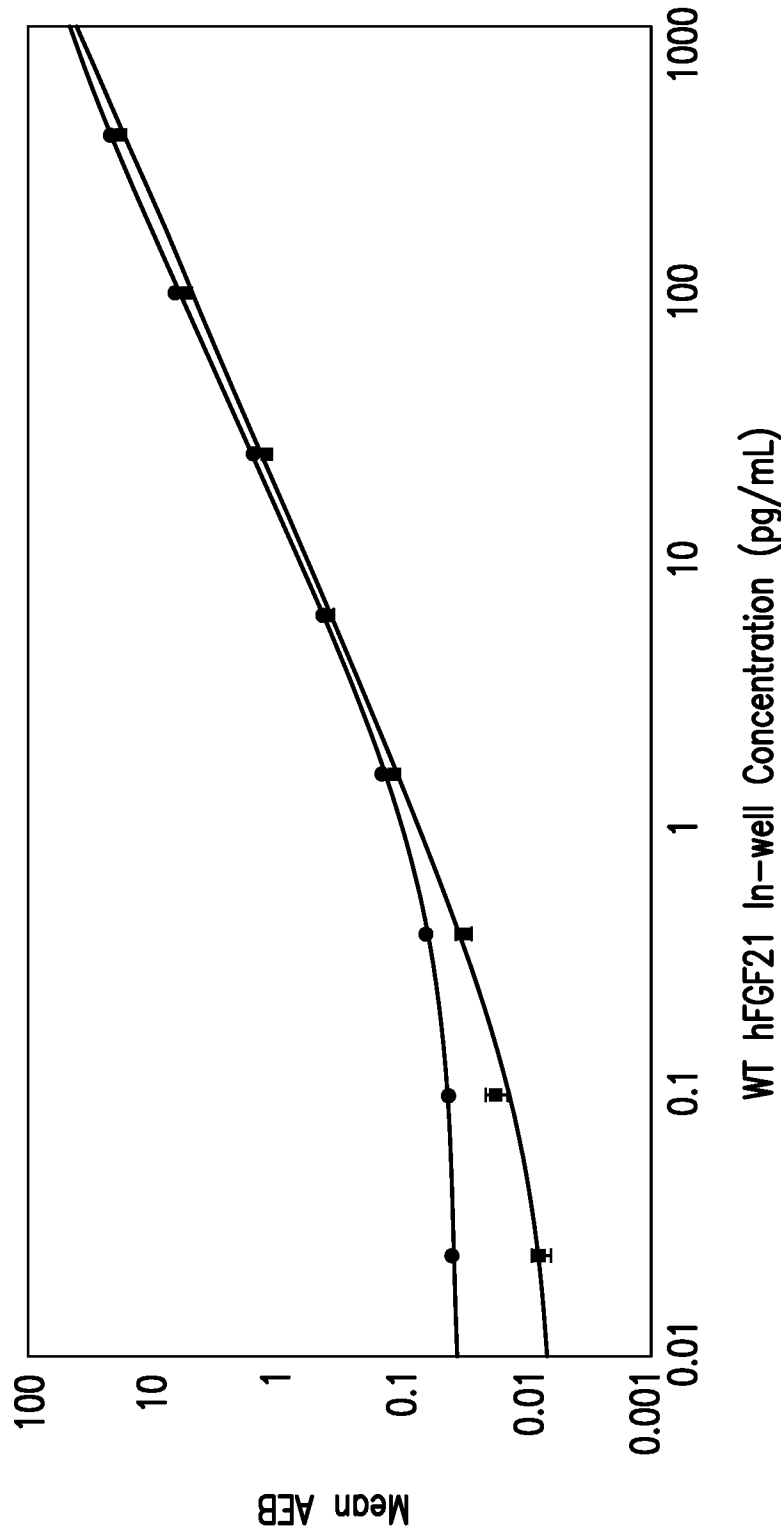


FIGURE 40

FIGURE 41A

Kabat number	CDR L3-Contact																			
	CDR L3-Choitia																			
	CDR L3-Kabat																			
	08	18	28	38	48	58	68	78	88	98	108	118	128	138	148	158	168	178	188	198
FGF21.GN36.4.	A	E	D	A	A	T	Y	Y	C	H	Q	Y	H	R	S	P	T	W	T	F
FGF21.GN36.9	P	E	D	F	G	S	Y	Y	C	Q	H	H	Y	D	S	P	.	W	T	F
FGF21.GN36.11	A	E	D	A	A	T	Y	Y	C	Q	Q	W	S	S	N	P	.	R	T	F
FGF21.GN36.15	A	E	D	L	G	V	Y	Y	C	F	Q	G	S	H	V	P	.	Y	T	F
FGF21.GN36.4	A	E	D	A	A	T	Y	Y	C	H	Q	Y	H	R	S	P	T	W	T	F
FGF21.GN36.9	P	E	D	F	G	S	Y	Y	C	Q	H	H	Y	D	S	P	.	W	T	F
FGF21.GN36.11	A	E	D	A	A	T	Y	Y	C	Q	Q	W	S	S	N	P	.	R	T	F
FGF21.GN36.15	A	E	D	L	G	V	Y	Y	C	F	Q	G	S	H	V	P	.	Y	T	F

FIGURE 41A continued

45/46

Heavy chain variable region																																											
CDR H1-Contact																CDR H1-Choithia																											
CDR H1-Kabat																																											
Kabat number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	
FGF21.GN36.4.	E	V	K	L	V	E	S	G	G	D	L	V	K	P	G	G	S	L	K	L	S	C	A	A	S	G	F	T	F	S	S	Y	G	M	S	W	V	R	Q	T	P	D	
FGF21.GN36.9	E	V	Q	L	Q	Q	S	G	P	E	L	V	K	P	G	A	S	V	K	I	P	C	K	A	S	G	Y	T	F	T	D	Y	Y	M	G	W	V	K	Q	S	H	G	
FGF21.GN36.11	Q	V	Q	L	Q	Q	S	G	A	E	L	A	R	P	G	A	S	V	K	L	S	C	K	A	S	G	Y	T	F	T	N	Y	G	I	S	W	V	K	Q	R	T	G	
FGF21.GN36.15	Q	V	Q	L	I	Q	S	G	P	G	L	V	Q	P	S	Q	S	L	S	I	T	C	T	V	S	G	F	S	L	T	G	Y	A	I	H	W	V	R	Q	S	P	G	
FGF21.GN36.4	E	V	K	L	V	E	S	G	G	D	L	V	K	P	G	G	S	L	K	L	S	C	A	A	S	G	F	T	F	S	S	Y	G	M	S	W	V	R	Q	T	P	D	
FGF21.GN36.9	E	V	Q	L	Q	Q	S	G	P	E	L	V	K	P	G	A	S	V	K	I	P	C	K	A	S	G	Y	T	F	T	D	Y	Y	M	G	W	V	K	Q	S	H	G	
FGF21.GN36.11	Q	V	Q	L	Q	Q	S	G	A	E	L	A	R	P	G	A	S	V	K	L	S	C	K	A	S	G	Y	T	F	T	N	Y	G	I	S	W	V	K	Q	R	T	G	
FGF21.GN36.15	Q	V	Q	L	I	Q	S	G	P	G	L	V	Q	P	S	Q	S	L	S	I	T	C	T	V	S	G	F	S	L	T	G	Y	A	I	H	W	V	R	Q	S	P	G	
CDR H2-Contact																																											
CDR H2-Choithia																CDR H2-Kabat																											
Kabat number	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83		
FGF21.GN36.4.	K	R	L	E	W	V	A	T	I	S	T	G	G	G	Y	T	Y	P	D	S	V	K	K	G	R	F	T	I	S	R	D	N	A	K	N	T	L	Y	L	Q	M	S	
FGF21.GN36.9	K	S	L	E	W	I	G	D	I	N	P	N	G	V	T	I	N	Q	N	S	N	F	K	K	G	R	F	T	I	S	R	D	N	A	K	N	T	L	Y	L	Q	M	S
FGF21.GN36.11	Q	G	L	E	W	I	G	E	I	Y	P	R	S	D	N	T	Y	N	E	K	F	K	K	G	R	F	T	I	S	R	D	N	A	K	N	T	L	Y	L	Q	M	S	
FGF21.GN36.15	K	G	L	E	W	L	G	M	I	W	.	K	S	G	N	T	D	Y	N	A	F	M	S	R	L	S	I	T	K	D	N	S	K	S	Q	V	F	F	K	M	N		
FGF21.GN36.4	K	R	L	E	W	V	A	T	I	S	T	G	G	G	Y	T	Y	P	D	S	V	K	K	G	R	F	T	I	S	R	D	N	A	K	N	T	L	Y	L	Q	M	S	
FGF21.GN36.9	K	S	L	E	W	I	G	D	I	N	P	N	G	V	T	I	N	Q	N	S	N	F	K	K	G	R	F	T	I	S	R	D	N	A	K	N	T	L	Y	L	Q	M	S
FGF21.GN36.11	Q	G	L	E	W	I	G	E	I	Y	P	R	S	D	N	T	Y	N	E	K	F	K	K	G	R	F	T	I	S	R	D	N	A	K	N	T	L	Y	L	Q	M	S	
FGF21.GN36.15	K	G	L	E	W	L	G	M	I	W	.	K	S	G	N	T	D	Y	N	A	F	M	S	R	L	S	I	T	K	D	N	S	K	S	Q	V	F	F	K	M	N		

FIGURE 41B

46/46

Kabat number	CDR H3-Contact																																		
	CDR H3-Choitia												CDR H3-Kabat																						
82b	82c	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	100a	100b	101	102	103	104	105	106	107	108	109	110	111	112	113	
FGF21.GN36.4.	S	L	R	S	E	D	T	A	M	Y	Y	C	A	R	H	D	L	V	D	W	Y	F	D	V	W	G	T	G	T	T	V	T	V	S	S
FGF21.GN36.9	S	L	A	S	E	D	T	A	V	Y	Y	C	T	R	G	Y	G	A	L	.	.	D	Y	W	G	Q	G	T	S	V	T	V	S	S	
FGF21.GN36.11	S	L	T	S	E	D	S	A	V	Y	F	C	T	R	S	D	Y	G	F	.	.	D	Y	W	G	Q	G	T	T	L	T	V	S	S	
FGF21.GN36.15	S	L	Q	A	D	D	T	A	I	Y	Y	C	A	R	N	G	Y	D	Y	E	F	.	V	Y	W	G	Q	G	T	L	V	T	V	S	A
FGF21.GN36.4	S	L	R	S	E	D	T	A	M	Y	Y	C	A	R	H	D	L	V	D	W	Y	F	D	V	W	G	T	G	T	T	V	T	V	S	S
FGF21.GN36.9	S	L	A	S	E	D	T	A	V	Y	Y	C	T	R	G	Y	G	A	L	.	.	D	Y	W	G	Q	G	T	S	V	T	V	S	S	
FGF21.GN36.11	S	L	T	S	E	D	S	A	V	Y	F	C	T	R	S	D	Y	G	F	.	.	D	Y	W	G	Q	G	T	T	L	T	V	S	S	
FGF21.GN36.15	S	L	Q	A	D	D	T	A	I	Y	Y	C	A	R	N	G	Y	D	Y	E	F	.	V	Y	W	G	Q	G	T	L	V	T	V	S	S

FIGURE 41B continued

00B206_0809_SL.TXT
SEQUENCE LISTING

<110> GENENTECH, INC.

<120> METHODS FOR DETECTING AND QUANTIFYING FGF21

<130> 00B206.0809

<150> 62/652,701

<151> 2018-04-04

<160> 77

<170> PatentIn version 3.5

<210> 1

<211> 181

<212> PRT

<213> Homo sapiens

<400> 1

His	Pro	Ile	Pro	Asp	Ser	Ser	Pro	Leu	Leu	Gln	Phe	Gly	Gly	Gln	Val
1				5					10					15	

Arg	Gln	Arg	Tyr	Leu	Tyr	Thr	Asp	Asp	Ala	Gln	Gln	Thr	Glu	Ala	His
			20					25					30		

Leu	Glu	Ile	Arg	Glu	Asp	Gly	Thr	Val	Gly	Gly	Ala	Ala	Asp	Gln	Ser
		35					40					45			

Pro	Glu	Ser	Leu	Leu	Gln	Leu	Lys	Ala	Leu	Lys	Pro	Gly	Val	Ile	Gln
	50					55					60				

Ile	Leu	Gly	Val	Lys	Thr	Ser	Arg	Phe	Leu	Cys	Gln	Arg	Pro	Asp	Gly
65					70					75					80

Ala	Leu	Tyr	Gly	Ser	Leu	His	Phe	Asp	Pro	Glu	Ala	Cys	Ser	Phe	Arg
				85					90					95	

Glu	Leu	Leu	Leu	Glu	Asp	Gly	Tyr	Asn	Val	Tyr	Gln	Ser	Glu	Ala	His
			100					105					110		

Gly	Leu	Pro	Leu	His	Leu	Pro	Gly	Asn	Lys	Ser	Pro	His	Arg	Asp	Pro
		115					120					125			

00B206_0809_SL.TXT

Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro
130 135 140

Ala Leu Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val
145 150 155 160

Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg Ser
165 170 175

Pro Ser Tyr Ala Ser
180

<210> 2

<211> 194

<212> PRT

<213> Homo sapiens

<400> 2

Arg Pro Leu Ala Phe Ser Asp Ala Gly Pro His Val His Tyr Gly Trp
1 5 10 15

Gly Asp Pro Ile Arg Leu Arg His Leu Tyr Thr Ser Gly Pro His Gly
20 25 30

Leu Ser Ser Cys Phe Leu Arg Ile Arg Ala Asp Gly Val Val Asp Cys
35 40 45

Ala Arg Gly Gln Ser Ala His Ser Leu Leu Glu Ile Lys Ala Val Ala
50 55 60

Leu Arg Thr Val Ala Ile Lys Gly Val His Ser Val Arg Tyr Leu Cys
65 70 75 80

Met Gly Ala Asp Gly Lys Met Gln Gly Leu Leu Gln Tyr Ser Glu Glu
85 90 95

Asp Cys Ala Phe Glu Glu Glu Ile Arg Pro Asp Gly Tyr Asn Val Tyr
100 105 110

Arg Ser Glu Lys His Arg Leu Pro Val Ser Leu Ser Ser Ala Lys Gln

115

120

125

Arg Gln Leu Tyr Lys Asn Arg Gly Phe Leu Pro Leu Ser His Phe Leu
 130 135 140

Pro Met Leu Pro Met Val Pro Glu Glu Pro Glu Asp Leu Arg Gly His
 145 150 155 160

Leu Glu Ser Asp Met Phe Ser Ser Pro Leu Glu Thr Asp Ser Met Asp
 165 170 175

Pro Phe Gly Leu Val Thr Gly Leu Glu Ala Val Arg Ser Pro Ser Phe
 180 185 190

Glu Lys

<210> 3

<211> 192

<212> PRT

<213> Homo sapiens

<400> 3

His Pro Ile Pro Asp Ser Ser Pro His Val His Tyr Gly Trp Gly Asp
 1 5 10 15

Pro Ile Arg Leu Arg His Leu Tyr Thr Ser Gly Pro His Gly Leu Ser
 20 25 30

Ser Cys Phe Leu Arg Ile Arg Ala Asp Gly Val Val Asp Cys Ala Arg
 35 40 45

Gly Gln Ser Ala His Ser Leu Leu Glu Ile Lys Ala Val Ala Leu Arg
 50 55 60

Thr Val Ala Ile Lys Gly Val His Ser Val Arg Tyr Leu Cys Met Gly
 65 70 75 80

Ala Asp Gly Lys Met Gln Gly Leu Leu Gln Tyr Ser Glu Glu Asp Cys
 85 90 95

00B206_0809_SL.TXT

Ala Phe Glu Glu Glu Ile Arg Pro Asp Gly Tyr Asn Val Tyr Arg Ser
100 105 110

Glu Lys His Arg Leu Pro Val Ser Leu Ser Ser Ala Lys Gln Arg Gln
115 120 125

Leu Tyr Lys Asn Arg Gly Phe Leu Pro Leu Ser His Phe Leu Pro Met
130 135 140

Leu Pro Met Val Pro Glu Glu Pro Glu Asp Leu Arg Gly His Leu Glu
145 150 155 160

Ser Asp Met Phe Ser Ser Pro Leu Glu Thr Asp Ser Met Asp Pro Phe
165 170 175

Gly Leu Val Thr Gly Leu Glu Ala Val Arg Ser Pro Ser Phe Glu Lys
180 185 190

<210> 4

<211> 189

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 4

His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val
1 5 10 15

Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His
20 25 30

Leu Glu Ile Arg Ala Asp Gly Val Val Asp Cys Ala Arg Gly Gln Ser
35 40 45

Ala His Ser Leu Leu Glu Ile Lys Ala Val Ala Leu Arg Thr Val Ala
50 55 60

00B206_0809_SL.TXT

Ile Lys Gly Val His Ser Val Arg Tyr Leu Cys Met Gly Ala Asp Gly
65 70 75 80

Lys Met Gln Gly Leu Leu Gln Tyr Ser Glu Glu Asp Cys Ala Phe Glu
85 90 95

Glu Glu Ile Arg Pro Asp Gly Tyr Asn Val Tyr Arg Ser Glu Lys His
100 105 110

Arg Leu Pro Val Ser Leu Ser Ser Ala Lys Gln Arg Gln Leu Tyr Lys
115 120 125

Asn Arg Gly Phe Leu Pro Leu Ser His Phe Leu Pro Met Leu Pro Met
130 135 140

Val Pro Glu Glu Pro Glu Asp Leu Arg Gly His Leu Glu Ser Asp Met
145 150 155 160

Phe Ser Ser Pro Leu Glu Thr Asp Ser Met Asp Pro Phe Gly Leu Val
165 170 175

Thr Gly Leu Glu Ala Val Arg Ser Pro Ser Phe Glu Lys
180 185

<210> 5

<211> 189

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 5

His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val
1 5 10 15

Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His
20 25 30

Leu Glu Ile Arg Glu Asp Gly Val Val Asp Cys Ala Arg Gly Gln Ser

35

40

45

Ala His Ser Leu Leu Glu Ile Lys Ala Val Ala Leu Arg Thr Val Ala
 50 55 60

Ile Lys Gly Val His Ser Val Arg Tyr Leu Cys Met Gly Ala Asp Gly
 65 70 75 80

Lys Met Gln Gly Leu Leu Gln Tyr Ser Glu Glu Asp Cys Ala Phe Glu
 85 90 95

Glu Glu Ile Arg Pro Asp Gly Tyr Asn Val Tyr Arg Ser Glu Lys His
 100 105 110

Arg Leu Pro Val Ser Leu Ser Ser Ala Lys Gln Arg Gln Leu Tyr Lys
 115 120 125

Asn Arg Gly Phe Leu Pro Leu Ser His Phe Leu Pro Met Leu Pro Met
 130 135 140

Val Pro Glu Glu Pro Glu Asp Leu Arg Gly His Leu Glu Ser Asp Met
 145 150 155 160

Phe Ser Ser Pro Leu Glu Thr Asp Ser Met Asp Pro Phe Gly Leu Val
 165 170 175

Thr Gly Leu Glu Ala Val Arg Ser Pro Ser Phe Glu Lys
 180 185

<210> 6

<211> 189

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic
 polypeptide"

<400> 6

His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val
 1 5 10 15

Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His
20 25 30

Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser
35 40 45

Ala His Ser Leu Leu Glu Ile Lys Ala Val Ala Leu Arg Thr Val Ala
50 55 60

Ile Lys Gly Val His Ser Val Arg Tyr Leu Cys Met Gly Ala Asp Gly
65 70 75 80

Lys Met Gln Gly Leu Leu Gln Tyr Ser Glu Glu Asp Cys Ala Phe Glu
85 90 95

Glu Glu Ile Arg Pro Asp Gly Tyr Asn Val Tyr Arg Ser Glu Lys His
100 105 110

Arg Leu Pro Val Ser Leu Ser Ser Ala Lys Gln Arg Gln Leu Tyr Lys
115 120 125

Asn Arg Gly Phe Leu Pro Leu Ser His Phe Leu Pro Met Leu Pro Met
130 135 140

Val Pro Glu Glu Pro Glu Asp Leu Arg Gly His Leu Glu Ser Asp Met
145 150 155 160

Phe Ser Ser Pro Leu Glu Thr Asp Ser Met Asp Pro Phe Gly Leu Val
165 170 175

Thr Gly Leu Glu Ala Val Arg Ser Pro Ser Phe Glu Lys
180 185

<210> 7

<211> 189

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 7

His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val
 1 5 10 15

Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His
 20 25 30

Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser
 35 40 45

Pro Glu Ser Leu Leu Glu Ile Lys Ala Val Ala Leu Arg Thr Val Ala
 50 55 60

Ile Lys Gly Val His Ser Val Arg Tyr Leu Cys Met Gly Ala Asp Gly
 65 70 75 80

Lys Met Gln Gly Leu Leu Gln Tyr Ser Glu Glu Asp Cys Ala Phe Glu
 85 90 95

Glu Glu Ile Arg Pro Asp Gly Tyr Asn Val Tyr Arg Ser Glu Lys His
 100 105 110

Arg Leu Pro Val Ser Leu Ser Ser Ala Lys Gln Arg Gln Leu Tyr Lys
 115 120 125

Asn Arg Gly Phe Leu Pro Leu Ser His Phe Leu Pro Met Leu Pro Met
 130 135 140

Val Pro Glu Glu Pro Glu Asp Leu Arg Gly His Leu Glu Ser Asp Met
 145 150 155 160

Phe Ser Ser Pro Leu Glu Thr Asp Ser Met Asp Pro Phe Gly Leu Val
 165 170 175

Thr Gly Leu Glu Ala Val Arg Ser Pro Ser Phe Glu Lys
 180 185

<210> 8

<211> 189

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 8

His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val
 1 5 10 15

Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His
 20 25 30

Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser
 35 40 45

Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln
 50 55 60

Ile Leu Gly Val His Ser Val Arg Tyr Leu Cys Met Gly Ala Asp Gly
 65 70 75 80

Lys Met Gln Gly Leu Leu Gln Tyr Ser Glu Glu Asp Cys Ala Phe Glu
 85 90 95

Glu Glu Ile Arg Pro Asp Gly Tyr Asn Val Tyr Arg Ser Glu Lys His
 100 105 110

Arg Leu Pro Val Ser Leu Ser Ser Ala Lys Gln Arg Gln Leu Tyr Lys
 115 120 125

Asn Arg Gly Phe Leu Pro Leu Ser His Phe Leu Pro Met Leu Pro Met
 130 135 140

Val Pro Glu Glu Pro Glu Asp Leu Arg Gly His Leu Glu Ser Asp Met
 145 150 155 160

00B206_0809_SL.TXT

Phe Ser Ser Pro Leu Glu Thr Asp Ser Met Asp Pro Phe Gly Leu Val
165 170 175

Thr Gly Leu Glu Ala Val Arg Ser Pro Ser Phe Glu Lys
180 185

<210> 9

<211> 189

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 9

His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val
1 5 10 15

Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His
20 25 30

Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser
35 40 45

Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln
50 55 60

Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Met Gly Ala Asp Gly
65 70 75 80

Lys Met Gln Gly Leu Leu Gln Tyr Ser Glu Glu Asp Cys Ala Phe Glu
85 90 95

Glu Glu Ile Arg Pro Asp Gly Tyr Asn Val Tyr Arg Ser Glu Lys His
100 105 110

Arg Leu Pro Val Ser Leu Ser Ser Ala Lys Gln Arg Gln Leu Tyr Lys
115 120 125

Asn Arg Gly Phe Leu Pro Leu Ser His Phe Leu Pro Met Leu Pro Met

130

135

140

Val Pro Glu Glu Pro Glu Asp Leu Arg Gly His Leu Glu Ser Asp Met
 145 150 155 160

Phe Ser Ser Pro Leu Glu Thr Asp Ser Met Asp Pro Phe Gly Leu Val
 165 170 175

Thr Gly Leu Glu Ala Val Arg Ser Pro Ser Phe Glu Lys
 180 185

<210> 10

<211> 187

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic
 polypeptide"

<400> 10

His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val
 1 5 10 15

Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His
 20 25 30

Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser
 35 40 45

Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln
 50 55 60

Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly
 65 70 75 80

Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg
 85 90 95

Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His
 100 105 110

Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro
115 120 125

Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Met Leu Pro Met Val Pro
130 135 140

Glu Glu Pro Glu Asp Leu Arg Gly His Leu Glu Ser Asp Met Phe Ser
145 150 155 160

Ser Pro Leu Glu Thr Asp Ser Met Asp Pro Phe Gly Leu Val Thr Gly
165 170 175

Leu Glu Ala Val Arg Ser Pro Ser Phe Glu Lys
180 185

<210> 11
<211> 183
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 11
Arg Pro Leu Ala Phe Ser Asp Ala Gly Pro Leu Leu Gln Phe Gly Gly
1 5 10 15

Gln Val Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu
20 25 30

Ala His Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp
35 40 45

Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val
50 55 60

Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro
65 70 75 80

00B206_0809_SL.TXT

Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser
85 90 95

Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu
100 105 110

Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg
115 120 125

Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu
130 135 140

Pro Pro Ala Leu Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro
145 150 155 160

Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro Ser Gln Gly
165 170 175

Arg Ser Pro Ser Tyr Ala Ser
180

<210> 12

<211> 186

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 12

Arg Pro Leu Ala Phe Ser Asp Ala Gly Pro His Val His Tyr Gly Trp
1 5 10 15

Gly Asp Pro Ile Arg Leu Arg His Leu Tyr Thr Ser Gly Pro His Gly
20 25 30

Leu Ser Ser Cys Phe Leu Arg Ile Arg Glu Asp Gly Thr Val Gly Gly
35 40 45

00B206_0809_SL.TXT

Ala Ala Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys
50 55 60

Pro Gly Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys
65 70 75 80

Gln Arg Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu
85 90 95

Ala Cys Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr
100 105 110

Gln Ser Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser
115 120 125

Pro His Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu
130 135 140

Pro Gly Leu Pro Pro Ala Leu Pro Glu Pro Pro Gly Ile Leu Ala Pro
145 150 155 160

Gln Pro Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro
165 170 175

Ser Gln Gly Arg Ser Pro Ser Tyr Ala Ser
180 185

<210> 13

<211> 186

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 13

Arg Pro Leu Ala Phe Ser Asp Ala Gly Pro His Val His Tyr Gly Trp
1 5 10 15

Gly Asp Pro Ile Arg Leu Arg His Leu Tyr Thr Ser Gly Pro His Gly

20

25

30

Leu Ser Ser Cys Phe Leu Arg Ile Arg Ala Asp Gly Thr Val Gly Gly
 35 40 45

Ala Ala Asp Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys
 50 55 60

Pro Gly Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys
 65 70 75 80

Gln Arg Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu
 85 90 95

Ala Cys Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr
 100 105 110

Gln Ser Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser
 115 120 125

Pro His Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu
 130 135 140

Pro Gly Leu Pro Pro Ala Leu Pro Glu Pro Pro Gly Ile Leu Ala Pro
 145 150 155 160

Gln Pro Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro
 165 170 175

Ser Gln Gly Arg Ser Pro Ser Tyr Ala Ser
 180 185

<210> 14

<211> 186

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic
 polypeptide"

<400> 14

Arg Pro Leu Ala Phe Ser Asp Ala Gly Pro His Val His Tyr Gly Trp
 1 5 10 15

Gly Asp Pro Ile Arg Leu Arg His Leu Tyr Thr Ser Gly Pro His Gly
 20 25 30

Leu Ser Ser Cys Phe Leu Arg Ile Arg Ala Asp Gly Val Val Asp Cys
 35 40 45

Ala Arg Gly Gln Ser Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys
 50 55 60

Pro Gly Val Ile Gln Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys
 65 70 75 80

Gln Arg Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu
 85 90 95

Ala Cys Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr
 100 105 110

Gln Ser Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser
 115 120 125

Pro His Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu
 130 135 140

Pro Gly Leu Pro Pro Ala Leu Pro Glu Pro Pro Gly Ile Leu Ala Pro
 145 150 155 160

Gln Pro Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro
 165 170 175

Ser Gln Gly Arg Ser Pro Ser Tyr Ala Ser
 180 185

<210> 15

<211> 186

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 15

Arg	Pro	Leu	Ala	Phe	Ser	Asp	Ala	Gly	Pro	His	Val	His	Tyr	Gly	Trp
1				5					10					15	

Gly	Asp	Pro	Ile	Arg	Leu	Arg	His	Leu	Tyr	Thr	Ser	Gly	Pro	His	Gly
			20					25					30		

Leu	Ser	Ser	Cys	Phe	Leu	Arg	Ile	Arg	Ala	Asp	Gly	Val	Val	Asp	Cys
		35					40					45			

Ala	Arg	Gly	Gln	Ser	Ala	His	Ser	Leu	Leu	Gln	Leu	Lys	Ala	Leu	Lys
	50					55					60				

Pro	Gly	Val	Ile	Gln	Ile	Leu	Gly	Val	Lys	Thr	Ser	Arg	Phe	Leu	Cys
65					70					75					80

Gln	Arg	Pro	Asp	Gly	Ala	Leu	Tyr	Gly	Ser	Leu	His	Phe	Asp	Pro	Glu
				85					90					95	

Ala	Cys	Ser	Phe	Arg	Glu	Leu	Leu	Leu	Glu	Asp	Gly	Tyr	Asn	Val	Tyr
			100					105					110		

Gln	Ser	Glu	Ala	His	Gly	Leu	Pro	Leu	His	Leu	Pro	Gly	Asn	Lys	Ser
		115					120					125			

Pro	His	Arg	Asp	Pro	Ala	Pro	Arg	Gly	Pro	Ala	Arg	Phe	Leu	Pro	Leu
	130					135					140				

Pro	Gly	Leu	Pro	Pro	Ala	Leu	Pro	Glu	Pro	Pro	Gly	Ile	Leu	Ala	Pro
145					150					155					160

Gln	Pro	Pro	Asp	Val	Gly	Ser	Ser	Asp	Pro	Leu	Ser	Met	Val	Gly	Pro
				165					170					175	

Ser Gln Gly Arg Ser Pro Ser Tyr Ala Ser
 180 185

<210> 16
 <211> 186
 <212> PRT
 <213> Artificial Sequence

<220>
 <221> source
 <223> /note="Description of Artificial Sequence: Synthetic
 polypeptide"

<400> 16
 Arg Pro Leu Ala Phe Ser Asp Ala Gly Pro His Val His Tyr Gly Trp
 1 5 10 15

Gly Asp Pro Ile Arg Leu Arg His Leu Tyr Thr Ser Gly Pro His Gly
 20 25 30

Leu Ser Ser Cys Phe Leu Arg Ile Arg Ala Asp Gly Val Val Asp Cys
 35 40 45

Ala Arg Gly Gln Ser Ala His Ser Leu Leu Glu Ile Lys Ala Val Ala
 50 55 60

Leu Arg Thr Val Ala Ile Lys Gly Val His Ser Val Arg Tyr Leu Cys
 65 70 75 80

Gln Arg Pro Asp Gly Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu
 85 90 95

Ala Cys Ser Phe Arg Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr
 100 105 110

Gln Ser Glu Ala His Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser
 115 120 125

Pro His Arg Asp Pro Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu
 130 135 140

00B206_0809_SL.TXT

Pro Gly Leu Pro Pro Ala Leu Pro Glu Pro Pro Gly Ile Leu Ala Pro
145 150 155 160

Gln Pro Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro
165 170 175

Ser Gln Gly Arg Ser Pro Ser Tyr Ala Ser
180 185

<210> 17

<211> 188

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 17

Arg Pro Leu Ala Phe Ser Asp Ala Gly Pro His Val His Tyr Gly Trp
1 5 10 15

Gly Asp Pro Ile Arg Leu Arg His Leu Tyr Thr Ser Gly Pro His Gly
20 25 30

Leu Ser Ser Cys Phe Leu Arg Ile Arg Ala Asp Gly Val Val Asp Cys
35 40 45

Ala Arg Gly Gln Ser Ala His Ser Leu Leu Glu Ile Lys Ala Val Ala
50 55 60

Leu Arg Thr Val Ala Ile Lys Gly Val His Ser Val Arg Tyr Leu Cys
65 70 75 80

Met Gly Ala Asp Gly Lys Met Gln Gly Leu Leu Gln Tyr Ser Glu Glu
85 90 95

Asp Cys Ala Phe Glu Glu Glu Ile Arg Pro Asp Gly Tyr Asn Val Tyr
100 105 110

Arg Ser Glu Lys His Arg Leu Pro Val Ser Leu Ser Ser Ala Lys Gln

115

120

125

Arg Gln Leu Tyr Lys Asn Arg Gly Phe Leu Pro Leu Ser His Phe Leu
 130 135 140

Pro Leu Pro Gly Leu Pro Pro Ala Leu Pro Glu Pro Pro Gly Ile Leu
 145 150 155 160

Ala Pro Gln Pro Pro Asp Val Gly Ser Ser Asp Pro Leu Ser Met Val
 165 170 175

Gly Pro Ser Gln Gly Arg Ser Pro Ser Tyr Ala Ser
 180 185

<210> 18

<211> 217

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic
 polypeptide"

<400> 18

Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Pro Leu Gly
 1 5 10 15

Glu Arg Val Thr Met Thr Cys Thr Ala Ser Ser Ser Val Ser Ser Ser
 20 25 30

Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Val Trp
 35 40 45

Ile Tyr Arg Thr Thr Asn Leu Ala Ser Gly Val Pro Thr Arg Phe Ser
 50 55 60

Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu
 65 70 75 80

Ala Glu Asp Ala Ala Thr Tyr Tyr Cys His Gln Tyr His Arg Ser Pro
 85 90 95

Pro Thr Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala
100 105 110

Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu
115 120 125

Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr Pro
130 135 140

Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln Asn
145 150 155 160

Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr Tyr
165 170 175

Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg His
180 185 190

Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro Ile
195 200 205

Val Lys Ser Phe Asn Arg Asn Glu Cys
210 215

<210> 19
<211> 214
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 19
Asp Ile Gln Met Thr Gln Ser Pro Ala Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Glu Thr Val Ile Ile Thr Cys Arg Ala Ser Glu Asn Ile Tyr Ser Tyr
20 25 30

00B206_0809_SL.TXT

Leu Ala Trp Tyr Gln Gln Lys Gln Gly Lys Ser Pro Gln Leu Leu Val
35 40 45

Tyr Asn Ile Arg Thr Leu Ala Glu Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Gln Phe Ser Leu Lys Ile Asn Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Gly Ser Tyr Tyr Cys Gln His His Tyr Asp Ser Pro Trp
85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala Asp Ala Ala
100 105 110

Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu Thr Ser Gly
115 120 125

Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr Pro Lys Asp Ile
130 135 140

Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln Asn Gly Val Leu
145 150 155 160

Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr Tyr Ser Met Ser
165 170 175

Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg His Asn Ser Tyr
180 185 190

Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro Ile Val Lys Ser
195 200 205

Phe Asn Arg Asn Glu Cys
210

<210> 20
<211> 213
<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 20

Gln Ile Val Leu Thr Gln Ser Pro Ala Leu Met Ser Ala Ser Pro Gly
1 5 10 15

Glu Arg Val Thr Met Thr Cys Ser Ala Gly Ser Ser Val Ser Tyr Met
20 25 30

Tyr Trp Tyr Gln Gln Lys Pro Arg Ser Ser Pro Lys Pro Trp Ile Tyr
35 40 45

Leu Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu
65 70 75 80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Arg Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala Asp Ala Ala Pro
100 105 110

Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu Thr Ser Gly Gly
115 120 125

Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr Pro Lys Asp Ile Asn
130 135 140

Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln Asn Gly Val Leu Asn
145 150 155 160

Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr Tyr Ser Met Ser Ser
165 170 175

00B206_0809_SL.TXT

Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg His Asn Ser Tyr Thr
180 185 190

Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro Ile Val Lys Ser Phe
195 200 205

Asn Arg Asn Glu Cys
210

<210> 21
<211> 219
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 21
Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
1 5 10 15

Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ile Ile Val His Asn
20 25 30

Asn Gly Asp Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Lys Leu Leu Ile Tyr Lys Ile Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu

115

120

125

Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe
 130 135 140

Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg
 145 150 155 160

Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser
 165 170 175

Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu
 180 185 190

Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser
 195 200 205

Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
 210 215

<210> 22

<211> 449

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic
 polypeptide"

<400> 22

Glu Val Lys Leu Val Glu Ser Gly Gly Asp Leu Val Lys Pro Gly Gly
 1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Gly Met Ser Trp Val Arg Gln Thr Pro Asp Lys Arg Leu Glu Trp Val
 35 40 45

Ala Thr Ile Ser Thr Gly Gly Gly Tyr Thr Tyr Tyr Pro Asp Ser Val
 50 55 60

00B206_0809_SL.TXT

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Ser Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Ala Arg His Asp Leu Val Asp Trp Tyr Phe Asp Val Trp Gly Thr Gly
100 105 110

Thr Thr Val Thr Val Ser Ser Ala Lys Thr Thr Ala Pro Ser Val Tyr
115 120 125

Pro Leu Ala Pro Val Cys Gly Asp Thr Thr Gly Ser Ser Val Thr Leu
130 135 140

Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Leu Thr Trp
145 150 155 160

Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175

Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Thr Ser Ser
180 185 190

Thr Trp Pro Ser Gln Ser Ile Thr Cys Asn Val Ala His Pro Ala Ser
195 200 205

Ser Thr Lys Val Asp Lys Lys Ile Glu Pro Arg Gly Pro Thr Ile Lys
210 215 220

Pro Cys Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Leu Gly Gly Pro
225 230 235 240

Ser Val Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu Met Ile Ser
245 250 255

Leu Ser Pro Ile Val Thr Cys Val Val Val Asp Val Ser Glu Asp Asp
260 265 270

Pro Asp Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu Val His Thr
 275 280 285

Ala Gln Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Leu Arg Val
 290 295 300

Val Ser Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly Lys Glu
 305 310 315 320

Phe Lys Cys Lys Val Asn Asn Lys Asp Leu Pro Ala Pro Ile Glu Arg
 325 330 335

Thr Ile Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln Val Tyr Val
 340 345 350

Leu Pro Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val Thr Leu Thr
 355 360 365

Cys Met Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val Glu Trp Thr
 370 375 380

Asn Asn Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu Pro Val Leu
 385 390 395 400

Asp Ser Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg Val Glu Lys
 405 410 415

Lys Asn Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val Val His Glu
 420 425 430

Gly Leu His Asn His His Thr Thr Lys Ser Phe Ser Arg Thr Pro Gly
 435 440 445

Lys

<210> 23
 <211> 447

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 23

Glu	Val	Gln	Leu	Gln	Gln	Ser	Gly	Pro	Glu	Leu	Val	Lys	Pro	Gly	Ala
1			5					10						15	

Ser	Val	Lys	Ile	Pro	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Asp	Tyr
		20					25						30		

Tyr	Met	Gly	Trp	Val	Lys	Gln	Ser	His	Gly	Lys	Ser	Leu	Glu	Trp	Ile
		35				40						45			

Gly	Asp	Ile	Asn	Pro	Asn	Asn	Gly	Val	Thr	Ile	Asn	Asn	Gln	Asn	Phe
	50					55					60				

Lys	Gly	Lys	Ala	Thr	Leu	Thr	Val	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Tyr
65					70				75					80	

Met	Glu	Leu	Arg	Ser	Leu	Ala	Ser	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
			85						90					95	

Thr	Arg	Gly	Tyr	Gly	Gly	Ala	Leu	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Ser
		100						105					110		

Val	Thr	Val	Ser	Ser	Ala	Lys	Thr	Thr	Ala	Pro	Ser	Val	Tyr	Pro	Leu
		115					120					125			

Ala	Pro	Val	Cys	Gly	Asp	Thr	Thr	Gly	Ser	Ser	Val	Thr	Leu	Gly	Cys
	130					135					140				

Leu	Val	Lys	Gly	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Leu	Thr	Trp	Asn	Ser
145					150					155					160

Gly	Ser	Leu	Ser	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser
				165					170					175	

00B206_0809_SL.TXT

Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Thr Ser Ser Thr Trp
180 185 190

Pro Ser Gln Ser Ile Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr
195 200 205

Lys Val Asp Lys Lys Ile Glu Pro Arg Gly Pro Thr Ile Lys Pro Cys
210 215 220

Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Leu Gly Gly Pro Ser Val
225 230 235 240

Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu Met Ile Ser Leu Ser
245 250 255

Pro Ile Val Thr Cys Val Val Val Asp Val Ser Glu Asp Asp Pro Asp
260 265 270

Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu Val His Thr Ala Gln
275 280 285

Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Leu Arg Val Val Ser
290 295 300

Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly Lys Glu Phe Lys
305 310 315 320

Cys Lys Val Asn Asn Lys Asp Leu Pro Ala Pro Ile Glu Arg Thr Ile
325 330 335

Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln Val Tyr Val Leu Pro
340 345 350

Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val Thr Leu Thr Cys Met
355 360 365

Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val Glu Trp Thr Asn Asn
370 375 380

00B206_0809_SL.TXT

Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu Pro Val Leu Asp Ser
385 390 395 400

Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg Val Glu Lys Lys Asn
405 410 415

Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val Val His Glu Gly Leu
420 425 430

His Asn His His Thr Thr Lys Ser Phe Ser Arg Thr Pro Gly Lys
435 440 445

<210> 24

<211> 447

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 24

Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala
1 5 10 15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Ile Ser Trp Val Lys Gln Arg Thr Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Tyr Pro Arg Ser Asp Asn Thr Tyr Tyr Asn Glu Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
85 90 95

00B206_0809_SL.TXT

Thr Arg Ser Asp Tyr Gly Phe Phe Asp Tyr Trp Gly Gln Gly Thr Thr
100 105 110

Leu Thr Val Ser Ser Ala Lys Thr Thr Ala Pro Ser Val Tyr Pro Leu
115 120 125

Ala Pro Val Cys Gly Asp Thr Thr Gly Ser Ser Val Thr Leu Gly Cys
130 135 140

Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Leu Thr Trp Asn Ser
145 150 155 160

Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
165 170 175

Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Thr Ser Ser Thr Trp
180 185 190

Pro Ser Gln Ser Ile Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr
195 200 205

Lys Val Asp Lys Lys Ile Glu Pro Arg Gly Pro Thr Ile Lys Pro Cys
210 215 220

Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Leu Gly Gly Pro Ser Val
225 230 235 240

Phe Ile Phe Pro Pro Lys Ile Lys Asp Val Leu Met Ile Ser Leu Ser
245 250 255

Pro Ile Val Thr Cys Val Val Val Asp Val Ser Glu Asp Asp Pro Asp
260 265 270

Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu Val His Thr Ala Gln
275 280 285

Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Leu Arg Val Val Ser
290 295 300

00B206_0809_SL.TXT

Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly Lys Glu Phe Lys
305 310 315 320

Cys Lys Val Asn Asn Lys Asp Leu Pro Ala Pro Ile Glu Arg Thr Ile
325 330 335

Ser Lys Pro Lys Gly Ser Val Arg Ala Pro Gln Val Tyr Val Leu Pro
340 345 350

Pro Pro Glu Glu Glu Met Thr Lys Lys Gln Val Thr Leu Thr Cys Met
355 360 365

Val Thr Asp Phe Met Pro Glu Asp Ile Tyr Val Glu Trp Thr Asn Asn
370 375 380

Gly Lys Thr Glu Leu Asn Tyr Lys Asn Thr Glu Pro Val Leu Asp Ser
385 390 395 400

Asp Gly Ser Tyr Phe Met Tyr Ser Lys Leu Arg Val Glu Lys Lys Asn
405 410 415

Trp Val Glu Arg Asn Ser Tyr Ser Cys Ser Val Val His Glu Gly Leu
420 425 430

His Asn His His Thr Thr Lys Ser Phe Ser Arg Thr Pro Gly Lys
435 440 445

<210> 25

<211> 447

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 25

Gln Val Gln Leu Ile Gln Ser Gly Pro Gly Leu Val Gln Pro Ser Gln
1 5 10 15

Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Gly Tyr

20

25

30

Ala Ile His Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Leu
 35 40 45

Gly Met Ile Trp Lys Ser Gly Asn Thr Asp Tyr Asn Ala Ala Phe Met
 50 55 60

Ser Arg Leu Ser Ile Thr Lys Asp Asn Ser Lys Ser Gln Val Phe Phe
 65 70 75 80

Lys Met Asn Ser Leu Gln Ala Asp Asp Thr Ala Ile Tyr Tyr Cys Ala
 85 90 95

Arg Asn Gly Tyr Asp Tyr Glu Phe Val Tyr Trp Gly Gln Gly Thr Leu
 100 105 110

Val Thr Val Ser Ala Ala Lys Thr Thr Ala Pro Ser Val Tyr Pro Leu
 115 120 125

Ala Pro Val Cys Gly Asp Thr Thr Gly Ser Ser Val Thr Leu Gly Cys
 130 135 140

Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Leu Thr Trp Asn Ser
 145 150 155 160

Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
 165 170 175

Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Thr Ser Ser Thr Trp
 180 185 190

Pro Ser Gln Ser Ile Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr
 195 200 205

Lys Val Asp Lys Lys Ile Glu Pro Arg Gly Pro Thr Ile Lys Pro Cys
 210 215 220

Pro Pro Cys Lys Cys Pro Ala Pro Asn Leu Leu Gly Gly Pro Ser Val

His Asn His His Thr Thr Lys Ser Phe Ser Arg Thr Pro Gly Lys

<210> 26
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
peptide"

<400> 26
Ser Tyr Gly Met Ser
1 5

<210> 27
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
peptide"

<400> 27
Asp Tyr Tyr Met Gly
1 5

<210> 28
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
peptide"

<400> 28
Asn Tyr Gly Ile Ser
1 5

<210> 29
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
 <221> source
 <223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 29
 Gly Tyr Ala Ile His
 1 5

<210> 30
 <211> 17
 <212> PRT
 <213> Artificial Sequence

<220>
 <221> source
 <223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 30
 Thr Ile Ser Thr Gly Gly Gly Tyr Thr Tyr Tyr Pro Asp Ser Val Lys
 1 5 10 15

Gly

<210> 31
 <211> 17
 <212> PRT
 <213> Artificial Sequence

<220>
 <221> source
 <223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 31
 Asp Ile Asn Pro Asn Asn Gly Val Thr Ile Asn Asn Gln Asn Phe Lys
 1 5 10 15

Gly

<210> 32
 <211> 17
 <212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 32

Glu	Ile	Tyr	Pro	Arg	Ser	Asp	Asn	Thr	Tyr	Tyr	Asn	Glu	Lys	Phe	Lys
1				5				10						15	

Gly

<210> 33

<211> 16

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 33

Met	Ile	Trp	Lys	Ser	Gly	Asn	Thr	Asp	Tyr	Asn	Ala	Ala	Phe	Met	Ser
1				5				10						15	

<210> 34

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 34

His	Asp	Leu	Val	Asp	Trp	Tyr	Phe	Asp	Val
1				5				10	

<210> 35

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<221> source
 <223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 35
 Gly Tyr Gly Gly Ala Leu Asp Tyr
 1 5

<210> 36
 <211> 8
 <212> PRT
 <213> Artificial Sequence

<220>
 <221> source
 <223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 36
 Ser Asp Tyr Gly Phe Phe Asp Tyr
 1 5

<210> 37
 <211> 9
 <212> PRT
 <213> Artificial Sequence

<220>
 <221> source
 <223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 37
 Asn Gly Tyr Asp Tyr Glu Phe Val Tyr
 1 5

<210> 38
 <211> 12
 <212> PRT
 <213> Artificial Sequence

<220>
 <221> source
 <223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 38
 Thr Ala Ser Ser Ser Val Ser Ser Ser Tyr Leu His
 1 5 10

<210> 39
 <211> 11
 <212> PRT
 <213> Artificial Sequence

<220>
 <221> source
 <223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 39
 Arg Ala Ser Glu Asn Ile Tyr Ser Tyr Leu Ala
 1 5 10

<210> 40
 <211> 10
 <212> PRT
 <213> Artificial Sequence

<220>
 <221> source
 <223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 40
 Ser Ala Gly Ser Ser Val Ser Tyr Met Tyr
 1 5 10

<210> 41
 <211> 16
 <212> PRT
 <213> Artificial Sequence

<220>
 <221> source
 <223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 41
 Arg Ser Ser Gln Ile Ile Val His Asn Asn Gly Asp Thr Tyr Leu Glu
 1 5 10 15

<210> 42
 <211> 7
 <212> PRT
 <213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 42

Arg Thr Thr Asn Leu Ala Ser
1 5

<210> 43

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 43

Asn Ile Arg Thr Leu Ala Glu
1 5

<210> 44

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 44

Leu Thr Ser Asn Leu Ala Ser
1 5

<210> 45

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 45

Lys Ile Ser Asn Arg Phe Ser

1

5

<210> 46

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 46

His Gln Tyr His Arg Ser Pro Pro Thr Trp Thr

1

5

10

<210> 47

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 47

Gln His His Tyr Asp Ser Pro Trp Thr

1

5

<210> 48

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 48

Gln Gln Trp Ser Ser Asn Pro Arg Thr

1

5

<210> 49

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 49

Phe	Gln	Gly	Ser	His	Val	Pro	Tyr	Thr
1				5				

<210> 50

<211> 110

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 50

Gln	Ile	Val	Leu	Thr	Gln	Ser	Pro	Ala	Ile	Met	Ser	Ala	Pro	Leu	Gly
1				5					10					15	

Glu	Arg	Val	Thr	Met	Thr	Cys	Thr	Ala	Ser	Ser	Ser	Val	Ser	Ser	Ser
			20					25					30		

Tyr	Leu	His	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Ser	Ser	Pro	Lys	Val	Trp
		35					40					45			

Ile	Tyr	Arg	Thr	Thr	Asn	Leu	Ala	Ser	Gly	Val	Pro	Thr	Arg	Phe	Ser
	50					55					60				

Gly	Ser	Gly	Ser	Gly	Thr	Ser	Tyr	Ser	Leu	Thr	Ile	Ser	Ser	Met	Glu
65					70					75					80

Ala	Glu	Asp	Ala	Ala	Thr	Tyr	Tyr	Cys	His	Gln	Tyr	His	Arg	Ser	Pro
			85						90					95	

Pro	Thr	Trp	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys
			100					105					110

<210> 51

<211> 107

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 51

Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ala	Ser	Leu	Ser	Ala	Ser	Val	Gly
1				5				10						15	

Glu	Thr	Val	Ile	Ile	Thr	Cys	Arg	Ala	Ser	Glu	Asn	Ile	Tyr	Ser	Tyr
			20					25					30		

Leu	Ala	Trp	Tyr	Gln	Gln	Lys	Gln	Gly	Lys	Ser	Pro	Gln	Leu	Leu	Val
			35				40					45			

Tyr	Asn	Ile	Arg	Thr	Leu	Ala	Glu	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly
	50					55					60				

Ser	Gly	Ser	Gly	Thr	Gln	Phe	Ser	Leu	Lys	Ile	Asn	Ser	Leu	Gln	Pro
65					70					75				80	

Glu	Asp	Phe	Gly	Ser	Tyr	Tyr	Cys	Gln	His	His	Tyr	Asp	Ser	Pro	Trp
				85					90					95	

Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Ile	Lys
			100					105		

<210> 52

<211> 106

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 52

Gln	Ile	Val	Leu	Thr	Gln	Ser	Pro	Ala	Leu	Met	Ser	Ala	Ser	Pro	Gly
1				5					10					15	

00B206_0809_SL.TXT

Glu Arg Val Thr Met Thr Cys Ser Ala Gly Ser Ser Val Ser Tyr Met
20 25 30

Tyr Trp Tyr Gln Gln Lys Pro Arg Ser Ser Pro Lys Pro Trp Ile Tyr
35 40 45

Leu Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu
65 70 75 80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Arg Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 53

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 53

Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
1 5 10 15

Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ile Ile Val His Asn
20 25 30

Asn Gly Asp Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Lys Leu Leu Ile Tyr Lys Ile Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

```
<220>  
<221> source  
<223> /note="Description of Artificial Sequence: Synthetic  
polypeptide"
```

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Thr Ile Ser Thr Gly Gly Gly Tyr Thr Tyr Tyr Pro Asp Ser Val
50 55 60

Leu Gln Met Ser Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Thr Thr Val Thr Val Ser Ser
115

<210> 55
 <211> 117
 <212> PRT
 <213> Artificial Sequence

<220>
 <221> source
 <223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 55
 Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
 1 5 10 15

Ser Val Lys Ile Pro Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
 20 25 30

Tyr Met Gly Trp Val Lys Gln Ser His Gly Lys Ser Leu Glu Trp Ile
 35 40 45

Gly Asp Ile Asn Pro Asn Asn Gly Val Thr Ile Asn Asn Gln Asn Phe
 50 55 60

Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
 65 70 75 80

Met Glu Leu Arg Ser Leu Ala Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Thr Arg Gly Tyr Gly Gly Ala Leu Asp Tyr Trp Gly Gln Gly Thr Ser
 100 105 110

Val Thr Val Ser Ser
 115

<210> 56
 <211> 117
 <212> PRT
 <213> Artificial Sequence

<220>
 <221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 56

Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala
1 5 10 15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Ile Ser Trp Val Lys Gln Arg Thr Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Tyr Pro Arg Ser Asp Asn Thr Tyr Tyr Asn Glu Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
85 90 95

Thr Arg Ser Asp Tyr Gly Phe Phe Asp Tyr Trp Gly Gln Gly Thr Thr
100 105 110

Leu Thr Val Ser Ser
115

<210> 57

<211> 117

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 57

Gln Val Gln Leu Ile Gln Ser Gly Pro Gly Leu Val Gln Pro Ser Gln
1 5 10 15

Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Gly Tyr

20

25

30

Ala Ile His Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Leu
 35 40 45

Gly Met Ile Trp Lys Ser Gly Asn Thr Asp Tyr Asn Ala Ala Phe Met
 50 55 60

Ser Arg Leu Ser Ile Thr Lys Asp Asn Ser Lys Ser Gln Val Phe Phe
 65 70 75 80

Lys Met Asn Ser Leu Gln Ala Asp Asp Thr Ala Ile Tyr Tyr Cys Ala
 85 90 95

Arg Asn Gly Tyr Asp Tyr Glu Phe Val Tyr Trp Gly Gln Gly Thr Leu
 100 105 110

Val Thr Val Ser Ala
 115

<210> 58

<211> 171

<212> PRT

<213> Homo sapiens

<400> 58

His Pro Ile Pro Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val
 1 5 10 15

Arg Gln Arg Tyr Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His
 20 25 30

Leu Glu Ile Arg Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser
 35 40 45

Pro Glu Ser Leu Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln
 50 55 60

Ile Leu Gly Val Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly
 65 70 75 80

00B206_0809_SL.TXT

Ala Leu Tyr Gly Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg
85 90 95

Glu Leu Leu Leu Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His
100 105 110

Gly Leu Pro Leu His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro
115 120 125

Ala Pro Arg Gly Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro
130 135 140

Ala Leu Pro Glu Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val
145 150 155 160

Gly Ser Ser Asp Pro Leu Ser Met Val Gly Pro
165 170

<210> 59

<211> 177

<212> PRT

<213> Homo sapiens

<400> 59

Asp Ser Ser Pro Leu Leu Gln Phe Gly Gly Gln Val Arg Gln Arg Tyr
1 5 10 15

Leu Tyr Thr Asp Asp Ala Gln Gln Thr Glu Ala His Leu Glu Ile Arg
20 25 30

Glu Asp Gly Thr Val Gly Gly Ala Ala Asp Gln Ser Pro Glu Ser Leu
35 40 45

Leu Gln Leu Lys Ala Leu Lys Pro Gly Val Ile Gln Ile Leu Gly Val
50 55 60

Lys Thr Ser Arg Phe Leu Cys Gln Arg Pro Asp Gly Ala Leu Tyr Gly
65 70 75 80

Ser Leu His Phe Asp Pro Glu Ala Cys Ser Phe Arg Glu Leu Leu Leu

85

90

95

Glu Asp Gly Tyr Asn Val Tyr Gln Ser Glu Ala His Gly Leu Pro Leu
 100 105 110

His Leu Pro Gly Asn Lys Ser Pro His Arg Asp Pro Ala Pro Arg Gly
 115 120 125

Pro Ala Arg Phe Leu Pro Leu Pro Gly Leu Pro Pro Ala Leu Pro Glu
 130 135 140

Pro Pro Gly Ile Leu Ala Pro Gln Pro Pro Asp Val Gly Ser Ser Asp
 145 150 155 160

Pro Leu Ser Met Val Gly Pro Ser Gln Gly Arg Ser Pro Ser Tyr Ala
 165 170 175

Ser

<210> 60

<211> 219

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic
 polypeptide"

<400> 60

Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
 1 5 10 15

Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ile Ile Val His Asn
 20 25 30

Asn Gly Asp Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
 35 40 45

Pro Lys Leu Leu Ile Tyr Lys Ile Ser Asn Arg Phe Ser Gly Val Pro
 50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
115 120 125

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
130 135 140

Tyr Pro Arg Glu Ala Lys Val Gln Trp Cys Val Asp Asn Ala Leu Gln
145 150 155 160

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
165 170 175

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
180 185 190

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
195 200 205

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
210 215

<210> 61

<211> 213

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

00B206_0809_SL.TXT

<400> 61

Gln Ile Val Leu Thr Gln Ser Pro Ala Leu Met Ser Ala Ser Pro Gly
1 5 10 15

Glu Arg Val Thr Met Thr Cys Ser Ala Gly Ser Ser Val Ser Tyr Met
20 25 30

Tyr Trp Tyr Gln Gln Lys Pro Arg Ser Ser Pro Lys Pro Trp Ile Tyr
35 40 45

Leu Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu
65 70 75 80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Arg Thr
85 90 95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140

Val Gln Trp Cys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
165 170 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
180 185 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
195 200 205

Asn Arg Gly Glu Cys
210

<210> 62
<211> 214
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 62
Asp Ile Gln Met Thr Gln Ser Pro Ala Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Glu Thr Val Ile Ile Thr Cys Arg Ala Ser Glu Asn Ile Tyr Ser Tyr
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Gln Gly Lys Ser Pro Gln Leu Leu Val
35 40 45

Tyr Asn Ile Arg Thr Leu Ala Glu Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Gln Phe Ser Leu Lys Ile Asn Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Gly Ser Tyr Tyr Cys Gln His His Tyr Asp Ser Pro Trp
85 90 95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

00B206_0809_SL.TXT

Lys Val Gln Trp Cys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205

Phe Asn Arg Gly Glu Cys
210

<210> 63

<211> 217

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 63

Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Pro Leu Gly
1 5 10 15

Glu Arg Val Thr Met Thr Cys Thr Ala Ser Ser Ser Val Ser Ser Ser
20 25 30

Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Val Trp
35 40 45

Ile Tyr Arg Thr Thr Asn Leu Ala Ser Gly Val Pro Thr Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu
65 70 75 80

Ala Glu Asp Ala Ala Thr Tyr Tyr Cys His Gln Tyr His Arg Ser Pro

85

90

95

Pro Thr Trp Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr
 100 105 110

Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu
 115 120 125

Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
 130 135 140

Arg Glu Ala Lys Val Gln Trp Cys Val Asp Asn Ala Leu Gln Ser Gly
 145 150 155 160

Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr
 165 170 175

Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His
 180 185 190

Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val
 195 200 205

Thr Lys Ser Phe Asn Arg Gly Glu Cys
 210 215

<210> 64

<211> 447

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic
 polypeptide"

<400> 64

Gln Val Gln Leu Ile Gln Ser Gly Pro Gly Leu Val Gln Pro Ser Gln
 1 5 10 15

Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Gly Tyr
 20 25 30

00B206_0809_SL.TXT

Ala Ile His Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45

Gly Met Ile Trp Lys Ser Gly Asn Thr Asp Tyr Asn Ala Ala Phe Met
50 55 60

Ser Arg Leu Ser Ile Thr Lys Asp Asn Ser Lys Ser Gln Val Phe Phe
65 70 75 80

Lys Met Asn Ser Leu Gln Ala Asp Asp Thr Ala Ile Tyr Tyr Cys Ala
85 90 95

Arg Asn Gly Tyr Asp Tyr Glu Phe Val Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
115 120 125

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
130 135 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145 150 155 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
165 170 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
180 185 190

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
195 200 205

Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
210 215 220

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
225 230 235 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
245 250 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
260 265 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
275 280 285

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
290 295 300

Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys
305					310					315					320

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
325 330 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
340 345 350

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
355 360 365

Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn
	370					375					380				

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385 390 395 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
405 410 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
420 425 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> 65

<211> 447

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 65

Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala
 1 5 10 15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
 20 25 30

Gly Ile Ser Trp Val Lys Gln Arg Thr Gly Gln Gly Leu Glu Trp Ile
 35 40 45

Gly Glu Ile Tyr Pro Arg Ser Asp Asn Thr Tyr Tyr Asn Glu Lys Phe
 50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
 65 70 75 80

Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
 85 90 95

Thr Arg Ser Asp Tyr Gly Phe Phe Asp Tyr Trp Gly Gln Gly Thr Thr
 100 105 110

Leu Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
 115 120 125

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
 130 135 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
 145 150 155 160

00B206_0809_SL.TXT

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
165 170 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
180 185 190

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
195 200 205

Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
210 215 220

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
225 230 235 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
245 250 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
260 265 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
275 280 285

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
290 295 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305 310 315 320

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
325 330 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
340 345 350

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
355 360 365

00B206_0809_SL.TXT

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
370 375 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385 390 395 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
405 410 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
420 425 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> 66

<211> 447

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 66

Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Ile Pro Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
20 25 30

Tyr Met Gly Trp Val Lys Gln Ser His Gly Lys Ser Leu Glu Trp Ile
35 40 45

Gly Asp Ile Asn Pro Asn Asn Gly Val Thr Ile Asn Asn Gln Asn Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

00B206_0809_SL.TXT

Met Glu Leu Arg Ser Leu Ala Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Arg Gly Tyr Gly Gly Ala Leu Asp Tyr Trp Gly Gln Gly Thr Ser
100 105 110

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
115 120 125

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
130 135 140

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
145 150 155 160

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
165 170 175

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
180 185 190

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
195 200 205

Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
210 215 220

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
225 230 235 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
245 250 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
260 265 270

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
275 280 285

00B206_0809_SL.TXT

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
290 295 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305 310 315 320

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
325 330 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
340 345 350

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
355 360 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
370 375 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385 390 395 400

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
405 410 415

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
420 425 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440 445

<210> 67

<211> 449

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 67

Glu Val Lys Leu Val Glu Ser Gly Gly Asp Leu Val Lys Pro Gly Gly

1	5	10	15
Ser	Leu	Lys	Leu
	20	Ser	Cys
		Ala	Ala
		25	Ser
		Gly	Phe
		Thr	Phe
		30	Ser
		Ser	Tyr
Gly	Met	Ser	Trp
	35	Val	Arg
		Gln	Thr
		40	Pro
		Asp	Lys
		Arg	Leu
		45	Glu
		Trp	Val
Ala	Thr	Ile	Ser
	50	Thr	Gly
		Gly	Gly
		55	Tyr
		Thr	Tyr
		60	Tyr
		Pro	Asp
		Ser	Val
Lys	Gly	Arg	Phe
65		Thr	Ile
		70	Ser
		Arg	Asp
		Asn	Ala
		75	Lys
		Asn	Thr
		Leu	Tyr
		80	
Leu	Gln	Met	Ser
		85	Ser
		Leu	Arg
		Ser	Glu
		90	Asp
		Thr	Ala
		Met	Tyr
		95	Tyr
		Cys	
Ala	Arg	His	Asp
		100	Leu
		Val	Asp
		Trp	Tyr
		105	Phe
		Asp	Val
		Trp	Gly
		110	Thr
		Gly	
Thr	Thr	Val	Thr
		115	Val
		Ser	Ser
		Ala	Ser
		120	Thr
		Lys	Gly
		125	Pro
		Ser	Val
		Phe	
Pro	Leu	Ala	Pro
	130	Ser	Ser
		Lys	Ser
		135	Thr
		Ser	Gly
		140	Gly
		Thr	Ala
		Ala	Leu
Gly	Cys	Leu	Val
145		Lys	Asp
		150	Tyr
		Phe	Pro
		155	Glu
		Pro	Val
		Thr	Val
		Ser	Trp
		160	
Asn	Ser	Gly	Ala
		165	Leu
		Thr	Ser
		Gly	Val
		170	His
		Thr	Phe
		Pro	Ala
		175	Val
		Leu	
Gln	Ser	Ser	Gly
		180	Leu
		Tyr	Ser
		Leu	Ser
		185	Ser
		Val	Val
		Thr	Val
		190	Pro
		Ser	
Ser	Ser	Leu	Gly
		195	Thr
		Gln	Thr
		200	Tyr
		Ile	Cys
		Asn	Val
		205	Asn
		His	Lys
		Pro	
Ser	Asn	Thr	Lys
		Val	Asp
		Lys	Lys
		Val	Glu
		Pro	Lys
		Ser	Cys
		Asp	Lys

210

215

220

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
 225 230 235 240

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
 245 250 255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
 260 265 270

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
 275 280 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
 290 295 300

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
 305 310 315 320

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
 325 330 335

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
 340 345 350

Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr
 355 360 365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
 370 375 380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
 385 390 395 400

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
 405 410 415

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu

420

425

430

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
 435 440 445

Lys

<210> 68

<211> 112

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic
 polypeptide"

<400> 68

Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
 1 5 10 15

Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ile Ile Val His Asn
 20 25 30

Asn Gly Asp Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
 35 40 45

Pro Lys Leu Leu Ile Tyr Lys Ile Ser Asn Arg Phe Ser Gly Val Pro
 50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
 65 70 75 80

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Phe Gln Gly
 85 90 95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
 100 105 110

<210> 69

<211> 98

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 69

Ala	Leu	Met	Ser	Ala	Ser	Pro	Gly	Glu	Arg	Val	Thr	Met	Thr	Cys	Ser
1				5					10					15	

Ala	Gly	Ser	Ser	Val	Ser	Tyr	Met	Tyr	Trp	Tyr	Gln	Gln	Lys	Pro	Arg
			20					25					30		

Ser	Ser	Pro	Lys	Pro	Trp	Ile	Tyr	Leu	Thr	Ser	Asn	Leu	Ala	Ser	Gly
		35					40					45			

Val	Pro	Ala	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Ser	Tyr	Ser	Leu
	50					55					60				

Thr	Ile	Ser	Ser	Met	Glu	Ala	Glu	Asp	Ala	Ala	Thr	Tyr	Tyr	Cys	Gln
65					70					75					80

Gln	Trp	Ser	Ser	Asn	Pro	Arg	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Val	Glu
				85					90					95	

Ile Lys

<210> 70

<211> 107

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 70

Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ala	Ser	Leu	Ser	Ala	Ser	Val	Gly
1				5					10					15	

00B206_0809_SL.TXT

Glu Thr Val Ile Ile Thr Cys Arg Ala Ser Glu Asn Ile Tyr Ser Tyr
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Gln Gly Lys Ser Pro Gln Leu Leu Val
35 40 45

Tyr Asn Ile Arg Thr Leu Ala Glu Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Gln Phe Ser Leu Lys Ile Asn Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Gly Ser Tyr Tyr Cys Gln His His Tyr Asp Ser Pro Trp
85 90 95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 71

<211> 110

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 71

Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Pro Leu Gly
1 5 10 15

Glu Arg Val Thr Met Thr Cys Thr Ala Ser Ser Ser Val Ser Ser Ser
20 25 30

Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys Val Trp
35 40 45

Ile Tyr Arg Thr Thr Asn Leu Ala Ser Gly Val Pro Thr Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu

Pro Thr Trp Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

```
<210> 72
<211> 117
<212> PRT
<213> Artificial Sequence
```

```
<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"
```

<400> 72
Gln Val Gln Leu Ile Gln Ser Gly Pro Gly Leu Val Gln Pro Ser Gln
1 5 10 15

Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu Thr Gly Tyr
20 25 30

Ala Ile His Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45

Gly Met Ile Trp Lys Ser Gly Asn Thr Asp Tyr Asn Ala Ala Phe Met
50 55 60

Ser Arg Leu Ser Ile Thr Lys Asp Asn Ser Lys Ser Gln Val Phe Phe
65 70 75 80

Lys Met Asn Ser Leu Gln Ala Asp Asp Thr Ala Ile Tyr Tyr Cys Ala
85 90 95

Arg Asn Gly Tyr Asp Tyr Glu Phe Val Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 73
 <211> 117
 <212> PRT
 <213> Artificial Sequence

<220>
 <221> source
 <223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 73
 Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala
 1 5 10 15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
 20 25 30

Gly Ile Ser Trp Val Lys Gln Arg Thr Gly Gln Gly Leu Glu Trp Ile
 35 40 45

Gly Glu Ile Tyr Pro Arg Ser Asp Asn Thr Tyr Tyr Asn Glu Lys Phe
 50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
 65 70 75 80

Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
 85 90 95

Thr Arg Ser Asp Tyr Gly Phe Phe Asp Tyr Trp Gly Gln Gly Thr Thr
 100 105 110

Leu Thr Val Ser Ser
 115

<210> 74
 <211> 117
 <212> PRT
 <213> Artificial Sequence

<220>
 <221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 74

Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Ile Pro Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
20 25 30

Tyr Met Gly Trp Val Lys Gln Ser His Gly Lys Ser Leu Glu Trp Ile
35 40 45

Gly Asp Ile Asn Pro Asn Asn Gly Val Thr Ile Asn Asn Gln Asn Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Arg Ser Leu Ala Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Arg Gly Tyr Gly Gly Ala Leu Asp Tyr Trp Gly Gln Gly Thr Ser
100 105 110

Val Thr Val Ser Ser
115

<210> 75

<211> 119

<212> PRT

<213> Artificial Sequence

<220>

<221> source

<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 75

Glu Val Lys Leu Val Glu Ser Gly Gly Asp Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr

20

25

30

Gly Met Ser Trp Val Arg Gln Thr Pro Asp Lys Arg Leu Glu Trp Val
 35 40 45

Ala Thr Ile Ser Thr Gly Gly Gly Tyr Thr Tyr Tyr Pro Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Ser Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys
 85 90 95

Ala Arg His Asp Leu Val Asp Trp Tyr Phe Asp Val Trp Gly Thr Gly
 100 105 110

Thr Thr Val Thr Val Ser Ser
 115

<210> 76
 <211> 4
 <212> PRT
 <213> Homo sapiens

<400> 76
 His Pro Ile Pro
 1

<210> 77
 <211> 10
 <212> PRT
 <213> Homo sapiens

<400> 77
 Ser Gln Gly Arg Ser Pro Ser Tyr Ala Ser
 1 5 10