(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
16 March 2023 (16.03.2023)

WIPO | PCT

(10) International Publication Number
WO 2023/039107 A2

(51) International Patent Classification:
G01N 33/53 (2006.01) *C07K 16/30* (2006.01)

(21) International Application Number:
PCT/US2022/042963

(22) International Filing Date:
08 September 2022 (08.09.2022)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/242,437 09 September 2021 (09.09.2021) US

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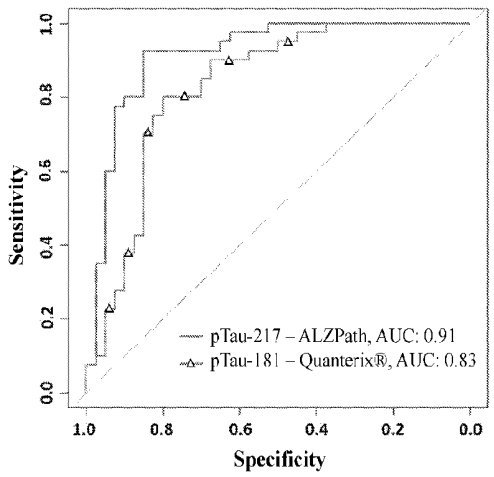
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, 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, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, 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, 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).

Published:
— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

(54) Title: PHOSPHO-TAU ANTIBODIES AND METHODS OF USE



	AD-dementia Median (IQR)	Controls Median	Differentiation Fold change	Differentiation AUC (95% CI)	AD-dementia vs Controls Cut off	AD-dementia vs Controls % Sensitivity	AD-dementia vs Controls % Specificity
pTau-217 ALZPath	5.2 (3.5 - 6.8)	1.3 (0.8 – 1.9)	4.2	0.91 (0.85 – 0.98)	2.26	92.5	85
pTau-181 Quanterix®	2.5 (2.2 - 3.3)	1.4 (1.1 – 1.9)	1.8	0.83 (0.74 – 0.920	2.14	80	80

FIG. 24

(57) Abstract: Provided herein are compositions and methods relating to improved assays for establishing Alzheimer's disease. Further provided herein are compositions and methods comprising improved antibodies for assays including immunoassays.

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PHOSPHO-TAU ANTIBODIES AND METHODS OF USE

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 63/242,437 filed on September 9, 2021, which is incorporated by reference in its entirety.

BACKGROUND

[0002] The discovery of biomarkers and screening techniques of Alzheimer's disease (AD) and other tauopathies is an ongoing area of development in which these tools may be applied to screening populations to determine which non-demented individuals are at greatest risk of developing AD dementia and also to assess disease progression in patients. Proteins that are reflective of AD pathology, including amyloid beta 42 ($A\beta_{42}$), neurofilament light chain, and various tau isoforms have been detected by a variety of means. Abnormal or excessive phosphorylation of tau has been associated with transformation of pathologically normal tau molecules into paired-helical-filament (PHF) tau and neurofibrillary tangles (NFTs) indicative of various tauopathy pathologies.

SUMMARY

[0003] Tau is an important microtubule-associated protein, abundantly expressed in CNS neurons, and serves critical roles in normal cellular physiology. Tau has also been found to be dysregulated in Alzheimer's disease and other tauopathies. Six isoforms of tau protein are generated from the *TAU* gene by alternative splicing. The isoforms differ from each other by the presence or absence of two N-terminal inserts and a repeat termed R2. All six protein isoforms of tau are highly soluble under normal and healthy cellular conditions and are typically regulated by phosphorylation and dephosphorylation. Tau has been demonstrated to interact with microtubules and promote microtubule assembly. In neurons, tau promotes the formation of axonal microtubules and stabilizes them. Tau has additional roles in driving neurite outgrowth. Impaired interaction of tau with microtubules may be an important component in the pathology, development, and progression of tauopathies. Hyperphosphorylation of tau is a hallmark feature of AD and other tauopathies and the extent of hyperphosphorylation is often correlated with disease progression. Hyperphosphorylation of tau protein can result in the self-assembly of insoluble tangles of paired helical filaments and straight filaments of tau. These insoluble

aggregates of tangles, termed neurofibrillary tangles (NFTs), are comprised of hyperphosphorylated tau and are considered to be pathological markers of tauopathies.

[0004] Phosphorylated tau (pTau), total tau, and $A\beta_{42}$ each detected from the cerebrospinal fluid (CSF) and/or the blood are individual biomarkers for Alzheimer's disease and several other related tauopathies. CSF pTau is increased in individuals later confirmed to have AD both at the prodromal stages and the dementia stages compared to age- and gender-matched controls. CSF pTau levels exhibit a strong degree of correlation to the extent of cognitive impairment in individuals with AD. In fact, CSF pTau levels may be used with some degree of precision as a biomarker to predict progression from cognitively unimpaired, to mild cognitive impairment (MCI) and then to AD dementia. In terms of utility as a biomarker to predict even relatively early stages of AD progression, CSF pTau has been shown to be significantly increased in samples from individuals with preclinical AD. Changes in the extent of pTau phosphorylation have been demonstrated in both preclinical sporadic cases of AD and in early stages of autosomal-dominant AD. Blood levels of pTau, total tau, and $A\beta_{42}$ are generally lower than CSF levels when assayed within the same individual and may be utilized as informative biomarkers for AD and other related tauopathies if blood levels of these biomarkers can be assayed with sufficient specificity and precision.

[0005] Several sites of phosphorylation contributing to hyperphosphorylated tau which aggregates into NFTs have been identified. In the longest tau isoform, 79 potential serine or threonine phosphorylation sites are present and at least 30 of these sites have been identified as phosphorylated in NFT aggregates. A common site used to assay tau molecules for phosphorylation status is at threonine-181. CSF fluid contains an array of tau fragments at various abundances. Fragments of tau from the N-terminal region and from the middle region of tau polypeptides are considerably more abundant in CSF samples than C-terminal tau fragments. Plasma samples from individuals also contain tau polypeptides and tau polypeptide fragments, however they tend to be present at lower concentrations that in matched CSF samples. Being able to detect tau phosphorylation at particular amino acid residues relevant for disease pathology and progression is a critical component of diagnosis, disease staging, and as a metric to measure treatment efficacy for AD and other tauopathies. Detection and measurement of pTau levels at particular disease-relevant residues from plasma samples would aid greatly to the development of more sensitive and finely-tuned diagnosis, prognosis, and disease analysis for individuals who may be at risk for developing or are at early stages of AD or other tauopathies. Phosphorylation of tau at threonine 217 (pTau 217) is one such residue of particular interest in development new biomarkers and diagnostic assays. Alterations in pTau biomarker

concentration in CSF and in plasma are thought to precede measurable behavioral or cognitive changes in AD and in other tauopathies. A development of new assays to enable a continuum of specific points and extents of tau phosphorylation of certain residues would undoubtedly aid in the clinically relevant medical diagnosis and treatment decisions. A comparison of results from new assays to results from existing assays can also yield further medically informative determinations. Results from plasma-based tau biomarker assays can be compared against matched CSF samples (detecting CSF pTau or CSF soluble Aβ) and also against positron emission tomography (PET) scans detecting an extent and locations of Aβ aggregates as metrics for their utility, especially for analysis at preclinical or early disease stages.

[0006] Provided herein are methods for detecting phosphorylated tau in a sample from an individual comprising: performing an immunoassay on the sample using an antibody or antibody fragment comprising a variable domain, heavy chain region (VH) and a variable domain, light chain region (VL), wherein the VH comprises an amino acid sequence at least about 90% identical to a sequence as set forth in any one of SEQ ID NOs: 30-34, and wherein the VL comprises an amino acid sequence at least about 90% identical to a sequence as set forth in any one of SEQ ID NOs: 35-40. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the phosphorylated tau is selected from the group consisting of pTau-181, pTau-212, pTau-217, pTau-231, pTau-214, and pTau-220. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the phosphorylated tau is pTau-217. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the phosphorylated tau is pTau-231. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the method detects pTau-217 and pTau-231. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the method detects pTau-212 and pTau-217. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the method detects pTau-212 and pTau-231. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the method detects pTau-181 and pTau-217. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the method detects pTau-181 and pTau-231. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the method detects pTau-181, pTau-217, and pTau-231. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the method detects pTau-212, pTau-217 and pTau-231. Further provided herein are methods for detecting phosphorylated tau in a sample from an

individual, wherein the method detects pTau-217 and pTau-231 in a sample selected from the group consisting of a plasma sample and serum sample. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the method detects pTau-212 and pTau-217 in a sample selected from the group consisting of a plasma sample and serum sample. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the method detects pTau-212 and pTau-231 in a sample selected from the group consisting of a plasma sample and serum sample. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the method detects pTau-181 and pTau-217 in a sample selected from the group consisting of a plasma sample and serum sample. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the method detects pTau-181 and pTau-231 in a sample selected from the group consisting of a plasma sample and serum sample. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the method detects pTau-181, pTau-217, and pTau-231 in a sample selected from the group consisting of a plasma sample and serum sample. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the method detects pTau-212, pTau-217, and pTau-231 in a sample selected from the group consisting of a plasma sample and serum sample. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the VH comprises an amino acid sequence according to any one of SEQ ID NOs: 30-34. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the VL comprises an amino acid sequence according to any one of SEQ ID NOs: 35-40. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the VH comprises an amino acid sequence according to any one of SEQ ID NOs: 30-34, and wherein the VL comprises an amino acid sequence according to any one of SEQ ID NOs: 35-40. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the VH comprises an amino acid sequence at least about 90% identical to SEQ ID NO: 30, and wherein the VL comprises an amino acid sequence at least about 90% identical to SEQ ID NO: 35. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the VH comprises an amino acid sequence at least about 90% identical to SEQ ID NO: 31, and wherein the VL comprises an amino acid sequence at least about 90% identical to SEQ ID NO: 36. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the VH comprises an amino acid sequence at least about 90% identical to SEQ ID NO: 31, and wherein the VL comprises an amino acid sequence at least about 90% identical to SEQ

ID NO: 37. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the VH comprises an amino acid sequence at least about 90% identical to SEQ ID NO: 32, and wherein the VL comprises an amino acid sequence at least about 90% identical to SEQ ID NO: 38. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the VH comprises an amino acid sequence at least about 90% identical to SEQ ID NO: 33, and wherein the VL comprises an amino acid sequence at least about 90% identical to SEQ ID NO: 39. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the VH comprises an amino acid sequence at least about 90% identical to SEQ ID NO: 34, and wherein the VL comprises an amino acid sequence at least about 90% identical to SEQ ID NO: 40. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the antibody or antibody fragment comprises an amino acid sequence at least about 90% identical to any one of SEQ ID NOs: 41-51. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, further comprising performing an assay on the sample to determine a level of a biomarker selected from the group consisting of Aβ42, Aβ40, Aβ38, BACE1, hFABP, TREM2, YKL-40, IP-10, neurogranin, SNAP-25, synaptotagmin, alpha-synuclein, TDP-43, ferritin, VILIP-1, NfL, GFAP, and combinations thereof. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the sample is selected from the group consisting of a blood sample, a plasma sample, a serum sample, and a cerebrospinal fluid (CSF) sample. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, further comprising establishing Alzheimer's disease in the individual based on detection of phosphorylated tau. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, further comprising establishing prognosis of the individual for developing Alzheimer's disease based on detection of phosphorylated tau. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, further determining the individual's age, genotype, or expression of a biomarker. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the biomarker is selected from the group consisting of Aβ42, Aβ40, Aβ38, BACE1, hFABP, TREM2, YKL-40, IP-10, neurogranin, SNAP-25, synaptotagmin, alpha-synuclein, TDP-43, ferritin, VILIP-1, NfL, GFAP, and combinations thereof. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the method has a specificity of at least about 80% for detecting phosphorylated tau. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the method

has a specificity of at least about 85% for detecting phosphorylated tau. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the method has a specificity of at least about 90% for detecting phosphorylated tau. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the method has a sensitivity of at least about 80% for detecting phosphorylated tau. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the method has a sensitivity of at least about 85% for detecting phosphorylated tau. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the method has a sensitivity of at least about 90% for detecting phosphorylated tau. Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the method is capable of detecting phosphorylated tau in the sample at a limit of detection of at least about 1.0 picogram per milliliter (pg/mL). Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the method is capable of detecting phosphorylated tau in the sample at a limit of detection of at least about 1.5 picogram per milliliter (pg/mL). Further provided herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the method is capable of detecting phosphorylated tau in the sample at a limit of detection of at least about 5 picogram per milliliter (pg/mL).

[0007] Also provided herein are, in certain embodiments, anti-tau antibodies comprising i) a heavy chain comprising variable heavy chain (VH) domain and ii) a light chain comprising a variable light chain (VL) domain, wherein the VH domain comprises HCDR1 sequence comprising a sequence selected from SEQ ID NOs: 1-5, HCDR2 sequence comprising a sequence selected from SEQ ID NOs: 6-9, and HCDR3 sequence comprising a sequence selected from SEQ ID NOs: 10-13, and VL domain comprises LCDR1 sequence comprising a sequence selected from SEQ ID NOs: 14-19, LCDR2 sequence comprising a sequence selected from SEQ ID NOs: 20-23, and LCDR3 sequence comprising a sequence selected from SEQ ID NOs: 24-29. In some embodiments, the HCDR1 sequence comprises SEQ ID NO: 1, the HCDR2 sequence comprises SEQ ID NO: 6, the HCDR3 sequence comprises SEQ ID NO: 10, the LCDR1 sequence comprises SEQ ID NO: 14, the LCDR2 sequence comprises SEQ ID NO: 20, and the LCDR3 sequence comprises SEQ ID NO: 24. In some embodiments, the HCDR1 sequence comprises SEQ ID NO: 2, the HCDR2 sequence comprises SEQ ID NO: 7, the HCDR3 sequence comprises SEQ ID NO: 11, the LCDR1 sequence comprises SEQ ID NO: 15, the LCDR2 sequence comprises SEQ ID NO: 21, and the LCDR3 sequence comprises SEQ ID NO: 25. In some embodiments, the HCDR1 sequence comprises SEQ ID NO: 2, the HCDR2 sequence comprises SEQ ID NO: 7, the HCDR3 sequence comprises SEQ ID NO: 11, the

LCDR1 sequence comprises SEQ ID NO: 16, the LCDR2 sequence comprises SEQ ID NO: 22, and the LCDR3 sequence comprises SEQ ID NO: 26. In some embodiments, the HCDR1 sequence comprises SEQ ID NO: 3, the HCDR2 sequence comprises SEQ ID NO: 8, the HCDR3 sequence comprises SEQ ID NO: 10, the LCDR1 sequence comprises SEQ ID NO: 17, the LCDR2 sequence comprises SEQ ID NO: 20, and the LCDR3 sequence comprises SEQ ID NO: 27. In some embodiments, the HCDR1 sequence comprises SEQ ID NO: 4, the HCDR2 sequence comprises SEQ ID NO: 7, the HCDR3 sequence comprises SEQ ID NO: 12, the LCDR1 sequence comprises SEQ ID NO: 18, the LCDR2 sequence comprises SEQ ID NO: 23, and the LCDR3 sequence comprises SEQ ID NO: 28. In some embodiments, the HCDR1 sequence comprises SEQ ID NO: 5, the HCDR2 sequence comprises SEQ ID NO: 9, the HCDR3 sequence comprises SEQ ID NO: 13, the LCDR1 sequence comprises SEQ ID NO: 19, the LCDR2 sequence comprises SEQ ID NO: 21, and the LCDR3 sequence comprises SEQ ID NO: 29. Further provided herein are, in some embodiments, anti-tau antibodies comprising i) a heavy chain comprising variable heavy chain (VH) domain and ii) a light chain comprising a variable light chain (VL) domain, wherein the VH domain comprises at least 80%, at least 85%, at least 90%, at least 95% sequence identity to a sequence selected from SEQ ID NOs: 30-34. Further provided herein are, in some embodiments, anti-tau antibodies comprising i) a heavy chain comprising variable heavy chain (VH) domain and ii) a light chain comprising a variable light chain (VL) domain, wherein the VL domain comprises at least 80%, at least 85%, at least 90%, at least 95% sequence identity to a sequence selected from SEQ ID NOs: 35-40. In some embodiments, the anti-tau antibody described herein is a chimeric antibody or antigen binding fragment thereof. In some embodiments, the anti-tau antibody described herein comprises an IgG-scFv, nanobody, BiTE, diabody, DART, TandAb, scDiabody, scDiabody-CH3, triple body, mini-antibody, minibody, TriBi minibody, scFv-CH3 KIH, Fab-scFv-Fc KIH, Fab-scFv, scFv-CH-CL-scFv, Fab', F(ab')2, F(ab')3,_F(ab')2-scFv2, scFv, scFv-KIH, Fab-scFv-Fc, tetravalent HCAb, scDiabody-Fc, diabody-Fc, tandem scFv-Fc, or intrabody. In some embodiments, the anti-tau antibody described herein is an IgG1 antibody. In some embodiments, the anti-tau antibody described herein is an IgG2 antibody. In some embodiments, the anti-tau antibody described herein is an IgG4 antibody. Further provided herein are, in some embodiments, anti-tau antibodies comprising i) a heavy chain comprising variable heavy chain (VH) domain and ii) a light chain comprising a variable light chain (VL) domain, wherein the light chain is a kappa chain. Further provided herein are, in some embodiments, anti-tau antibodies comprising i) a heavy chain comprising variable heavy chain (VH) domain and ii) a light chain comprising a variable light chain (VL) domain, wherein the anti-tau antibody has a binding affinity to human

tau of about 100 pM to about 3 nM. Provided herein are, in some embodiments, anti-tau antibodies comprising a VH domain that is encoded by a nucleic acid comprising at least 80%, at least 85%, at least 90%, at least 95% sequence identity to a sequence selected from SEQ ID NOs: 52-56. Provided herein are, in some embodiments, anti-tau antibodies comprising a VL domain that is encoded by a nucleic acid comprising at least 80%, at least 85%, at least 90%, at least 95% sequence identity to a sequence selected from SEQ ID NOs: 57-62. Provided herein are, in some embodiments, anti-tau antibodies comprising a VH domain that is encoded by a nucleic acid comprising at least 80%, at least 85%, at least 90%, at least 95% sequence identity to a sequence selected from SEQ ID NOs: 52-56 and a VL domain that is encoded by a nucleic acid comprising at least 80%, at least 85%, at least 90%, at least 95% sequence identity to a sequence selected from SEQ ID NOs: 57-62. Provided herein are, in some embodiments, anti-tau antibodies comprising a VH domain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NOs: 52-56. Provided herein are, in some embodiments, anti-tau antibodies comprising a VL domain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NOs: 57-62. Provided herein are, in some embodiments, anti-tau antibodies comprising a VH domain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NOs: 52-56 and a VL domain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NOs: 57-62.

INCORPORATION BY REFERENCE

[0008] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0010] **FIG. 1** depicts a schema of the single molecule array (Simoa®) method used herein for assaying tau antibodies described herein. After substrate is added to sample (sandwich ELISA on bead, 1.1), sample is added to Simoa® Disk (1.2). Beads are given time to settle into microarray wells on disk (one bead per well) (1.3). Then, sealing oil is used to remove excess beads to allow for imaging (1.4). Beads that have sandwich complex (positive beads) will

fluoresce with the substrate and show up during imaging; beads without sandwich complex (negative) will still show up in imaging but will not fluoresce (1.5). The percentage of positive beads is converted to an AEB (average enzymes per bead) value.

[0011] **FIGS. 2A-2D** depict data for Antibody 1, Antibody 2, Antibody 3, Antibody 4, Antibody 5, and Antibody 6 in the Simoa® assay.

[0012] **FIG. 3** depict ELISA data.

[0013] **FIGS. 4A-4G** depict data for immunohistochemistry staining of Antibody 6.

[0014] **FIGS. 5A-5G** depict data for immunohistochemistry staining of Antibody 5.

[0015] **FIGS. 6A-6G** depict data for immunohistochemistry staining of Antibody 2.

[0016] **FIG. 7** depicts a diagram of an indirect ELISA assay and graphs of ELISA data assaying antibody binding to pTau-217 peptides.

[0017] **FIG. 8** depicts a graph of signal/noise (S/N) analysis of ELISA assay for Antibody 2 binding to a pTau-217 peptide for 120 clinic samples derived from plasma and a graph of a coefficient of variation (CV%) for ELISA assay for Antibody 2 binding to a pTau-217 peptide for 120 clinic samples derived from plasma.

[0018] **FIG. 9** depicts graphs of calibration curves (Cal curves) for a Simoa®-based pTau-217 assay using Antibody 2 on groups (plates) designated QTx of clinical samples derived from cerebrospinal fluid (68 CSF samples) and plasma (120 plasma samples) compared to the assay using ADx p204 antibody.

[0019] **FIG. 10** depicts a graph of Simoa®-based pTau assay-217 results using Antibody 2 in matched samples from the sample individual derived from either plasma (Y-axis) or CSF (X-axis) and statistical analysis of correlated results in individual with a clinical diagnosis of either non-Alzheimer's disease, an uncertain diagnosis, or Alzheimer's disease.

[0020] **FIG. 11** depicts a graph of Simoa®-based pTau assay-217 results using Antibody 2 per sample vs Simoa®-based pTau assay-181 results using Antibody 2 per sample and statistical analysis of correlated results.

[0021] **FIG. 12** depicts a graph of Simoa®-based pTau assay-217 results using Antibody 2 per sample vs Simoa®-based Tau assay results using Innotest pTau 181 antibody per sample and statistical analysis of correlated results.

[0022] **FIG. 13** depicts a graph of Simoa®-based pTau assay results using Antibody 2 as a capture antibody, antibody ADx p204 as a detector antibody and a peptide as calibrator and statistical analysis of correlated results.

[0023] **FIG. 14** depicts a graph of Simoa®-based pTau assay-217 results using Antibody 2 grouping together samples from individuals with a clinical diagnosis of Alzheimer's disease and samples from control individuals derived from either CSF or plasma.

[0024] **FIG. 15** depicts a graph of Simoa®-based pTau assay-217 results using Antibody 2 on various concentrations of EDTA plasma samples and a chart of listing coefficient of variation for each sample concentration to illustrate the precision of the assay.

[0025] **FIG. 16** depicts graphs of Simoa®-based pTau assay-217 results using Antibody 2 graphed as coefficient of variation (CV%) vs measured concentration.

[0026] **FIG. 17** depicts a graph of Simoa®-based pTau assay-217 results using Antibody 2 and statistical analysis of parallelism with determines whether actual samples containing high endogenous analyte concentrations provide a similar degree of detection in a standard curve after dilutions.

[0027] **FIG. 18** depicts a graph of Simoa®-based pTau assay-217 results using Antibody 2 and statistical analysis of linearity with determines whether sample matrices spiked with detection analyte above an upper limit of detect can still provide reliable quantification after dilution within standard curve ranges for four samples plus a buffer spike.

[0028] **FIG. 19** depicts a graph of Simoa®-based pTau assay-217 results using Antibody 2 and statistical analysis of linearity with determines whether sample matrices spiked with detection analyte above an upper limit of detect can still provide reliable quantification after dilution within standard curve ranges for three samples plus a calibration sample.

[0029] **FIG. 20** depicts a graph of Simoa®-based pTau assay-217 results using Antibody 2 in a clinical validation of a memory clinic cohort and a graph of receiver-operating characteristic (ROC) analysis graphed against pTau-217 assay sensitivity.

[0030] **FIG. 21** depicts a graph of Simoa®-based pTau assay-217 results using Antibody 2 on groups from Control and AD dementia individuals.

[0031] **FIG. 22** depicts a graph of Simoa®-based pTau assay-181 results using an antibody from Quanterix® on groups from Control and AD dementia individuals and a chart of sample stratification.

[0032] **FIG. 23** depicts graphs of Simoa®-based pTau assay-217 results using Antibody 2, and Simoa®-based pTau assay-181 results using an antibody from Quanterix® showing precision plots with calculated coefficient of variation.

[0033] **FIG. 24** depicts a graph of clinical performance of various pTau Simoa®-based assays comparing sensitivity and specificity and a chart with a statistical analysis of results.

[0034] **FIG. 25** depicts a schematic diagram of Tau indicating the relative location of various protein domains and the locations of threonine residues which can be assayed for phosphorylation status using methods disclosed herein.

[0035] **FIG. 26** depicts graphs of reactivity to a Tau fragment with non-phosphorylated T217 (Bio-pt654) and full length Tau (Tau441) in indirect ELISA for various antibodies.

[0036] **FIG. 27** depicts graphs of reactivity to Tau fragments with phosphorylated T181 (Bio-pt126) and phosphorylated T231 (Bio-pt146) in indirect ELISA for various antibodies.

[0037] **FIG. 28** depicts a diagram of an assay utilizing a pTau217 monoclonal antibody as a capture tool for various synthetic peptides and a graph of results for this assay using Antibody 2 as the capture tool.

[0038] **FIG. 29** depicts Western blot analysis using various Tau antibodies on brain lysate samples from AD patients or control subjects.

DETAILED DESCRIPTION

[0039] Alzheimer's disease (AD) is a complex disease and effective treatment requires accurate diagnosis. Described herein are improved compositions and methods for detecting AD that comprises improved antibodies for use in diagnostic and/or prognostic assays.

[0040] **Certain terminologies**

[0041] Throughout this disclosure, various embodiments are presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of any embodiments. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range to the tenth of the unit of the lower limit unless the context clearly dictates otherwise. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual values within that range, for example, 1.1, 2, 2.3, 5, and 5.9. This applies regardless of the breadth of the range. The upper and lower limits of these intervening ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention, unless the context clearly dictates otherwise.

[0042] The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting of any embodiment. As used herein, the singular forms "a," "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. It will be further understood that the terms "comprises" and/or "comprising," when used in this specification, specify the presence of stated features, integers, steps, operations, elements, and/or components, but do not preclude the presence or addition of one or more other features, integers, steps, operations, elements, components, and/or groups thereof. As used herein, the term "and/or" includes any and all combinations of one or more of the associated listed items.

[0043] Unless specifically stated or obvious from context, as used herein, the term "about" in reference to a number or range of numbers is understood to mean the stated number and numbers +/- 10% thereof, or 10% below the lower listed limit and 10% above the higher listed limit for the values listed for a range.

[0044] The terms "individual," "patient," or "subject" are used interchangeably. None of the terms require or are limited to a situation characterized by the supervision (e.g., constant or intermittent) of a health care worker (e.g., a doctor, a registered nurse, a nurse practitioner, a physician's assistant, an orderly, or a hospice worker). Further, these terms refer to human or animal subjects.

[0045] The term "antibody" herein is used in the broadest sense and includes monoclonal antibodies, including intact antibodies and functional (antigen-binding) antibody fragments thereof, including fragment antigen binding (Fab) fragments, F(ab')2 fragments, Fab' fragments, Fv fragments, recombinant IgG (rIgG) fragments, single chain antibody fragments, including single chain variable fragments (sFv or scFv), and single domain antibodies (e.g., sdAb, sdFv, nanobody) fragments. The term encompasses genetically engineered and/or otherwise modified forms of immunoglobulins, such as intrabodies, peptibodies, chimeric antibodies, and heteroconjugate antibodies, tandem di-scFv, tandem tri-scFv. Unless otherwise stated, the term "antibody" should be understood to encompass functional antibody fragments thereof. The term also encompasses intact or full- length antibodies, including antibodies of any class or sub-class, including IgG and sub-classes thereof, IgM, IgE, IgA, and IgD. The antibody can comprise a rabbit IgG1 constant region. The antibody can comprise a rabbit IgG4 constant region. An antibody includes, but is not limited to, full-length and native antibodies, as well as fragments and portion thereof retaining the binding specificities thereof, such as any specific binding portion thereof including those having any number of, immunoglobulin classes and/or isotypes (e.g., IgGl, IgG2, IgG3, IgG4, IgM, IgA, IgD, IgE and IgM); and biologically relevant (antigen-

binding) fragments or specific binding portions thereof, including but not limited to Fab, F(ab')2, Fv, and scFv (single chain or related entity). A monoclonal antibody is generally one within a composition of substantially homogeneous antibodies; thus, any individual antibodies comprised within the monoclonal antibody composition are identical except for possible naturally occurring mutations that may be present in minor amounts. A monoclonal antibody can comprise a rabbit IgG1 constant region or a rabbit IgG4 constant region.

[0046] The term "complementarity determining region" or "CDR" is a segment of the variable region of an antibody that is complementary in structure to the epitope to which the antibody binds and is more variable than the rest of the variable region. Accordingly, a CDR is sometimes referred to as hypervariable region. A variable region comprises three CDRs. CDR peptides can be obtained by constructing genes encoding the CDR of an antibody of interest. Such genes are prepared, for example, by using the polymerase chain reaction to synthesize the variable region from RNA of antibody-producing cells. See, for example, Larrick et al., *Methods: A Companion to Methods in Enzymology* 2: 106 (1991); Courtenay-Luck, "Genetic Manipulation of Monoclonal Antibodies," in *Monoclonal Antibodies: Production, Engineering and Clinical Application*, Ritter et al. (eds.), pages 166-179 (Cambridge University Press 1995); and Ward et al., "Genetic Manipulation and Expression of Antibodies," in *Monoclonal Antibodies: Principles and Applications*, Birch et al., (eds.), pages 137-185 (Wiley-Liss, Inc. 1995).

[0047] The term "Fab" refers to a protein that contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab fragments differ from Fab′ fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab′ in which the cysteine residue(s) of the constant domains bear a free thiol group. Fab′ fragments are produced by reducing the F(ab')2 fragment's heavy chain disulfide bridge. Other chemical couplings of antibody fragments are also known.

[0048] A "single-chain variable fragment (scFv)" is a fusion protein of the variable regions of the heavy (VH) and light chains (VL) of an antibody, connected with a short linker peptide of ten to about 25 amino acids. The linker is usually rich in glycine for flexibility, as well as serine or threonine for solubility, and can either connect the N-terminus of the VH with the C-terminus of the VL, or vice versa. This protein retains the specificity of the original antibody, despite removal of the constant regions and the introduction of the linker. scFv antibodies are, e.g. described in Houston, J. S., Methods in Enzymol. 203 (1991) 46-96). In addition, antibody fragments comprise single chain polypeptides having the characteristics of a VH domain, namely

being able to assemble together with a VL domain, or of a VL domain, namely being able to assemble together with a VH domain to a functional antigen binding site and thereby providing the antigen binding property of full length antibodies.

[0049] As used herein, the term "percent (%) amino acid sequence identity" with respect to a sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific 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 EMBOSS MATCHER, EMBOSS WATER, EMBOSS STRETCHER, EMBOSS NEEDLE, EMBOSS LALIGN, BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

[0050] 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 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.

[0051] The terms "complementarity determining region," and "CDR," which are synonymous with "hypervariable region" or "HVR," are known in the art to refer to non-contiguous sequences of amino acids within antibody variable regions, which confer antigen specificity and/or binding affinity. In general, there are three CDRs in each heavy chain variable region (CDR-H1, CDR-H2, CDR-H3) and three CDRs in each light chain variable region (CDR-L1, CDR-L2, CDR-L3). "Framework regions" and "FR" are known in the art to refer to the non-CDR portions of the variable regions of the heavy and light chains. In general, there are four FRs

in each full-length heavy chain variable region (FR-H1, FR-H2, FR-H3, and FR-H4), and four FRs in each full-length light chain variable region (FR-L1, FR-L2, FR-L3, and FR-L4). The precise amino acid sequence boundaries of a given CDR or FR can be readily determined using any of a number of well-known schemes, including those described by Kabat et al. (1991), "Sequences of Proteins of Immunological Interest," 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD ("Kabat" numbering scheme), Al-Lazikani et al., (1997) JMB 273,927-948 ("Chothia" numbering scheme); MacCallum et al., J. Mol. Biol. 262:732-745 (1996), "Antibody-antigen interactions: Contact analysis and binding site topography," J. Mol. Biol. 262, 732-745." ("Contact" numbering scheme); Lefranc MP et al., "IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains," Dev Comp Immunol, 2003 Jan;27(1):55-77 ("IMGT" numbering scheme); Honegger A and Plückthun A, "Yet another numbering scheme for immunoglobulin variable domains: an automatic modeling and analysis tool," J Mol Biol, 2001 Jun 8;309(3):657-70, ("Aho" numbering scheme); and Whitelegg NR and Rees AR, "WAM: an improved algorithm for modelling antibodies on the WEB," Protein Eng. 2000 Dec;13(12):819-24 ("AbM" numbering scheme. In certain embodiments the CDRs of the antibodies described herein can be defined by a method selected from Kabat, Chothia, IMGT, Aho, AbM, or combinations thereof.

[0052] The boundaries of a given CDR or FR may vary depending on the scheme used for identification. For example, the Kabat scheme is based on structural alignments, while the Chothia scheme is based on structural information. Numbering for both the Kabat and Chothia schemes is based upon the most common antibody region sequence lengths, with insertions accommodated by insertion letters, for example, "30a," and deletions appearing in some antibodies. The two schemes place certain insertions and deletions ("indels") at different positions, resulting in differential numbering. The Contact scheme is based on analysis of complex crystal structures and is similar in many respects to the Chothia numbering scheme.

[0053] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the methods and compositions described herein belong. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the methods and compositions described herein, representative illustrative methods and materials are now described.

Tau Antibodies

[0054] Provided herein are antibodies that bind to tau. In some instances, the antibodies that bind to tau are monoclonal antibodies. In certain aspects, disclosed herein is an anti-tau antibody. In some instances, the anti-tau antibody specifically binds to mammalian tau. In some instances, the anti-tau antibody specifically binds to a human tau. In some instances, the anti-tau antibody specifically binds to an N-terminal portion of tau. In some instances, the anti-tau antibody specifically binds to an N-terminal portion of human tau. In some instances, the anti-tau antibody specifically binds to an portion of tau comprising protein domain P2. In some instances, the anti-tau antibody specifically binds to an portion of human tau comprising protein domain P2. In some instances, the anti-tau antibody specifically binds to an portion of tau comprising protein domain P1. In some instances, the anti-tau antibody specifically binds to an portion of human tau comprising protein domain P1. In some instances, the anti-tau antibody specifically binds to an portion of tau comprising protein domains P1 and P2. In some instances, the anti-tau antibody specifically binds to an portion of human tau comprising protein domains P1 and P2.

[0055] In some embodiments, the anti-tau antibody comprises i) a heavy chain comprising a variable heavy chain (VH) domain and ii) a light chain comprising a variable light chain (VL) domain. In some embodiments, VH domain comprises heavy chain CDR1 (HCDR1) sequence comprising a sequence selected from SEQ ID NOs: 1-5, heavy chain CDR2 (HCDR2) sequence comprising a sequence selected from SEQ ID NOs: 6-9, and heavy chain CDR3 (HCDR3) sequence comprising a sequence selected from SEQ ID NOs: 10-13. In some embodiments, VL domain comprises light chain CDR1 (LCDR1) sequence comprising a sequence selected from SEQ ID NOs: 14-19, light chain CDR2 (LCDR2) sequence comprising a sequence selected from SEQ ID NOs: 20-23, and light chain CDR3 (LCDR3) sequence comprising a sequence selected from SEQ ID NOs: 24-29.

[0056] In some embodiments, the VH region of the anti-tau antibody comprises HCDR1, HCDR2, and HCDR3 sequences selected from Table 1.

Table 1. HCDR Amino Acid Sequences

SEQ ID NO:	HCDR1 Sequence
1	SQKVG
2	SYAMI
3	NYKVG
4	NYAMS
5	THAMT

SEQ ID NO:	HCDR2 Sequence
6	IINNYGSTYYASWAKG
7	FISRSGITYYASWAKG
8	IINYYSQTYYASWAKG
9	VINPSGSAYYATWVNG
SEQ ID NO:	**HCDR3 Sequence**
10	DPDGSIVFDI
11	EFGAVGSDYYRDAFNL
12	EFGAVGSDYYRDALRL
13	DYITAGDYYMDAFDP

[0057] In some embodiments, the VH region comprises HCDR1 sequence comprising SEQ ID NO: 1; HCDR2 sequence comprising SEQ ID NO: 6; and HCDR3 sequence comprising SEQ ID NO: 10. In some embodiments, the VH region comprises HCDR1 sequence comprising SEQ ID NO: 2; HCDR2 sequence comprising SEQ ID NO: 7; and HCDR3 sequence comprising SEQ ID NO: 11. In some embodiments, the VH region comprises HCDR1 sequence comprising SEQ ID NO: 3; HCDR2 sequence comprising SEQ ID NO: 8; and HCDR3 sequence comprising SEQ ID NO: 10. In some embodiments, the VH region comprises HCDR1 sequence comprising SEQ ID NO: 4; HCDR2 sequence comprising SEQ ID NO: 7; and HCDR3 sequence comprising SEQ ID NO: 12. In some embodiments, the VH region comprises HCDR1 sequence comprising SEQ ID NO: 5; HCDR2 sequence comprising SEQ ID NO: 9; and HCDR3 sequence comprising SEQ ID NO: 13.

[0058] In some embodiments, the VL region of the anti-tau antibody comprises LCDR1, LCDR2, and LCDR3 sequences selected from Table 2.

Table 2. LCDR Amino Acid Sequences

SEQ ID NO:	LCDR1 Sequence
14	QSSQSVVYNNRLS
15	QASESINSWLS
16	QASQNIYSNLA
17	QSSQSVYSNKRLA
18	QASQSIGSNLA
19	QASQSISNQLS
SEQ ID NO:	**LCDR2 Sequence**

20	GASTLAS
21	RASTLAS
22	GASNLAS
23	GASTLES
SEQ ID NO:	**LCDR3 Sequence**
24	LGSYDCSSGDCHA
25	QSYYEEDGIGYA
26	QGYDYSTAGAYP
27	AGGYDCSTGDCWT
28	QSYYEGSDIGYA
29	QQGYNRDNVDNL

[0059] In some embodiments, the VL region comprises LCDR1 sequence comprising SEQ ID NO: 14; LCDR2 sequence comprising SEQ ID NO: 20; and LCDR3 sequence comprising SEQ ID NO: 24. In some embodiments, the VL region comprises LCDR1 sequence comprising SEQ ID NO: 15; LCDR2 sequence comprising SEQ ID NO: 21; and LCDR3 sequence comprising SEQ ID NO: 25. In some embodiments, the VL region comprises LCDR1 sequence comprising SEQ ID NO: 16; LCDR2 sequence comprising SEQ ID NO: 22; and LCDR3 sequence comprising SEQ ID NO: 26. In some embodiments, the VL region comprises LCDR1 sequence comprising SEQ ID NO: 17; LCDR2 sequence comprising SEQ ID NO: 20; and LCDR3 sequence comprising SEQ ID NO: 27. In some embodiments, the VL region comprises LCDR1 sequence comprising SEQ ID NO: 18; LCDR2 sequence comprising SEQ ID NO: 23; and LCDR3 sequence comprising SEQ ID NO: 28. In some embodiments, the VL region comprises LCDR1 sequence comprising SEQ ID NO: 19; LCDR2 sequence comprising SEQ ID NO: 21; and LCDR3 sequence comprising SEQ ID NO: 29.

[0060] In some embodiments, the anti-tau antibody is an antigen binding fragment thereof. In some embodiments, the anti-tau antibody is a chimeric antibody or antigen binding fragment thereof. In some embodiments, the anti-tau antibody comprises an IgG-scFv, nanobody, mini-antibody, minibody, scFv-CH3 KIH, Fab-scFv-Fc KIH, Fab-scFv, scFv-CH-CL-scFv, Fab', F(ab')2, F(ab')3, F(ab')2-scFv2, scFv, scFv-KIH, Fab-scFv-Fc, or intrabody. In some embodiments, the anti-tau antibody comprises a bispecific antibody. In some embodiments, the anti-tau antibody comprises a multispecific antibody. In some embodiments, the anti-tau antibody is an IgG1 antibody. In some embodiments, the anti-tau antibody is an IgG2 antibody.

In some embodiments, the anti-tau antibody is an IgG4 antibody. In some embodiments, the anti-tau antibody comprises a light chain wherein the light chain is a kappa chain.

[0061] In some embodiments, the anti-tau antibody has a binding affinity to human tau of about 100 pM to about 3 nM. In some embodiments, the anti-tau antibody has a binding affinity to human tau of about 100 pM to 300 pM. In some embodiments, the anti-tau antibody has a binding affinity to human tau of about 100 pM to 500 pM. In some embodiments, the anti-tau antibody has a binding affinity to human tau of about 100 pM to 800 pM. In some embodiments, the anti-tau antibody has a binding affinity to human tau of about 300 pM to 600 pM. In some embodiments, the anti-tau antibody has a binding affinity to human tau of about 300 pM to 900 pM. In some embodiments, the anti-tau antibody has a binding affinity to human tau of about 400 pM to 1 nM. In some embodiments, the anti-tau antibody has a binding affinity to human tau of about 500 pM to 1.5 nM. In some embodiments, the anti-tau antibody has a binding affinity to human tau of about 500 pM to 2 nM. In some embodiments, the anti-tau antibody has a binding affinity to human tau of about 600 pM to 3 nM. In some embodiments, the anti-tau antibody has a binding affinity to human tau of about 100 pM to about 3 nM.

[0062] In some embodiments, the anti-tau antibody has a binding affinity to phosphorylated human tau of about 100 pM to 300 pM. In some embodiments, the anti-tau antibody has a binding affinity to phosphorylated human tau of about 100 pM to 500 pM. In some embodiments, the anti-tau antibody has a binding affinity to phosphorylated human tau of about 100 pM to 800 pM. In some embodiments, the anti-tau antibody has a binding affinity to phosphorylated human tau of about 300 pM to 600 pM. In some embodiments, the anti-tau antibody has a binding affinity to phosphorylated human tau of about 300 pM to 900 pM. In some embodiments, the anti-tau antibody has a binding affinity to phosphorylated human tau of about 400 pM to 1 nM. In some embodiments, the anti-tau antibody has a binding affinity to phosphorylated human tau of about 500 pM to 1.5 nM. In some embodiments, the anti-tau antibody has a binding affinity to phosphorylated human tau of about 500 pM to 2 nM. In some embodiments, the anti-tau antibody has a binding affinity to phosphorylated human tau of about 600 pM to 3 nM.

[0063] Described herein are antibodies comprising a sequence of any sequence set forth in **Table 3** or **Table 4**.

Table 3. Variable Domain, Heavy Chain

Name	SEQ ID NO:	Amino Acid Sequence
Antibody 1 Variable Domain, Heavy Chain	30	METGLRWLLLVAVLKGVQCQSLEESGGRLVTPGTPLTLT CTVSGFSLSSQKVGWVRQAPGKGLEWIGIINNYGSTYYAS WAKGRFTISKTSTTVDLRITSLTAEDTATYFCARDPDGSIV FDIWGPGTLVTVSL
Antibody 2 and Antibody 3 Variable Domain, Heavy Chain	31	METGLRWLLLVAVLKGVQCQSVEESGGRLVTPGTPLTLT CTVSGFSLSSYAMIWVRQAPGKGLEWIGFISRSGITYYASW AKGRFTISKTSTTVDLKMTSLTTEDTATYFCAREFGAVGS DYYRDAFNLWGPGTLVTVSS
Antibody 4 Variable Domain, Heavy Chain	32	METGLRWLLLVAVLKGVQCQSLEESGGRLVTPGTPLTLT CTVSGFSLNNYKVGWVRQAPGKGLEWIGIINYYSQTYYA SWAKGRFTISKTSTTVDLKLTSPTTEDTATYFCARDPDGS IVFDIWGPGTLVTVSL
Antibody 5 Variable Domain, Heavy Chain	33	METGLRWLLLVAVLKGVQCQSVEESGGGLVTPGGTLTLT CTVSGFSLSNYAMSWVRQAPGKGLEWIGFISRSGITYYAS WAKGRFTISKTSTTVDLKITSPTTEDTAAYFCAREFGAVGS DYYRDALRLWGPGTLVTVSS
Antibody 6 Variable Domain, Heavy Chain	34	METGLRWLLLVAVLKGVQCQSLEESGGRLVTPGTPLTLT CTVSGIDLSTHAMTWVRQAPGKGLEWIGVINPSGSAYYA TWVNGRFTISKTSTTVDLKITSPTTGDTAKYFCARDYITA GDYYMDAFDPWGPGTLVTVSS

Table 4. Variable Domain, Light Chain

Name	SEQ ID NO:	Amino Acid Sequence
Antibody 1 Variable Domain, Light Chain	35	MDTRAPTQLLGLLLLWLPGATFAQVLTQTASPVSAAVG GTVTINCQSSQSVVYNNRLSWFQQKPGQPPKLLIYGAST LASGVPSRFKGSGSGTQFTLTISDVQCDDAATYYCLGSY DCSSGDCHAFGGGTEVVVK
Antibody 2 Variable Domain, Light Chain	36	MDMRAPTQLLGLLLLWLPGARCADIVMTQTPASVEAA VGGTVTINCQASESINSWLSWYQQKPGQPPNLLIYRAST LASGVPSRFSGGGSGTEYTLTISDLECADAVTYYCQSYY EEDGIGYAFGGGTEVVVE
Antibody 3 Variable	37	MDMRAPTQLLGLLLLWLPGARCADIVMTQTPSSVSAA VGGTVTINCQASQNIYSNLAWYQQKPGQRPRLLIYGAS

Domain, Light Chain		NLASGVPSRFKGSRSGTEFTLTISDLECADAATYYCQGY DYSTAGAYPFGGGTAVVVK
Antibody 4 Variable Domain, Light Chain	38	MDTRAPTQLLGLLLLWLPGATFAQVLTQTASPVSAAV GSTVTINCQSSQSVYSNKRLAWFQLKPGQPPKLLIYGAS TLASGVPSRFKGSGSGTQFTLTISDVQCDDAATYYCAGG YDCSTGDCWTFGGGTEVVVT
Antibody 5 Variable Domain, Light Chain	39	MDMRAPTQLLGLLLLWLPGARCADIVMTQTPSSVSAA VGGTVTIKCQASQSIGSNLAWYQQKPGQPPKLLIYGAS TLESGVPSRFKGSGSGTEYTLTISDLECADAATYYCQSY YEGSDIGYAFGGGTEVVVE
Antibody 6 Variable Domain, Light Chain	40	MDTRAPTQLLGLLLLWLPGARCADIVMTQTPASVSAA VGGTVTIKCQASQSISNQLSWYQQKSGQPPKLLIYRAS TLASGVPSRFKGSGSGTEFTLTISDLECADAATYYCQQ GYNRDNVDNLFGGGTEVVVK

[0064] In some embodiments, the variable domain, heavy chain region (VH) comprises an amino acid sequence that has at least 70% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence that has at least 80% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence that has at least 85% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence that has at least 90% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence that has at least 91% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence that has at least 92% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence that has at least 93% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence that has at least 94% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence that has at least 95% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence that has at least 96% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence that has at least 97% sequence identity to the amino acid sequence

according to any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence that has at least 98% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence that has at least 99% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence according to any one of SEQ ID NOs: 30-34.

[0065] In some embodiments, the VH comprises an amino acid sequence of at least 50 consecutive amino acid residues of any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence of at least 60 consecutive amino acid residues of any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence of at least 70 consecutive amino acid residues of any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence of at least 80 consecutive amino acid residues of any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence of at least 90 consecutive amino acid residues of any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence of at least 100 consecutive amino acid residues of any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence of at least 105 consecutive amino acid residues of any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence of at least 110 consecutive amino acid residues of any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence of at least 115 consecutive amino acid residues of any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence of at least 120 consecutive amino acid residues of any one of SEQ ID NOs: 30-34.

[0066] In some embodiments, the VH comprises an amino acid sequence of at least 50 consecutive amino acid residues of any one of SEQ ID NOs: 30-34, and has at least 80% sequence identity to the at least 50 consecutive amino acid residues of any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence of at least 60 consecutive amino acid residues of any one of SEQ ID NOs: 30-34, and has at least 80% sequence identity to the at least 60 consecutive amino acid residues of any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence of at least 70 consecutive amino acid residues of any one of SEQ ID NOs: 30-34, and has at least 80% sequence identity to the at least 70 consecutive amino acid residues of any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence of at least 80 consecutive amino acid residues of any one of SEQ ID NOs: 30-34, and has at least 80% sequence identity to the at least 80 consecutive amino acid residues of any one of SEQ ID NOs:

30-34. In some embodiments, the VH comprises an amino acid sequence of at least 90 consecutive amino acid residues of any one of SEQ ID NOs: 30-34, and has at least 80% sequence identity to the at least 90 consecutive amino acid residues of any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence of at least 100 consecutive amino acid residues of any one of SEQ ID NOs: 30-34, and has at least 80% sequence identity to the at least 100 consecutive amino acid residues of any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence of at least 105 consecutive amino acid residues of any one of SEQ ID NOs: 30-34, and has at least 80% sequence identity to the at least 105 consecutive amino acid residues of any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence of at least 110 consecutive amino acid residues of any one of SEQ ID NOs: 30-34, and has at least 80% sequence identity to the at least 110 consecutive amino acid residues of any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence of at least 115 consecutive amino acid residues of any one of SEQ ID NOs: 30-34, and has at least 80% sequence identity to the at least 115 consecutive amino acid residues of any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence of at least 120 consecutive amino acid residues of any one of SEQ ID NOs: 30-34, and has at least 80% sequence identity to the at least 120 consecutive amino acid residues of any one of SEQ ID NOs: 30-34.

[0067] In some embodiments, the VH comprises an amino acid sequence of at least 50 consecutive amino acid residues of any one of SEQ ID NOs: 30-34, and has at least 90% sequence identity to the at least 50 consecutive amino acid residues of any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence of at least 60 consecutive amino acid residues of any one of SEQ ID NOs: 30-34, and has at least 90% sequence identity to the at least 60 consecutive amino acid residues of any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence of at least 70 consecutive amino acid residues of any one of SEQ ID NOs: 30-34, and has at least 90% sequence identity to the at least 70 consecutive amino acid residues of any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence of at least 80 consecutive amino acid residues of any one of SEQ ID NOs: 30-34, and has at least 90% sequence identity to the at least 80 consecutive amino acid residues of any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence of at least 90 consecutive amino acid residues of any one of SEQ ID NOs: 30-34, and has at least 90% sequence identity to the at least 90 consecutive amino acid residues of any one of SEQ ID NOs:

30-34. In some embodiments, the VH comprises an amino acid sequence of at least 100 consecutive amino acid residues of any one of SEQ ID NOs: 30-34, and has at least 90% sequence identity to the at least 100 consecutive amino acid residues of any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence of at least 105 consecutive amino acid residues of any one of SEQ ID NOs: 30-34, and has at least 90% sequence identity to the at least 105 consecutive amino acid residues of any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence of at least 110 consecutive amino acid residues of any one of SEQ ID NOs: 30-34, and has at least 90% sequence identity to the at least 110 consecutive amino acid residues of any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence of at least 115 consecutive amino acid residues of any one of SEQ ID NOs: 30-34, and has at least 90% sequence identity to the at least 115 consecutive amino acid residues of any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence of at least 120 consecutive amino acid residues of any one of SEQ ID NOs: 30-34, and has at least 90% sequence identity to the at least 120 consecutive amino acid residues of any one of SEQ ID NOs: 30-34.

[0068] In some embodiments, the VH comprises an amino acid sequence of at least 50 consecutive amino acid residues of any one of SEQ ID NOs: 30-34, and has at least 95% sequence identity to the at least 50 consecutive amino acid residues of any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence of at least 60 consecutive amino acid residues of any one of SEQ ID NOs: 30-34, and has at least 95% sequence identity to the at least 60 consecutive amino acid residues of any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence of at least 70 consecutive amino acid residues of any one of SEQ ID NOs: 30-34, and has at least 95% sequence identity to the at least 70 consecutive amino acid residues of any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence of at least 80 consecutive amino acid residues of any one of SEQ ID NOs: 30-34, and has at least 95% sequence identity to the at least 80 consecutive amino acid residues of any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence of at least 90 consecutive amino acid residues of any one of SEQ ID NOs: 30-34, and has at least 95% sequence identity to the at least 90 consecutive amino acid residues of any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence of at least 100 consecutive amino acid residues of any one of SEQ ID NOs: 30-34, and has at least 95% sequence identity to the at least 100 consecutive amino acid residues of any one of SEQ ID NOs:

30-34. In some embodiments, the VH comprises an amino acid sequence of at least 105 consecutive amino acid residues of any one of SEQ ID NOs: 30-34, and has at least 95% sequence identity to the at least 105 consecutive amino acid residues of any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence of at least 110 consecutive amino acid residues of any one of SEQ ID NOs: 30-34, and has at least 95% sequence identity to the at least 110 consecutive amino acid residues of any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence of at least 115 consecutive amino acid residues of any one of SEQ ID NOs: 30-34, and has at least 95% sequence identity to the at least 115 consecutive amino acid residues of any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence of at least 120 consecutive amino acid residues of any one of SEQ ID NOs: 30-34, and has at least 95% sequence identity to the at least 120 consecutive amino acid residues of any one of SEQ ID NOs: 30-34.

[0069] In some embodiments, the VH comprises an amino acid sequence of at least 100 consecutive amino acid residues of any one of SEQ ID NOs: 30-34, and has at least 99% sequence identity to the at least 100 consecutive amino acid residues of any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence of at least 105 consecutive amino acid residues of any one of SEQ ID NOs: 30-34, and has at least 99% sequence identity to the at least 105 consecutive amino acid residues of any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence of at least 110 consecutive amino acid residues of any one of SEQ ID NOs: 30-34, and has at least 99% sequence identity to the at least 110 consecutive amino acid residues of any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence of at least 115 consecutive amino acid residues of any one of SEQ ID NOs: 30-34, and has at least 99% sequence identity to the at least 115 consecutive amino acid residues of any one of SEQ ID NOs: 30-34. In some embodiments, the VH comprises an amino acid sequence of at least 120 consecutive amino acid residues of any one of SEQ ID NOs: 30-34, and has at least 99% sequence identity to the at least 120 consecutive amino acid residues of any one of SEQ ID NOs: 30-34.

[0070] In some embodiments, the variable domain, light chain region (VL) comprises an amino acid sequence that has at least 70% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 35-40. In some embodiments, the VL comprises an amino acid sequence that has at least 80% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 35-40. In some embodiments, the VL comprises an amino acid

sequence that has at least 85% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 35-40. In some embodiments, the VL comprises an amino acid sequence that has at least 90% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 35-40. In some embodiments, the VL comprises an amino acid sequence that has at least 91% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 35-40. In some embodiments, the VL comprises an amino acid sequence that has at least 92% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 35-40. In some embodiments, the VL comprises an amino acid sequence that has at least 93% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 35-40. In some embodiments, the VL comprises an amino acid sequence that has at least 94% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 35-40. In some embodiments, the VL comprises an amino acid sequence that has at least 95% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 35-40. In some embodiments, the VL comprises an amino acid sequence that has at least 96% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 35-40. In some embodiments, the VL comprises an amino acid sequence that has at least 97% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 35-40. In some embodiments, the VL comprises an amino acid sequence that has at least 98% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 35-40. In some embodiments, the VL comprises an amino acid sequence that has at least 99% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 35-40. In some embodiments, the VL comprises an amino acid sequence according to any one of SEQ ID NOs: 35-40.

[0071] In some embodiments, the VL comprises an amino acid sequence of at least 50 consecutive amino acid residues of any one of SEQ ID NOs: 35-40. In some embodiments, the VL comprises an amino acid sequence of at least 60 consecutive amino acid residues of any one of SEQ ID NOs: 35-40. In some embodiments, the VL comprises an amino acid sequence of at least 70 consecutive amino acid residues of any one of SEQ ID NOs: 35-40. In some embodiments, the VL comprises an amino acid sequence of at least 80 consecutive amino acid residues of any one of SEQ ID NOs: 35-40. In some embodiments, the VL comprises an amino acid sequence of at least 90 consecutive amino acid residues of any one of SEQ ID NOs: 35-40. In some embodiments, the VL comprises an amino acid sequence of at least 100 consecutive amino acid residues of any one of SEQ ID NOs: 35-40. In some embodiments, the VL comprises an amino acid sequence of at least 105 consecutive amino acid residues of any one of SEQ ID NOs: 35-40.

[0072] In some embodiments, the VL comprises an amino acid sequence of at least 50 consecutive amino acid residues of any one of SEQ ID NOs: 35-40, and has at least 80% sequence identity to the at least 50 consecutive amino acid residues of any one of SEQ ID NOs: 35-40. In some embodiments, the VL comprises an amino acid sequence of at least 60 consecutive amino acid residues of any one of SEQ ID NOs: 35-40, and has at least 80% sequence identity to the at least 60 consecutive amino acid residues of any one of SEQ ID NOs: 35-40. In some embodiments, the VL comprises an amino acid sequence of at least 70 consecutive amino acid residues of any one of SEQ ID NOs: 35-40, and has at least 80% sequence identity to the at least 70 consecutive amino acid residues of any one of SEQ ID NOs: 35-40. In some embodiments, the VL comprises an amino acid sequence of at least 80 consecutive amino acid residues of any one of SEQ ID NOs: 35-40, and has at least 80% sequence identity to the at least 80 consecutive amino acid residues of any one of SEQ ID NOs: 35-40. In some embodiments, the VL comprises an amino acid sequence of at least 90 consecutive amino acid residues of any one of SEQ ID NOs: 35-40, and has at least 80% sequence identity to the at least 90 consecutive amino acid residues of any one of SEQ ID NOs: 35-40. In some embodiments, the VL comprises an amino acid sequence of at least 100 consecutive amino acid residues of any one of SEQ ID NOs: 35-40, and has at least 80% sequence identity to the at least 100 consecutive amino acid residues of any one of SEQ ID NOs: 35-40. In some embodiments, the VL comprises an amino acid sequence of at least 105 consecutive amino acid residues of any one of SEQ ID NOs: 35-40, and has at least 80% sequence identity to the at least 105 consecutive amino acid residues of any one of SEQ ID NOs: 35-40.

[0073] In some embodiments, the VL comprises an amino acid sequence of at least 50 consecutive amino acid residues of any one of SEQ ID NOs: 35-40, and has at least 90% sequence identity to the at least 50 consecutive amino acid residues of any one of SEQ ID NOs: 35-40. In some embodiments, the VL comprises an amino acid sequence of at least 60 consecutive amino acid residues of any one of SEQ ID NOs: 35-40, and has at least 90% sequence identity to the at least 60 consecutive amino acid residues of any one of SEQ ID NOs: 35-40. In some embodiments, the VL comprises an amino acid sequence of at least 70 consecutive amino acid residues of any one of SEQ ID NOs: 35-40, and has at least 90% sequence identity to the at least 70 consecutive amino acid residues of any one of SEQ ID NOs: 35-40. In some embodiments, the VL comprises an amino acid sequence of at least 80 consecutive amino acid residues of any one of SEQ ID NOs: 35-40, and has at least 90% sequence identity to the at least 80 consecutive amino acid residues of any one of SEQ ID NOs:

35-40. In some embodiments, the VL comprises an amino acid sequence of at least 90 consecutive amino acid residues of any one of SEQ ID NOs: 35-40, and has at least 90% sequence identity to the at least 90 consecutive amino acid residues of any one of SEQ ID NOs: 35-40. In some embodiments, the VL comprises an amino acid sequence of at least 100 consecutive amino acid residues of any one of SEQ ID NOs: 35-40, and has at least 90% sequence identity to the at least 100 consecutive amino acid residues of any one of SEQ ID NOs: 35-40. In some embodiments, the VL comprises an amino acid sequence of at least 105 consecutive amino acid residues of any one of SEQ ID NOs: 35-40, and has at least 90% sequence identity to the at least 105 consecutive amino acid residues of any one of SEQ ID NOs: 35-40.

[0074] In some embodiments, the VL comprises an amino acid sequence of at least 50 consecutive amino acid residues of any one of SEQ ID NOs: 35-40, and has at least 95% sequence identity to the at least 50 consecutive amino acid residues of any one of SEQ ID NOs: 35-40. In some embodiments, the VL comprises an amino acid sequence of at least 60 consecutive amino acid residues of any one of SEQ ID NOs: 35-40, and has at least 95% sequence identity to the at least 60 consecutive amino acid residues of any one of SEQ ID NOs: 35-40. In some embodiments, the VL comprises an amino acid sequence of at least 70 consecutive amino acid residues of any one of SEQ ID NOs: 35-40, and has at least 95% sequence identity to the at least 70 consecutive amino acid residues of any one of SEQ ID NOs: 35-40. In some embodiments, the VL comprises an amino acid sequence of at least 80 consecutive amino acid residues of any one of SEQ ID NOs: 35-40, and has at least 95% sequence identity to the at least 80 consecutive amino acid residues of any one of SEQ ID NOs: 35-40. In some embodiments, the VL comprises an amino acid sequence of at least 90 consecutive amino acid residues of any one of SEQ ID NOs: 35-40, and has at least 95% sequence identity to the at least 90 consecutive amino acid residues of any one of SEQ ID NOs: 35-40. In some embodiments, the VL comprises an amino acid sequence of at least 100 consecutive amino acid residues of any one of SEQ ID NOs: 35-40, and has at least 95% sequence identity to the at least 100 consecutive amino acid residues of any one of SEQ ID NOs: 35-40. In some embodiments, the VL comprises an amino acid sequence of at least 105 consecutive amino acid residues of any one of SEQ ID NOs: 35-40, and has at least 95% sequence identity to the at least 105 consecutive amino acid residues of any one of SEQ ID NOs: 35-40. In some embodiments, the VL comprises an amino acid sequence of at least 100 consecutive amino acid residues of any one of SEQ ID NOs: 35-40, and has at least 99% sequence identity to the at least 100 consecutive amino acid residues of any one of SEQ ID NOs:

35-40. In some embodiments, the VL comprises an amino acid sequence of at least 105 consecutive amino acid residues of any one of SEQ ID NOs: 35-40, and has at least 99% sequence identity to the at least 105 consecutive amino acid residues of any one of SEQ ID NOs: 35-40.

[0075] In some embodiments, the VH comprises an amino acid sequence that has at least 70% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 30-34; and the VL comprises an amino acid sequence that has at least 70% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 35-40. In some embodiments, the VH comprises an amino acid sequence that has at least 80% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 30-34; and the VL comprises an amino acid sequence that has at least 80% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 35-40. In some embodiments, the VH comprises an amino acid sequence that has at least 85% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 30-34; and the VL comprises an amino acid sequence that has at least 85% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 35-40. In some embodiments, the VH comprises an amino acid sequence that has at least 90% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 30-34; and the VL comprises an amino acid sequence that has at least 90% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 35-40. In some embodiments, the VH comprises an amino acid sequence that has at least 91% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 30-34; and the VL comprises an amino acid sequence that has at least 91% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 35-40. In some embodiments, the VH comprises an amino acid sequence that has at least 92% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 30-34; and the VL comprises an amino acid sequence that has at least 92% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 35-40. In some embodiments, the VH comprises an amino acid sequence that has at least 93% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 30-34; and the VL comprises an amino acid sequence that has at least 93% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 35-40. In some embodiments, the VH comprises an amino acid sequence that has at least 94% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 30-34; and the VL comprises an amino acid sequence that has at least 94% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 35-40. In some embodiments, the VH comprises an amino acid sequence that has at least 95% sequence identity

to the amino acid sequence according to any one of SEQ ID NOs: 30-34; and the VL comprises an amino acid sequence that has at least 95% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 35-40. In some embodiments, the VH comprises an amino acid sequence that has at least 96% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 30-34; and the VL comprises an amino acid sequence that has at least 96% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 35-40. In some embodiments, the VH comprises an amino acid sequence that has at least 97% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 30-34; and the VL comprises an amino acid sequence that has at least 97% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 35-40. In some embodiments, the VH comprises an amino acid sequence that has at least 98% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 30-34; and the VL comprises an amino acid sequence that has at least 98% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 35-40. In some embodiments, the VH comprises an amino acid sequence that has at least 99% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 30-34; and the VL comprises an amino acid sequence that has at least 99% sequence identity to the amino acid sequence according to any one of SEQ ID NOs: 35-40.

[0076] In some embodiments, the VH comprises an amino acid sequence that has at least 70% sequence identity to the amino acid sequence according to SEQ ID NO: 30; and the VL comprises an amino acid sequence that has at least 70% sequence identity to the amino acid sequence according to SEQ ID NO: 35. In some embodiments, the VH comprises an amino acid sequence that has at least 80% sequence identity to the amino acid sequence according to SEQ ID NO: 30; and the VL comprises an amino acid sequence that has at least 80% sequence identity to the amino acid sequence according to SEQ ID NO: 35. In some embodiments, the VH comprises an amino acid sequence that has at least 85% sequence identity to the amino acid sequence according to SEQ ID NO: 30; and the VL comprises an amino acid sequence that has at least 85% sequence identity to the amino acid sequence according to SEQ ID NO: 35. In some embodiments, the VH comprises an amino acid sequence that has at least 90% sequence identity to the amino acid sequence according to SEQ ID NO: 30; and the VL comprises an amino acid sequence that has at least 90% sequence identity to the amino acid sequence according to SEQ ID NO: 35. In some embodiments, the VH comprises an amino acid sequence that has at least 91% sequence identity to the amino acid sequence according to SEQ ID NO: 30; and the VL comprises an amino acid sequence that has at least 91% sequence identity to the amino acid sequence according to SEQ ID NO: 35. In some embodiments, the VH comprises an amino acid

sequence that has at least 92% sequence identity to the amino acid sequence according to SEQ ID NO: 30; and the VL comprises an amino acid sequence that has at least 92% sequence identity to the amino acid sequence according to SEQ ID NO: 35. In some embodiments, the VH comprises an amino acid sequence that has at least 93% sequence identity to the amino acid sequence according to SEQ ID NO: 30; and the VL comprises an amino acid sequence that has at least 93% sequence identity to the amino acid sequence according to SEQ ID NO: 35. In some embodiments, the VH comprises an amino acid sequence that has at least 94% sequence identity to the amino acid sequence according to SEQ ID NO: 30; and the VL comprises an amino acid sequence that has at least 94% sequence identity to the amino acid sequence according to SEQ ID NO: 35. In some embodiments, the VH comprises an amino acid sequence that has at least 95% sequence identity to the amino acid sequence according to SEQ ID NO: 30; and the VL comprises an amino acid sequence that has at least 95% sequence identity to the amino acid sequence according to SEQ ID NO: 35. In some embodiments, the VH comprises an amino acid sequence that has at least 96% sequence identity to the amino acid sequence according to SEQ ID NO: 30; and the VL comprises an amino acid sequence that has at least 96% sequence identity to the amino acid sequence according to SEQ ID NO: 35. In some embodiments, the VH comprises an amino acid sequence that has at least 97% sequence identity to the amino acid sequence according to SEQ ID NO: 30; and the VL comprises an amino acid sequence that has at least 97% sequence identity to the amino acid sequence according to SEQ ID NO: 35. In some embodiments, the VH comprises an amino acid sequence that has at least 98% sequence identity to the amino acid sequence according to SEQ ID NO: 30; and the VL comprises an amino acid sequence that has at least 98% sequence identity to the amino acid sequence according to SEQ ID NO: 35. In some embodiments, the VH comprises an amino acid sequence that has at least 99% sequence identity to the amino acid sequence according to SEQ ID NO: 30; and the VL comprises an amino acid sequence that has at least 99% sequence identity to the amino acid sequence according to SEQ ID NO: 35.

[0077] In some embodiments, the VH comprises an amino acid sequence that has at least 70% sequence identity to the amino acid sequence according to SEQ ID NO: 31; and the VL comprises an amino acid sequence that has at least 70% sequence identity to the amino acid sequence according to SEQ ID NO: 36. In some embodiments, the VH comprises an amino acid sequence that has at least 80% sequence identity to the amino acid sequence according to SEQ ID NO: 31; and the VL comprises an amino acid sequence that has at least 80% sequence identity to the amino acid sequence according to SEQ ID NO: 36. In some embodiments, the VH comprises an amino acid sequence that has at least 85% sequence identity to the amino acid

sequence according to SEQ ID NO: 31; and the VL comprises an amino acid sequence that has at least 85% sequence identity to the amino acid sequence according to SEQ ID NO: 36. In some embodiments, the VH comprises an amino acid sequence that has at least 90% sequence identity to the amino acid sequence according to SEQ ID NO: 31; and the VL comprises an amino acid sequence that has at least 90% sequence identity to the amino acid sequence according to SEQ ID NO: 36. In some embodiments, the VH comprises an amino acid sequence that has at least 91% sequence identity to the amino acid sequence according to SEQ ID NO: 31; and the VL comprises an amino acid sequence that has at least 91% sequence identity to the amino acid sequence according to SEQ ID NO: 36. In some embodiments, the VH comprises an amino acid sequence that has at least 92% sequence identity to the amino acid sequence according to SEQ ID NO: 31; and the VL comprises an amino acid sequence that has at least 92% sequence identity to the amino acid sequence according to SEQ ID NO: 36. In some embodiments, the VH comprises an amino acid sequence that has at least 93% sequence identity to the amino acid sequence according to SEQ ID NO: 31; and the VL comprises an amino acid sequence that has at least 93% sequence identity to the amino acid sequence according to SEQ ID NO: 36. In some embodiments, the VH comprises an amino acid sequence that has at least 94% sequence identity to the amino acid sequence according to SEQ ID NO: 31; and the VL comprises an amino acid sequence that has at least 94% sequence identity to the amino acid sequence according to SEQ ID NO: 36. In some embodiments, the VH comprises an amino acid sequence that has at least 95% sequence identity to the amino acid sequence according to SEQ ID NO: 31; and the VL comprises an amino acid sequence that has at least 95% sequence identity to the amino acid sequence according to SEQ ID NO: 36. In some embodiments, the VH comprises an amino acid sequence that has at least 96% sequence identity to the amino acid sequence according to SEQ ID NO: 31; and the VL comprises an amino acid sequence that has at least 96% sequence identity to the amino acid sequence according to SEQ ID NO: 36. In some embodiments, the VH comprises an amino acid sequence that has at least 97% sequence identity to the amino acid sequence according to SEQ ID NO: 31; and the VL comprises an amino acid sequence that has at least 97% sequence identity to the amino acid sequence according to SEQ ID NO: 36. In some embodiments, the VH comprises an amino acid sequence that has at least 98% sequence identity to the amino acid sequence according to SEQ ID NO: 31; and the VL comprises an amino acid sequence that has at least 98% sequence identity to the amino acid sequence according to SEQ ID NO: 36. In some embodiments, the VH comprises an amino acid sequence that has at least 99% sequence identity to the amino acid sequence according to SEQ ID NO: 31; and the VL

comprises an amino acid sequence that has at least 99% sequence identity to the amino acid sequence according to SEQ ID NO: 36.

[0078] In some embodiments, the VH comprises an amino acid sequence that has at least 70% sequence identity to the amino acid sequence according to SEQ ID NO: 31; and the VL comprises an amino acid sequence that has at least 70% sequence identity to the amino acid sequence according to SEQ ID NO: 37. In some embodiments, the VH comprises an amino acid sequence that has at least 80% sequence identity to the amino acid sequence according to SEQ ID NO: 31; and the VL comprises an amino acid sequence that has at least 80% sequence identity to the amino acid sequence according to SEQ ID NO: 37. In some embodiments, the VH comprises an amino acid sequence that has at least 85% sequence identity to the amino acid sequence according to SEQ ID NO: 31; and the VL comprises an amino acid sequence that has at least 85% sequence identity to the amino acid sequence according to SEQ ID NO: 37. In some embodiments, the VH comprises an amino acid sequence that has at least 90% sequence identity to the amino acid sequence according to SEQ ID NO: 31; and the VL comprises an amino acid sequence that has at least 90% sequence identity to the amino acid sequence according to SEQ ID NO: 37. In some embodiments, the VH comprises an amino acid sequence that has at least 91% sequence identity to the amino acid sequence according to SEQ ID NO: 31; and the VL comprises an amino acid sequence that has at least 91% sequence identity to the amino acid sequence according to SEQ ID NO: 37. In some embodiments, the VH comprises an amino acid sequence that has at least 92% sequence identity to the amino acid sequence according to SEQ ID NO: 31; and the VL comprises an amino acid sequence that has at least 92% sequence identity to the amino acid sequence according to SEQ ID NO: 37. In some embodiments, the VH comprises an amino acid sequence that has at least 93% sequence identity to the amino acid sequence according to SEQ ID NO: 31; and the VL comprises an amino acid sequence that has at least 93% sequence identity to the amino acid sequence according to SEQ ID NO: 37. In some embodiments, the VH comprises an amino acid sequence that has at least 94% sequence identity to the amino acid sequence according to SEQ ID NO: 31; and the VL comprises an amino acid sequence that has at least 94% sequence identity to the amino acid sequence according to SEQ ID NO: 37. In some embodiments, the VH comprises an amino acid sequence that has at least 95% sequence identity to the amino acid sequence according to SEQ ID NO: 31; and the VL comprises an amino acid sequence that has at least 95% sequence identity to the amino acid sequence according to SEQ ID NO: 37. In some embodiments, the VH comprises an amino acid sequence that has at least 96% sequence identity to the amino acid sequence according to SEQ ID NO: 31; and the VL comprises an amino acid sequence that has at least 96% sequence

identity to the amino acid sequence according to SEQ ID NO: 37. In some embodiments, the VH comprises an amino acid sequence that has at least 97% sequence identity to the amino acid sequence according to SEQ ID NO: 31; and the VL comprises an amino acid sequence that has at least 97% sequence identity to the amino acid sequence according to SEQ ID NO: 37. In some embodiments, the VH comprises an amino acid sequence that has at least 98% sequence identity to the amino acid sequence according to SEQ ID NO: 31; and the VL comprises an amino acid sequence that has at least 98% sequence identity to the amino acid sequence according to SEQ ID NO: 37. In some embodiments, the VH comprises an amino acid sequence that has at least 99% sequence identity to the amino acid sequence according to SEQ ID NO: 31; and the VL comprises an amino acid sequence that has at least 99% sequence identity to the amino acid sequence according to SEQ ID NO: 37.

[0079] In some embodiments, the VH comprises an amino acid sequence that has at least 70% sequence identity to the amino acid sequence according to SEQ ID NO: 32; and the VL comprises an amino acid sequence that has at least 70% sequence identity to the amino acid sequence according to SEQ ID NO: 38. In some embodiments, the VH comprises an amino acid sequence that has at least 80% sequence identity to the amino acid sequence according to SEQ ID NO: 32; and the VL comprises an amino acid sequence that has at least 80% sequence identity to the amino acid sequence according to SEQ ID NO: 38. In some embodiments, the VH comprises an amino acid sequence that has at least 85% sequence identity to the amino acid sequence according to SEQ ID NO: 32; and the VL comprises an amino acid sequence that has at least 85% sequence identity to the amino acid sequence according to SEQ ID NO: 38. In some embodiments, the VH comprises an amino acid sequence that has at least 90% sequence identity to the amino acid sequence according to SEQ ID NO: 32; and the VL comprises an amino acid sequence that has at least 90% sequence identity to the amino acid sequence according to SEQ ID NO: 38. In some embodiments, the VH comprises an amino acid sequence that has at least 91% sequence identity to the amino acid sequence according to SEQ ID NO: 32; and the VL comprises an amino acid sequence that has at least 91% sequence identity to the amino acid sequence according to SEQ ID NO: 38. In some embodiments, the VH comprises an amino acid sequence that has at least 92% sequence identity to the amino acid sequence according to SEQ ID NO: 32; and the VL comprises an amino acid sequence that has at least 92% sequence identity to the amino acid sequence according to SEQ ID NO: 38. In some embodiments, the VH comprises an amino acid sequence that has at least 93% sequence identity to the amino acid sequence according to SEQ ID NO: 32; and the VL comprises an amino acid sequence that has at least 93% sequence identity to the amino acid sequence according to SEQ ID NO: 38. In some

embodiments, the VH comprises an amino acid sequence that has at least 94% sequence identity to the amino acid sequence according to SEQ ID NO: 32; and the VL comprises an amino acid sequence that has at least 94% sequence identity to the amino acid sequence according to SEQ ID NO: 38. In some embodiments, the VH comprises an amino acid sequence that has at least 95% sequence identity to the amino acid sequence according to SEQ ID NO: 32; and the VL comprises an amino acid sequence that has at least 95% sequence identity to the amino acid sequence according to SEQ ID NO: 38. In some embodiments, the VH comprises an amino acid sequence that has at least 96% sequence identity to the amino acid sequence according to SEQ ID NO: 32; and the VL comprises an amino acid sequence that has at least 96% sequence identity to the amino acid sequence according to SEQ ID NO: 38. In some embodiments, the VH comprises an amino acid sequence that has at least 97% sequence identity to the amino acid sequence according to SEQ ID NO: 32; and the VL comprises an amino acid sequence that has at least 97% sequence identity to the amino acid sequence according to SEQ ID NO: 38. In some embodiments, the VH comprises an amino acid sequence that has at least 98% sequence identity to the amino acid sequence according to SEQ ID NO: 32; and the VL comprises an amino acid sequence that has at least 98% sequence identity to the amino acid sequence according to SEQ ID NO: 38. In some embodiments, the VH comprises an amino acid sequence that has at least 99% sequence identity to the amino acid sequence according to SEQ ID NO: 32; and the VL comprises an amino acid sequence that has at least 99% sequence identity to the amino acid sequence according to SEQ ID NO: 38.

[0080] In some embodiments, the VH comprises an amino acid sequence that has at least 70% sequence identity to the amino acid sequence according to SEQ ID NO: 33; and the VL comprises an amino acid sequence that has at least 70% sequence identity to the amino acid sequence according to SEQ ID NO: 39. In some embodiments, the VH comprises an amino acid sequence that has at least 80% sequence identity to the amino acid sequence according to SEQ ID NO: 33; and the VL comprises an amino acid sequence that has at least 80% sequence identity to the amino acid sequence according to SEQ ID NO: 39. In some embodiments, the VH comprises an amino acid sequence that has at least 85% sequence identity to the amino acid sequence according to SEQ ID NO: 33; and the VL comprises an amino acid sequence that has at least 85% sequence identity to the amino acid sequence according to SEQ ID NO: 39. In some embodiments, the VH comprises an amino acid sequence that has at least 90% sequence identity to the amino acid sequence according to SEQ ID NO: 33; and the VL comprises an amino acid sequence that has at least 90% sequence identity to the amino acid sequence according to SEQ ID NO: 39. In some embodiments, the VH comprises an amino acid sequence that has at least

91% sequence identity to the amino acid sequence according to SEQ ID NO: 33; and the VL comprises an amino acid sequence that has at least 91% sequence identity to the amino acid sequence according to SEQ ID NO: 39. In some embodiments, the VH comprises an amino acid sequence that has at least 92% sequence identity to the amino acid sequence according to SEQ ID NO: 33; and the VL comprises an amino acid sequence that has at least 92% sequence identity to the amino acid sequence according to SEQ ID NO: 39. In some embodiments, the VH comprises an amino acid sequence that has at least 93% sequence identity to the amino acid sequence according to SEQ ID NO: 33; and the VL comprises an amino acid sequence that has at least 93% sequence identity to the amino acid sequence according to SEQ ID NO: 39. In some embodiments, the VH comprises an amino acid sequence that has at least 94% sequence identity to the amino acid sequence according to SEQ ID NO: 33; and the VL comprises an amino acid sequence that has at least 94% sequence identity to the amino acid sequence according to SEQ ID NO: 39. In some embodiments, the VH comprises an amino acid sequence that has at least 95% sequence identity to the amino acid sequence according to SEQ ID NO: 33; and the VL comprises an amino acid sequence that has at least 95% sequence identity to the amino acid sequence according to SEQ ID NO: 39. In some embodiments, the VH comprises an amino acid sequence that has at least 96% sequence identity to the amino acid sequence according to SEQ ID NO: 33; and the VL comprises an amino acid sequence that has at least 96% sequence identity to the amino acid sequence according to SEQ ID NO: 39. In some embodiments, the VH comprises an amino acid sequence that has at least 97% sequence identity to the amino acid sequence according to SEQ ID NO: 33; and the VL comprises an amino acid sequence that has at least 97% sequence identity to the amino acid sequence according to SEQ ID NO: 39. In some embodiments, the VH comprises an amino acid sequence that has at least 98% sequence identity to the amino acid sequence according to SEQ ID NO: 33; and the VL comprises an amino acid sequence that has at least 98% sequence identity to the amino acid sequence according to SEQ ID NO: 39. In some embodiments, the VH comprises an amino acid sequence that has at least 99% sequence identity to the amino acid sequence according to SEQ ID NO: 33; and the VL comprises an amino acid sequence that has at least 99% sequence identity to the amino acid sequence according to SEQ ID NO: 39.

[0081] In some embodiments, the VH comprises an amino acid sequence that has at least 70% sequence identity to the amino acid sequence according to SEQ ID NO: 34; and the VL comprises an amino acid sequence that has at least 70% sequence identity to the amino acid sequence according to SEQ ID NO: 40. In some embodiments, the VH comprises an amino acid sequence that has at least 80% sequence identity to the amino acid sequence according to SEQ

ID NO: 34; and the VL comprises an amino acid sequence that has at least 80% sequence identity to the amino acid sequence according to SEQ ID NO: 40. In some embodiments, the VH comprises an amino acid sequence that has at least 85% sequence identity to the amino acid sequence according to SEQ ID NO: 34; and the VL comprises an amino acid sequence that has at least 85% sequence identity to the amino acid sequence according to SEQ ID NO: 40. In some embodiments, the VH comprises an amino acid sequence that has at least 90% sequence identity to the amino acid sequence according to SEQ ID NO: 34; and the VL comprises an amino acid sequence that has at least 90% sequence identity to the amino acid sequence according to SEQ ID NO: 40. In some embodiments, the VH comprises an amino acid sequence that has at least 91% sequence identity to the amino acid sequence according to SEQ ID NO: 34; and the VL comprises an amino acid sequence that has at least 91% sequence identity to the amino acid sequence according to SEQ ID NO: 40. In some embodiments, the VH comprises an amino acid sequence that has at least 92% sequence identity to the amino acid sequence according to SEQ ID NO: 34; and the VL comprises an amino acid sequence that has at least 92% sequence identity to the amino acid sequence according to SEQ ID NO: 40. In some embodiments, the VH comprises an amino acid sequence that has at least 93% sequence identity to the amino acid sequence according to SEQ ID NO: 34; and the VL comprises an amino acid sequence that has at least 93% sequence identity to the amino acid sequence according to SEQ ID NO: 40. In some embodiments, the VH comprises an amino acid sequence that has at least 94% sequence identity to the amino acid sequence according to SEQ ID NO: 34; and the VL comprises an amino acid sequence that has at least 94% sequence identity to the amino acid sequence according to SEQ ID NO: 40. In some embodiments, the VH comprises an amino acid sequence that has at least 95% sequence identity to the amino acid sequence according to SEQ ID NO: 34; and the VL comprises an amino acid sequence that has at least 95% sequence identity to the amino acid sequence according to SEQ ID NO: 40. In some embodiments, the VH comprises an amino acid sequence that has at least 96% sequence identity to the amino acid sequence according to SEQ ID NO: 34; and the VL comprises an amino acid sequence that has at least 96% sequence identity to the amino acid sequence according to SEQ ID NO: 40. In some embodiments, the VH comprises an amino acid sequence that has at least 97% sequence identity to the amino acid sequence according to SEQ ID NO: 34; and the VL comprises an amino acid sequence that has at least 97% sequence identity to the amino acid sequence according to SEQ ID NO: 40. In some embodiments, the VH comprises an amino acid sequence that has at least 98% sequence identity to the amino acid sequence according to SEQ ID NO: 34; and the VL comprises an amino acid sequence that has at least 98% sequence identity to the amino acid sequence according to SEQ

ID NO: 40. In some embodiments, the VH comprises an amino acid sequence that has at least 99% sequence identity to the amino acid sequence according to SEQ ID NO: 34; and the VL comprises an amino acid sequence that has at least 99% sequence identity to the amino acid sequence according to SEQ ID NO: 40.

[0082] Described herein, in some embodiments, are antibodies or antibody fragments comprising a heavy chain sequence at least about 90% identical to a sequence as set forth in any one of SEQ ID NOs: 41, 43, 46, 48, and 50. In some instances, the antibodies or antibody fragments comprise a heavy chain sequence at least or about 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NOs: 41, 43, 46, 48, and 50.

[0083] Described herein, in some embodiments, are antibodies or antibody fragments comprising a light chain sequence at least about 90% identical to a sequence as set forth in any one of SEQ ID NOs: 42, 44, 45, 47, 49, and 51. In some instances, the antibodies or antibody fragments comprise a light chain sequence at least or about 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NOs: 42, 44, 45, 47, 49, and 51.

[0084] Described herein, in some embodiments, are antibodies or antibody fragments comprising a heavy chain sequence at least about 90% identical to a sequence as set forth in any one of SEQ ID NOs: 41, 43, 46, 48, and 50 and a light chain sequence at least about 90% identical to a sequence as set forth in any one of SEQ ID NOs: 42, 44, 45, 47, 49, and 51. In some instances, the antibodies or antibody fragments comprise a heavy chain sequence at least or about 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NOs: 41, 43, 46, 48, and 50 and a light chain sequence at least or about 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to any one of SEQ ID NOs: 42, 44, 45, 47, 49, and 51.

Table 5. Heavy Chain and Light Chain Sequences

Name	SEQ ID NO:	Amino Acid Sequence
Antibody 1 Heavy Chain	41	METGLRWLLLVAVLKGVQCQSLEESGGRLVTPGTPLTLTCTVSG FSLSSQKVGWVRQAPGKGLEWIGIINNYGSTYYASWAKGRFTIS KTSTTVDLRITSLTAEDTATYFCARDPDGSIVFDIWGPGTLVTVSL GQPKAPSVFPLAPCCGDTPSSTVTLGCLVKGYLPEPVTVTWNSG TLTNGVRTFPSVRQSSGLYSLSSVVSVTSSSQPVTCNVAHPATNT KVDKTVAPSTCSKPTCPPPELLGRSSVFIFPPKPKDTLMISRTPEV TCVVVDVSQDDPEVQFTWYINNEQVRTARPPLREQQFNSTIRVV STLPIAHQDWLRGKEFKCKVHNKALPAPIEKTISKARGQPLEPKV YTMGPPREELSSRSVSLTCMINGFYPSDISVEWEKNGKAEDNYK TTPAVLDSDGSYFLYSKLSVPTSEWQRGDVFTCSVMHEALHNH YTQKSISRSPGK
Antibody 1 Light Chain	42	MDTRAPTQLLGLLLLWLPGATFAQVLTQTASPVSAAVGGTVTI NCQSSQSVVYNNRLSWFQQKPGQPPKLLIYGASTLASGVPSRF KGSGSGTQFTLTISDVQCDDAATYYCLGSYDCSSGDCHAFGGG TEVVVKGDPVAPTVLIFPPAADQVATGTVTIVCVANKYFPDVT VTWEVDGTTQTTGIENSKTPQNSADCTYNLSSTLTLTSTQYNSH KEYTCKVTQGTTSVVQSFNRGDC
Antibody 2 and Antibody 3 Heavy Chain	43	METGLRWLLLVAVLKGVQCQSVEESGGRLVTPGTPLTLTCTVSG FSLSSYAMIWVRQAPGKGLEWIGFISRSGITYYASWAKGRFTISK TSTTVDLKMTSLTTEDTATYFCAREFGAVGSDYYRDAFNLWGP GTLVTVSSGQPKAPSVFPLAPCCGDTPSSTVTLGCLVKGYLPEPV TVTWNSGTLTNGVRTFPSVRQSSGLYSLSSVVSVTSSSQPVTCNV AHPATNTKVDKTVAPSTCSKPTCPPPELLGRSSVFIFPPKPKDTL MISRTPEVTCVVVDVSQDDPEVQFTWYINNEQVRTARPPLREQQ FNSTIRVVSTLPIAHQDWLRGKEFKCKVHNKALPAPIEKTISKAR GQPLEPKVYTMGPPREELSSRSVSLTCMINGFYPSDISVEWEKNG KAEDNYKTTPAVLDSDGSYFLYSKLSVPTSEWQRGDVFTCSVM HEALHNHYTQKSISRSPGK
Antibody 2 Light Chain	44	MDMRAPTQLLGLLLLWLPGARCADIVMTQTPASVEAAVGGTV TINCQASESINSWLSWYQQKPGQPPNLLIYRASTLASGVPSRFSG GGSGTEYTLTISDLECADAVTYYCQSYYEEDGIGYAFGGGTEVV VEGDPVAPTVLIFPPAADQVATGTVTIVCVANKYFPDVTVTWE VDGTTQTTGIENSKTPQNSADCTYNLSSTLTLTSTQYNSHKEYT CKVTQGTTSVVQSFNRGDC
Antibody 3 Light Chain	45	MDMRAPTQLLGLLLLWLPGARCADIVMTQTPSSVSAAVGGTVT INCQASQNIYSNLAWYQQKPGQRPRLLIYGASNLASGVPSRFKG SRSGTEFTLTISDLECADAATYYCQGYDYSTAGAYPFGGGTAVV VKGDPVAPTVLIFPPAADQVATGTVTIVCVANKYFPDVTVTWEV DGTTQTTGIENSKTPQNSADCTYNLSSTLTLTSTQYNSHKEYTCK VTQGTTSVVQSFNRGDC

Antibody 4 Heavy Chain	46	METGLRWLLLVAVLKGVQCQSLEESGGRLVTPGTPLTLTCTVS GFSLNNYKVGWVRQAPGKGLEWIGIINYYSQTYYASWAKGRF TISKTSTTVDLKLTSPTTEDTATYFCARDPDGSIVFDIWGPGTLV TVSLGQPKAPSVFPLAPCCGDTPSSTVTLGCLVKGYLPEPVTVT WNSGTLTNGVRTFPSVRQSSGLYSLSSVVSVTSSSQPVTCNVAH PATNTKVDKTVVPSTCSKPTCPPPELLGRSSVFIFPPKPKDTLMI SRTPEVTCVVVDVSQDDPEVQFTWYINNEQVRTARPPLREQQF NSTIRVVSTLPIAHQDWLRGKEFKCKVHNKALPAPIEKTISKAR GQPLEPKVYTMGPPREELSSRSVSLTCMINGFYPSDISVEWEKN GKAEDNYKTTPAVLDSDGSYFLYSKLSVPTSEWQRGDVFTCSV MHEALHNHYTQKSISRSPGK
Antibody 4 Light Chain	47	MDTRAPTQLLGLLLLWLPGATFAQVLTQTASPVSAAVGSTVTIN CQSSQSVYSNKRLAWFQLKPGQPPKLLIYGASTLASGVPSRFKG SGSGTQFTLTISDVQCDDAATYYCAGGYDCSTGDCWTFGGGTE VVVTGDPVAPTVLIFPPAADQVATGTVTIVCVANKYFPDVTVT WEVDGTTQTTGIENSKTPQNSADCTYNLSSTLTLTSTQYNSHKE YTCKVTQGTTSVVQSFNRGDC
Antibody 5 Heavy Chain	48	METGLRWLLLVAVLKGVQCQSVEESGGGLVTPGGTLTLTCTVS GFSLSNYAMSWVRQAPGKGLEWIGFISRSGITYYASWAKGRFT ISKTSTTVDLKITSPTTEDTAAYFCAREFGAVGSDYYRDALRLW GPGTLVTVSSGQPKAPSVFPLAPCCGDTPSSTVTLGCLVKGYLP EPVTVTWNSGTLTNGVRTFPSVRQSSGLYSLSSVVSVTSSSQPVT CNVAHPATNTKVDKTVAPSTCSKPTCPPPELLGRSSVFIFPPKPK DTLMISRTPEVTCVVVDVSQDDPEVQFTWYINNEQVRTARPPL REQQFNSTIRVVSTLPIAHQDWLRGKEFKCKVHNKALPAPIEKT ISKARGQPLEPKVYTMGPPREELSSRSVSLTCMINGFYPSDISVE WEKNGKAEDNYKTTPAVLDSDGSYFLYSKLSVPTSEWQRGDV FTCSVMHEALHNHYTQKSISRSPGK
Antibody 5 Light Chain	49	MDMRAPTQLLGLLLLWLPGARCADIVMTQTPSSVSAAVGGTVT IKCQASQSIGSNLAWYQQKPGQPPKLLIYGASTLESGVPSRFKGS GSGTEYTLTISDLECADAATYYCQSYYEGSDIGYAFGGGTEVVV EGDPVAPTVLIFPPAADQVATGTVTIVCVANKYFPDVTVTWEVD GTTQTTGIENSKTPQNSADCTYNLSSTLTLTSTQYNSHKEYTCKV TQGTTSVVQSFNRGDC
Antibody 6 Heavy Chain	50	METGLRWLLLVAVLKGVQCQSLEESGGRLVTPGTPLTLTCTVS GIDLSTHAMTWVRQAPGKGLEWIGVINPSGSAYYATWVNGRF TISKTSTTVDLKITSPTTGDTAKYFCARDYITAGDYYMDAFDPW GPGTLVTVSSGQPKAPSVFPLAPCCGDTPSSTVTLGCLVKGYLP EPVTVTWNSGTLTNGVRTFPSVRQSSGLYSLSSVVSVTSSSQPV TCNVAHPATNTKVDKTVAPSTCSKPTCPPPELLGRSSVFIFPPKP KDTLMISRTPEVTCVVVDVSQDDPEVQFTWYINNEQVRTARPP LREQQFNSTIRVVSTLPIAHQDWLRGKEFKCKVHNKALPAPIEK TISKARGQPLEPKVYTMGPPREELSSRSVSLTCMINGFYPSDISV EWEKNGKAEDNYKTTPAVLDSDGSYFLYSKLSVPTSEWQRGD VFTCSVMHEALHNHYTQKSISRSPGK
Antibody 6 Light Chain	51	MDTRAPTQLLGLLLLWLPGARCADIVMTQTPASVSAAVGGTV TIKCQASQSISNQLSWYQQKSGQPPKLLIYRASTLASGVPSRFK GSGSGTEFTLTISDLECADAATYYCQQGYNRDNVDNLFGGGT EVVVKGDPVAPTVLIFPPAADQVATGTVTIVCVANKYFPDVTV TWEVDGTTQTTGIENSKTPQNSADCTYNLSSTLTLTSTQYNSH KEYTCKVTQGTTSVVQSFNRGDC

[0085] In some embodiments, the anti-tau antibody comprises a VH domain that is encoded by a nucleic acid comprising at least 80%, at least 85%, at least 90%, at least 95% sequence identity to a sequence selected from SEQ ID NOs: 52-56. In some embodiments, the anti-tau antibody comprises a VL domain that is encoded by a nucleic acid comprising at least 80%, at least 85%, at least 90%, at least 95% sequence identity to a sequence selected from SEQ ID NOs: 57-62. Nucleic acid sequences for VH domains for anti-tau-tau antibodies described here are listed in Table 6 and nucleic acid sequences for VL domains for anti-tau-tau antibodies described here are listed in Table 7. In some embodiments, the anti-tau-tau antibody comprises a VH domain that is encoded by a nucleic acid comprising at least 90% sequence identity to SEQ ID NO: 52. In some embodiments, the anti-tau-tau antibody comprises a VH domain that is encoded by a nucleic acid comprising at least 90% sequence identity to SEQ ID NO: 53. In some embodiments, the anti-tau-tau antibody comprises a VH domain that is encoded by a nucleic acid comprising at least 90% sequence identity to SEQ ID NO: 54. In some embodiments, the anti-tau-tau antibody comprises a VH domain that is encoded by a nucleic acid comprising at least 90% sequence identity to SEQ ID NO: 55. In some embodiments, the anti-tau-tau antibody comprises a VH domain that is encoded by a nucleic acid comprising at least 90% sequence identity to SEQ ID NO: 56. In some embodiments, the anti-tau-tau antibody comprises a VL domain that is encoded by a nucleic acid comprising at least 90% sequence identity to SEQ ID NO: 57. In some embodiments, the anti-tau-tau antibody comprises a VL domain that is encoded by a nucleic acid comprising at least 90% sequence identity to SEQ ID NO: 58. In some embodiments, the anti-tau antibody comprises a VL domain that is encoded by a nucleic acid comprising at least 90% sequence identity to SEQ ID NO: 59. In some embodiments, the anti-tau antibody comprises a VL domain that is encoded by a nucleic acid comprising at least 90% sequence identity to SEQ ID NO: 60. In some embodiments, the anti-tau antibody comprises a VL domain that is encoded by a nucleic acid comprising at least 90% sequence identity to SEQ ID NO: 61. In some embodiments, the anti-tau antibody comprises a VL domain that is encoded by a nucleic acid comprising at least 90% sequence identity to SEQ ID NO: 62. In some embodiments, the anti-tau antibody comprises a VH domain that is encoded by a nucleic acid comprising at least 90% sequence identity to SEQ ID NO: 52 and a VL domain that is encoded by a nucleic acid comprising at least 90% sequence identity to SEQ ID NO: 57. In some embodiments, the anti-tau antibody comprises a VH domain that is encoded by a nucleic acid comprising at least 90% sequence identity to SEQ ID NO: 53 and a VL domain that is encoded by a nucleic acid comprising at least 90% sequence identity to SEQ ID NO: 58. In some

embodiments, the anti-tau antibody comprises a VH domain that is encoded by a nucleic acid comprising at least 90% sequence identity to SEQ ID NO: 53 and a VL domain that is encoded by a nucleic acid comprising at least 90% sequence identity to SEQ ID NO: 59. In some embodiments, the anti-tau antibody comprises a VH domain that is encoded by a nucleic acid comprising at least 90% sequence identity to SEQ ID NO: 54 and a VL domain that is encoded by a nucleic acid comprising at least 90% sequence identity to SEQ ID NO: 60. In some embodiments, the anti-tau antibody comprises a VH domain that is encoded by a nucleic acid comprising at least 90% sequence identity to SEQ ID NO: 55 and a VL domain that is encoded by a nucleic acid comprising at least 90% sequence identity to SEQ ID NO: 61. In some embodiments, the anti-tau antibody comprises a VH domain that is encoded by a nucleic acid comprising at least 90% sequence identity to SEQ ID NO: 563 and a VL domain that is encoded by a nucleic acid comprising at least 90% sequence identity to SEQ ID NO: 62. In some embodiments, the anti-tau antibody comprises a VH domain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 52. In some embodiments, the anti-tau antibody comprises a VH domain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 53. In some embodiments, the anti-tau antibody comprises a VH domain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 54. In some embodiments, the anti-tau antibody comprises a VH domain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 55. In some embodiments, the anti-tau antibody comprises a VH domain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 56. In some embodiments, the anti-tau antibody comprises a VL domain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 57. In some embodiments, the anti-tau antibody comprises a VL domain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 58. In some embodiments, the anti-tau antibody comprises a VL domain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 59. In some embodiments, the anti-tau antibody comprises a VL domain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 60. In some embodiments, the anti-tau antibody comprises a VL domain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 61. In some embodiments, the anti-tau antibody comprises a VL domain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 62. In some embodiments, the anti-tau antibody comprises a VH domain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 52 and a VL domain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 57. In some embodiments, the anti-tau antibody comprises a VH domain that is encoded by a nucleic acid

comprising a sequence identical to SEQ ID NO: 53 and a VL domain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 58. In some embodiments, the anti-tau antibody comprises a VH domain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 53 and a VL domain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 59. In some embodiments, the anti-tau antibody comprises a VH domain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 54 and a VL domain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 60. In some embodiments, the anti-tau antibody comprises a VH domain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 55 and a VL domain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 61. In some embodiments, the anti-tau antibody comprises a VH domain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 56 and a VL domain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 62.

Table 6. Nucleic acid sequences encoding VH domains

SEQ ID NO:	Nucleic acid sequences encoding VH domains
52	ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGG TGTCCAGTGTCAGTCGCTGGAGGAGTCCGGGGGTCGCCTGGTCACG CCTGGGACACCCCTGACACTCACCTGCACAGTCTCTGGATTTTCCCT CAGTAGCCAGAAAGTGGGCTGGGTCCGCCAGGCTCCAGGGAAGGGG CTGGAATGGATCGGAATCATTAATAATTATGGTAGCACATACTACGC GAGCTGGGCGAAAGGCCGATTCACCATCTCGAAAACCTCGACCACA GTGGATCTGAGAATCACCAGTCTGACGGCCGAGGACACGGCCACCT ATTTCTGTGCCCGTGATCCTGATGGTAGTATTGTCTTTGACATCTGGG GCCCAGGCACCCTTGTCACCGTCTCCTTG
53	ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGG TGTCCAGTGTCAGTCGGTGGAGGAGTCCGGGGGTCGCCTGGTCACGC CTGGGACACCCCTGACACTCACCTGCACCGTCTCTGGATTCTCCCTC AGTAGCTATGCAATGATCTGGGTCCGCCAGGCTCCAGGGAAGGGGC TGGAATGGATCGGATTCATTAGTCGTAGTGGTATCACATACTACGCG AGCTGGGCAAAAGGCCGATTCACCATCTCCAAAACCTCGACCACGG TGGATCTGAAAATGACCAGTCTGACAACCGAGGACACGGCCACCTA TTTCTGTGCCAGAGAATTCGGTGCTGTTGGTAGTGATTATTATAGGG ACGCCTTTAACTTGTGGGGCCCAGGCACCCTGGTCACCGTCTCCTCA
54	ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGG TGTCCAGTGTCAGTCGCTGGAGGAGTCCGGGGGTCGCCTGGTCACG CCTGGGACACCCCTGACACTCACCTGCACAGTCTCTGGATTTTCCCT AAATAACTACAAAGTGGGCTGGGTCCGCCAGGCTCCAGGAAAGGG GCTGGAATGGATCGGAATCATTAACTATTATAGTCAGACATACTAC GCGAGCTGGGCCAAAGGCCGATTCACCATCTCGAAAACCTCGACC ACGGTG GATCTGAAGCTCACCAGTCCGACAACCGAAGACACGGCC ACCTATTTCTGTGCCCGTGATCCTGATGGTAGTATTGTCTTTGACAT CTGGGGCCCAGGCACCCTTGTCACCGTCTCCTTG
55	ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGG TGTCCAGTGTCAGTCGGTGGAGGAGTCCGGAGGAGGCCTGGTAACG

	CCTGGAGGAACCCTGACACTCACCTGCACCGTCTCTGGATTCTCCCT CAGTAACTATGCAATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGG CTGGAATGGATCGGATTCATTAGTCGTAGTGGTATTACATACTACGC GAGCTGGGCAAAAGGCCGATTCACCATCTCCAAAACCTCGACCACG GTGGATCTGAAAATCACCAGTCCGACGACCGAGGACACGGCCGCCT ATTTCTGTGCCAGAGAATTCGGTGCTGTTGGTAGTGATTATTATAGG GACGCCTTGAGGTTGTGGGGCCCAGGCACCCTGGTCACCGTCTCCT CA
56	ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGG TGTCCAGTGTCAGTCGCTGGAGGAGTCCGGGGGTCGCCTGGTAACG CCTGGGACACCCCTGACACTCACCTGCACAGTCTCTGGAATCGACCT CAGTACCCATGCAATGACCTGGGTCCGCCAGGCTCCAGGAAAGGGG CTGGAATGGATCGGAGTCATTAATCCTAGTGGTAGCGCATACTACG CGACCTGGGTGAATGGCCGATTCACCATCTCCAAAACCTCGACCACG GTGGATCTGAAAATCACCAGTCCGACAACCGGGGACACGGCCAAGT ATTTCTGTGCCAGAGATTATATTACTGCGGGTGATTATTATATGGAT GCTTTTGATCCCTGGGGCCCAGGCACCCTGGTCACCGTCTCCTCA

Table 7. Nucleic acid sequences encoding VL domains

SEQ ID NO:	Nucleic acid sequences encoding VL domains
57	ATGGACACGAGGGCCCCCACTCAGCTGCTGGGGCTCCTGCTGCTC TGGCTCCCAGGTGCCACATTTGCCCAAGTGCTGACCCAGACTGCA TCCCCCGTGTCTGCGGCTGTTGGAGGCACAGTCACCATCAATTGC CAGTCCAGTCAGAGTGTTGTATATAACAACCGCTTATCCTGGTTT CAACAGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTATGGTGCA TCCACTCTGGCATCTGGGGTCCCATCGCGGTTCAAAGGCAGTGGA TCTGGGACACAGTTCACTCTCACCATCAGCGACGTGCAGTGTGAC GATGCTGCCACTTACTACTGTCTAGGCTCCTATGATTGTAGTAGT GGTGATTGCCATGCTTTCGGCGGAGGGACCGAGGTGGTGGTCAAA
58	ATGGACATGAGGGCCCCCACTCAGCTGCTGGGGCTCCTACTGCTC TGGCTCCCAGGTGCCAGATGTGCTGACATTGTGATGACCCAGACT CCAGCCTCCGTGGAGGCAGCTGTGGGAGGCACAGTCACCATCAA TTGCCAAGCCAGTGAGAGCATTAATAGTTGGTTGTCCTGGTATCA GCAGAAACCAGGGCAGCCTCCCAACCTCCTGATCTACAGGGCATC CACTCTGGCATCTGGGGTCCCATCGCGGTTCAGTGGCGGTGGATC TGGGACAGAGTACACTCTCACCATCAGCGACCTGGAGTGTGCCGA TGCTGTCACTTATTACTGTCAAAGCTATTATGAGGAGGATGGTAT TGGTTATGCTTTCGGCGGAGGGACCGAGGTGGTGGTCGAA
59	ATGGACATGAGGGCCCCCACTCAGCTGCTGGGGCTCCTGCTGCTCTG GCTCCCAGGTGCCAGATGTGCTGACATTGTGATGACCCAGACTCCAT CCTCCGTGTCTGCAGCTGTGGGAGGCACAGTCACCATCAATTGCCAG GCCAGTCAGAACATTTACAGCAATTTAGCCTGGTATCAGCAGAAAC CAGGGCAGCGTCCCAGGCTCCTGATCTATGGCGCATCCAATCTGGCA TCTGGGGTCCCATCGCGGTTCAAAGGCAGTAGATCTGGGACAGAGTT CACTCTCACCATCAGCGACCTGGAGTGTGCCGATGCTGCCACTTACT ACTGTCAAGGCTATGATTATAGTACTGCTGGTGCCTATCCTTTCGGC GGAGGGACCGCGGTGGTGGTCAAA
60	ATGGACACGAGGGCCCCCACTCAGCTGCTGGGGCTCCTGCTGCTCTG GCTCCCAGGTGCCACATTTGCCCAAGTGCTGACCCAGACTGCATCGC CCGTGTCTGCGGCTGTGGGAAGCACAGTCACCATCAATTGCCAGTCC AGTCAGAGCGTTTATAGTAACAAGCGCTTAGCCTGGTTTCAGCTGAA ACCAGGGCAGCCTCCCAAGCTCCTGATCTATGGTGCATCCACACTGG CATCTGGGGTCCCATCGCGATTCAAGGGCAGTGGATCTGGGACACAG TTCACTCTCACCATCAGCGACGTGCAGTGTGACGATGCTGCCACTTA

	CTACTGTGCAGGCGGTTATGATTGTAGTACTGGTGATTGTTGGACTTT CGGCGGAGGGACCGAGGTGGTGGTCACA
61	ATGGACATGAGGGCCCCCACTCAGCTGCTGGGGCTCCTGCTGCTC TGGCTCCCAGGTGCCAGATGTGCTGACATCGTGATGACCCAGACT CCATCCTCCGTGTCTGCAGCTGTGGGAGGCACAGTCACCATCAAG TGCCAGGCCAGTCAGAGCATTGGTAGTAATTTAGCCTGGTATCAG CAGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTATGGTGCATCC ACTCTGGAATCTGGGGTCCCATCGCGGTTTAAAGGCAGTGGATCT GGGACAGAGTACACTCTCACCATCAGCGACCTGGAGTGTGCCGAT GCTGCCACTTACTACTGTCAAAGCTATTATGAGGGTAGTGATATT GGTTATGCTTTCGGCGGAGGGACCGAGGTGGTGGTCGAA
62	ATGGACACGAGGGCCCCCACTCAGCTGCTGGGGCTCCTGCTGCTC TGGCTCCCAGGTGCCAGATGTGCTGACATCGTGATGACCCAGACT CCAGCCTCTGTGTCTGCAGCTGTGGGAGGCACAGTCACCATCAAG TGCCAGGCCAGTCAGAGCATTAGCAACCAACTATCCTGGTATCAG CAGAAATCAGGGCAGCCTCCCAAGCTCCTGATCTACAGGGCATCT ACTCTGGCATCTGGGGTCCCATCGCGGTTCAAAGGCAGTGGATCT GGGACAGAGTTCACTCTCACCATCAGCGACCTGGAGTGTGCCGAT GCTGCCACTTACTACTGTCAACAGGGTTATAATAGAGATAATGTT GATAATCTTTTCGGCGGAGGGACCGAGGTGGTGGTCAAA

[0086] In some embodiments, the anti-tau antibody comprises a heavy chain that is encoded by a nucleic acid comprising a sequence identical to a sequence selected from SEQ ID NOs: 63-67. In some embodiments, the anti-tau antibody comprises a light chain that is encoded by a nucleic acid comprising a sequence identical to a sequence selected from SEQ ID NOs: 68-73. Nucleic acid sequences for heavy chains for anti-tau antibodies described here are listed in Table 8 and nucleic acid sequences for light chains for anti-tau antibodies described here are listed in Table 9. Nucleic acid sequences listed in Table 8 and Table 9 may be used in the process of in vitro production of antibodies described herein. In some embodiments, the anti-tau antibody comprises a heavy chain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 63. In some embodiments, the anti-tau antibody comprises a heavy chain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 64. In some embodiments, the anti-tau antibody comprises a heavy chain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 65. In some embodiments, the anti-tau antibody comprises a heavy chain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 66. In some embodiments, the anti-tau antibody comprises a heavy chain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 67. In some embodiments, the anti-tau antibody comprises a light chain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 68. In some embodiments, the anti-tau antibody comprises a light chain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 69. In some embodiments, the anti-tau antibody comprises a light chain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 70. In some embodiments, the anti-tau antibody comprises a light chain that is encoded by a nucleic acid comprising a sequence

identical to SEQ ID NO: 71. In some embodiments, the anti-tau antibody comprises a light chain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 72. In some embodiments, the anti-tau antibody comprises a light chain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 73. In some embodiments, the anti-tau antibody comprises a heavy chain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 63 and a light chain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 68. In some embodiments, the anti-tau antibody comprises a heavy chain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 64 and a light chain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 69. In some embodiments, the anti-tau antibody comprises a heavy chain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 64 and a light chain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 70. In some embodiments, the anti-tau antibody comprises a heavy chain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 65 and a light chain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 71. In some embodiments, the anti-tau antibody comprises a heavy chain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 66 and a light chain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 72. In some embodiments, the anti-tau antibody comprises a heavy chain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 67 and a light chain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NO: 73.

Table 8. Nucleic acid sequences encoding heavy chains

SEQ ID NO:	Nucleic acid sequences encoding heavy chains
63	ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGGTG TCCAGTGTCAGTCGCTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGG GACACCCCTGACACTCACCTGCACAGTCTCTGGATTTTCCCTCAGTAGC CAGAAAGTGGGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATGG ATCGGAATCATTAATAATTATGGTAGCACATACTACGCGAGCTGGGCG AAAGGCCGATTCACCATCTCGAAAACCTCGACCACAGTGGATCTGAGA ATCACCAGTCTGACGGCCGAGGACACGGCCACCTATTTCTGTGCCCGTG ATCCTGATGGTAGTATTGTCTTTGACATCTGGGGCCCAGGCACCCTTGTC ACCGTCTCCTTGGGGCAACCTAAGGCTCCATCAGTCTTCCCACTGGCCC CCTGCTGCGGGGACACACCCAGCTCCACGGTGACCCTGGGCTGCCTGGT CAAAGGCTACCTCCCGGAGCCAGTGACCGTGACCTGGAACTCGGGCAC

	CCTCACCAATGGGGTACGCACCTTCCCGTCCGTCCGGCAGTCCTCAGGC CTCTACTCGCTGAGCAGCGTGGTGAGCGTGACCTCAAGCAGCCAGCCC GTCACCTGCAACGTGGCCCACCCAGCCACCAACACCAAAGTGGACAAG ACCGTTGCGCCCTCGACATGCAGCAAGCCCACGTGCCCACCCCCTGAA CTCCTGGGGCGATCCTCTGTCTTCATCTTCCCCCCAAAACCCAAGGACA CCCTCATGATCTCACGCACCCCCGAGGTCACATGCGTGGTGGTGGACG TGAGCCAGGATGACCCCGAGGTGCAGTTCACATGGTACATAAACAAC GAGCAGGTGCGCACCGCCCGGCCGCCGCTACGGGAGCAGCAGTTCAAC AGCACGATCCGCGTGGTCAGCACCCTCCCCATCGCGCACCAGGACTGG CTGAGGGGCAAGGAGTTCAAGTGCAAAGTCCACAACAAGGCACTCCC GGCCCCCATCGAGAAAACCATCTCCAAAGCCAGAGGGCAGCCCCTGG AGCCGAAGGTCTACACCATGGGCCCTCCCCGGGAGGAGCTGAGCAGC AGGTCGGTCAGCCTGACCTGCATGATCAACGGCTTCTACCCTTCCGAC ATCTCGGTGGAGTGGGAGAAGAACGGGAAGGCAGAGGACAACTACAA GACCACGCCGGCCGTGCTGGACAGCGACGGCTCCTACTTCCTCTACAG CAAGCTCTCAGTGCCCACGAGTGAGTGGCAGCGGGGCGACGTCTTCAC CTGCTCCGTGATGCACGAGGCCTTGCACAACCACTACACGCAGAAGTC CATCTCCCGCTCTCCGGGTAAATGA
64	ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGG TGTCCAGTGTCAGTCGGTGGAGGAGTCCGGGGGTCGCCTGGTCACGC CTGGGACACCCCTGACACTCACCTGCACCGTCTCTGGATTCTCCCTC AGTAGCTATGCAATGATCTGGGTCCGCCAGGCTCCAGGGAAGGGGC TGGAATGGATCGGATTCATTAGTCGTAGTGGTATCACATACTACGCG AGCTGGGCAAAAGGCCGATTCACCATCTCCAAAACCTCGACCACGG TGGATCTGAAAATGACCAGTCTGACAACCGAGGACACGGCCACCTA TTTCTGTGCCAGAGAATTCGGTGCTGTTGGTAGTGATTATTATAGGGA CGCCTTTAACTTGTGGGGCCCAGGCACCCTGGTCACCGTCTCCTCAGG GCAACCTAAGGCTCCATCAGTCTTCCCACTGGCCCCCTGCTGCGGGGA CACACCCAGCTCCACGGTGACCCTGGGCTGCCTGGTCAAAGGCTACCT CCCGGAGCCAGTGACCGTGACCTGGAACTCGGGCACCCTCACCAATGG GGTACGCACCTTCCCGTCCGTCCGGCAGTCCTCAGGCCTCTACTCGCTG AGCAGCGTGGTGAGCGTGACCTCAAGCAGCCAGCCCGTCACCTGCAAC GTGGCCCACCCAGCCACCAACACCAAAGTGGACAAGACCGTTGCGCCC TCGACATGCAGCAAGCCCACGTGCCCACCCCCTGAACTCCTGGGGCGA TCCTCTGTCTTCATCTTCCCCCCAAAACCCAAGGACACCCTCATGATCT CACGCACCCCCGAGGTCACATGCGTGGTGGTGGACGTGAGCCAGGAT GACCCCGAGGTGCAGTTCACATGGTACATAAACAACGAGCAGGTGCG CACCGCCCGGCCGCCGCTACGGGAGCAGCAGTTCAACAGCACGATCC GCGTGGTCAGCACCCTCCCCATCGCGCACCAGGACTGGCTGAGGGGC AAGGAGTTCAAGTGCAAAGTCCACAACAAGGCACTCCCGGCCCCCAT CGAGAAAACCATCTCCAAAGCCAGAGGGCAGCCCCTGGAGCCGAAG GTCTACACCATGGGCCCTCCCCGGGAGGAGCTGAGCAGCAGGTCGGT CAGCCTGACCTGCATGATCAACGGCTTCTACCCTTCCGACATCTCGGT GGAGTGGGAGAAGAACGGGAAGGCAGAGGACAACTACAAGACCACG CCGGCCGTGCTGGACAGCGACGGCTCCTACTTCCTCTACAGCAAGCTC TCAGTGCCCACGAGTGAGTGGCAGCGGGGCGACGTCTTCACCTGCTCC GTGATGCACGAGGCCTTGCACAACCACTACACGCAGAAGTCCATCTC CCGCTCTCCGGGTAAATGA

65	ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGG TGTCCAGTGTCAGTCGCTGGAGGAGTCCGGGGGTCGCCTGGTCACG CCTGGGACACCCCTGACACTCACCTGCACAGTCTCTGGATTTTCCCT AAATAACTACAAAGTGGGCTGGGTCCGCCAGGCTCCAGGAAAGGG GCTGGAATGGATCGGAATCATTAACTATTATAGTCAGACATACTAC GCGAGCTGGGCCAAAGGCCGATTCACCATCTCGAAAACCTCGACC ACGGTG GATCTGAAGCTCACCAGTCCGACAACCGAAGACACGGCC ACCTATTTCTGTGCCCGTGATCCTGATGGTAGTATTGTCTTTGACAT CTGGGGCCCAGGCACCCTTGTCACCGTCTCCTTGGGGCAACCTAAGG CTCCATCAGTCTTCCCACTGGCCCCCTGCTGCGGGGACACACCCAGC TCCACGGTGACCCTGGGCTGCCTGGTCAAAGGCTACCTCCCGGAGCC AGTGACCGTGACCTGGAACTCGGGCACCCTCACCAATGGGGTACGCA CCTTCCCGTCCGTCCGGCAGTCCTCAGGCCTCTACTCGCTGAGCAGCG TGGTGAGCGTGACCTCAAGCAGCCAGCCCGTCACCTGCAACGTGGCC CACCCAGCCACCAACACCAAAGTGGACAAGACCGTTGTGCCCTCGAC ATGCAGCAAGCCCACGTGCCCACCCCCTGAACTCCTGGGGCGATCCT CTGTCTTCATCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCAC GCACCCCCGAGGTCACATGCGTGGTGGTGGACGTGAGCCAGGATGAC CCCGAGGTGCAGTTCACATGGTACATAAACAACGAGCAGGTGCGCAC CGCCCGGCCGCCGCTACGGGAGCAGCAGTTCAACAGCACGATCCGCG TGGTCAGCACCCTCCCCATCGCGCACCAGGACTGGCTGAGGGGCAAG GAGTTCAAGTGCAAAGTCCACAACAAGGCACTCCCGGCCCCCATCGA GAAAACCATCTCCAAAGCCAGAGGGCAGCCCCTGGAGCCGAAGGTCT ACACCATGGGCCCTCCCCGGGAGGAGCTGAGCAGCAGGTCGGTCAGC CTGACCTGCATGATCAACGGCTTCTACCCTTCCGACATCTCGGTGGAG TGGGAGAAGAACGGGAAGGCAGAGGACAACTACAAGACCACGCCGG CCGTGCTGGACAGCGACGGCTCCTACTTCCTCTACAGCAAGCTCTCAG TGCCCACGAGTGAGTGGCAGCGGGGCGACGTCTTCACCTGCTCCGTGA TGCACGAGGCCTTGCACAACCACTACACGCAGAAGTCCATCTCCCGCT CTCCGGGTAAATGA

66	ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGG TGTCCAGTGTCAGTCGGTGGAGGAGTCCGGAGGAGGCCTGGTAACG CCTGGAGGAACCCTGACACTCACCTGCACCGTCTCTGGATTCTCCCT CAGTAACTATGCAATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGG CTGGAATGGATCGGATTCATTAGTCGTAGTGGTATTACATACTACGC GAGCTGGGCAAAAGGCCGATTCACCATCTCCAAAACCTCGACCACG GTGGATCTGAAAATCACCAGTCCGACGACCGAGGACACGGCCGCCT ATTTCTGTGCCAGAGAATTCGGTGCTGTTGGTAGTGATTATTATAGG GACGCCTTGAGGTTGTGGGGCCCAGGCACCCTGGTCACCGTCTCCTC AGGGCAACCTAAGGCTCCATCAGTCTTCCCACTGGCCCCCTGCTGCG GGGACACACCCAGCTCCACGGTGACCCTGGGCTGCCTGGTCAAAGG CTACCTCCCGGAGCCAGTGACCGTGACCTGGAACTCGGGCACCCTCA CCAATGGGGTACGCACCTTCCCGTCCGTCCGGCAGTCCTCAGGCCTC TACTCGCTGAGCAGCGTGGTGAGCGTGACCTCAAGCAGCCAGCCCGT CACCTGCAACGTGGCCCACCCAGCCACCAACACCAAAGTGGACAAGA CCGTTGCGCCCTCGACATGCAGCAAGCCCACGTGCCCACCCCCTGAAC TCCTGGGGCGATCCTCTGTCTTCATCTTCCCCCCAAAACCCAAGGACA CCCTCATGATCTCACGCACCCCCGAGGTCACATGCGTGGTGGTGGACG TGAGCCAGGATGACCCCGAGGTGCAGTTCACATGGTACATAAACAAC GAGCAGGTGCGCACCGCCCGGCCGCCGCTACGGGAGCAGCAGTTCAA CAGCACGATCCGCGTGGTCAGCACCCTCCCCATCGCGCACCAGGACTG GCTGAGGGGCAAGGAGTTCAAGTGCAAAGTCCACAACAAGGCACTCC CGGCCCCCATCGAGAAAACCATCTCCAAAGCCAGAGGGCAGCCCCTG GAGCCGAAGGTCTACACCATGGGCCCTCCCCGGGAGGAGCTGAGCAG CAGGTCGGTCAGCCTGACCTGCATGATCAACGGCTTCTACCCTTCCGA CATCTCGGTGGAGTGGGAGAAGAACGGGAAGGCAGAGGACAACTAC AAGACCACGCCGGCCGTGCTGGACAGCGACGGCTCCTACTTCCTCTA CAGCAAGCTCTCAGTGCCCACGAGTGAGTGGCAGCGGGGCGACGTC TTCACCTGCTCCGTGATGCACGAGGCCTTGCACAACCACTACACGCA GAAGTCCATCTCCCGCTCTCCGGGTAAATGA

67	ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGG TGTCCAGTGTCAGTCGCTGGAGGAGTCCGGGGGTCGCCTGGTAACG CCTGGGACACCCCTGACACTCACCTGCACAGTCTCTGGAATCGACCT CAGTACCCATGCAATGACCTGGGTCCGCCAGGCTCCAGGAAAGGGG CTGGAATGGATCGGAGTCATTAATCCTAGTGGTAGCGCATACTACG CGACCTGGGTGAATGGCCGATTCACCATCTCCAAAACCTCGACCACG GTGGATCTGAAAATCACCAGTCCGACAACCGGGGACACGGCCAAGT ATTTCTGTGCCAGAGATTATATTACTGCGGGTGATTATTATATGGAT GCTTTTGATCCCTGGGGCCCAGGCACCCTGGTCACCGTCTCCTCAGG GCAACCTAAGGCTCCATCAGTCTTCCCACTGGCCCCCTGCTGCGGGG ACACACCCAGCTCCACGGTGACCCTGGGCTGCCTGGTCAAAGGCTAC CTCCCGGAGCCAGTGACCGTGACCTGGAACTCGGGCACCCTCACCAA TGGGGTACGCACCTTCCCGTCCGTCCGGCAGTCCTCAGGCCTCTACTC GCTGAGCAGCGTGGTGAGCGTGACCTCAAGCAGCCAGCCCGTCACCT GCAACGTGGCCCACCCAGCCACCAACACCAAAGTGGACAAGACCGTT GCGCCCTCGACATGCAGCAAGCCCACGTGCCCACCCCCTGAACTCCT GGGGCGATCCTCTGTCTTCATCTTCCCCCCAAAACCCAAGGACACCC TCATGATCTCACGCACCCCCGAGGTCACATGCGTGGTGGTGGACGTG AGCCAGGATGACCCCGAGGTGCAGTTCACATGGTACATAAACAACG AGCAGGTGCGCACCGCCCGGCCGCCGCTACGGGAGCAGCAGTTCAA CAGCACGATCCGCGTGGTCAGCACCCTCCCCATCGCGCACCAGGACT GGCTGAGGGGCAAGGAGTTCAAGTGCAAAGTCCACAACAAGGCACT CCCGGCCCCCATCGAGAAAACCATCTCCAAAGCCAGAGGGCAGCCC CTGGAGCCGAAGGTCTACACCATGGGCCCTCCCCGGGAGGAGCTGA GCAGCAGGTCGGTCAGCCTGACCTGCATGATCAACGGCTTCTACCC TTCCGACATCTCGGTGGAGTGGGAGAAGAACGGGAAGGCAGAGGA CAACTACAAGACCACGCCGGCCGTGCTGGACAGCGACGGCTCCTA CTTCCTCTACAGCAAGCTCTCAGTGCCCACGAGTGAGTGGCAGCGG GGCGACGTCTTCACCTGCTCCGTGATGCACGAGGCCTTGCACAACC ACTACACGCAGAAGTCCATCTCCCGCTCTCCGGGTAAATGA

Table 9. Nucleic acid sequences encoding light chains

SEQ ID NO:	Nucleic acid sequences encoding light chains
68	ATGGACACGAGGGCCCCCACTCAGCTGCTGGGGCTCCTGCTGCTCTG GCTCCCAGGTGCCACATTTGCCCAAGTGCTGACCCAGACTGCATCCC CCGTGTCTGCGGCTGTTGGAGGCACAGTCACCATCAATTGCCAGTCC AGTCAGAGTGTTGTATATAACAACCGCTTATCCTGGTTTCAACAGAA ACCAGGGCAGCCTCCCAAGCTCCTGATCTATGGTGCATCCACTCTGG CATCTGGGGTCCCATCGCGGTTCAAAGGCAGTGGATCTGGGACACA GTTCACTCTCACCATCAGCGACGTGCAGTGTGACGATGCTGCCACTT ACTACTGTCTAGGCTCCTATGATTGTAGTAGTGGTGATTGCCATGCT TTCGGCGGAGGGACCGAGGTGGTGGTCAAAGGTGATCCAGTTGCAC CTACTGTCCTCATCTTCCCACCAGCTGCTGATCAGGTGGCAACTGGA ACAGTCACCATCGTGTGTGTGGCGAATAAATACTTTCCCGATGTCAC CGTCACCTGGGAGGTGGATGGCACCACCCAAACAACTGGCATCGAG AACAGTAAAACACCGCAGAATTCTGCAGATTGTACCTACAACCTCA GCAGCACTCTGACACTGACCAGCACACAGTACAACAGCCACAAAG AGTACACCTGCAAGGTGACCCAGGGCACGACCTCAGTCGTCCAGAG CTTCAATAGGGGTGACTGTTAG
69	ATGGACATGAGGGCCCCCACTCAGCTGCTGGGGCTCCTACTGCTCTG

	GCTCCCAGGTGCCAGATGTGCTGACATTGTGATGACCCAGACTCCAG CCTCCGTGGAGGCAGCTGTGGGAGGCACAGTCACCATCAATTGCCAA GCCAGTGAGAGCATTAATAGTTGGTTGTCCTGGTATCAGCAGAAACC AGGGCAGCCTCCCAACCTCCTGATCTACAGGGCATCCACTCTGGCAT CTGGGGTCCCATCGCGGTTCAGTGGCGGTGGATCTGGGACAGAGTAC ACTCTCACCATCAGCGACCTGGAGTGTGCCGATGCTGTCACTTATTA CTGTCAAAGCTATTATGAGGAGGATGGTATTGGTTATGCTTTCGGCG GAGGGACCGAGGTGGTGGTCGAAGGTGATCCAGTTGCACCTACTGT CCTCATCTTCCCACCAGCTGCTGATCAGGTGGCAACTGGAACAGTCA CCATCGTGTGTGTGGCGAATAAATACTTTCCCGATGTCACCGTCACC TGGGAGGTGGATGGCACCACCCAAACAACTGGCATCGAGAACAGTA AAACACCGCAGAATTCTGCAGATTGTACCTACAACCTCAGCAGCACT CTGACACTGACCAGCACACAGTACAACAGCCACAAAGAGTACACCT GCAAGGTGACCCAGGGCACGACCTCAGTCGTCCAGAGCTTCAATAG GGGTGACTGTTAG
70	ATGGACATGAGGGCCCCCACTCAGCTGCTGGGGCTCCTGCTGCTCTG GCTCCCAGGTGCCAGATGTGCTGACATTGTGATGACCCAGACTCCAT CCTCCGTGTCTGCAGCTGTGGGAGGCACAGTCACCATCAATTGCCAG GCCAGTCAGAACATTTACAGCAATTTAGCCTGGTATCAGCAGAAACC AGGGCAGCGTCCCAGGCTCCTGATCTATGGCGCATCCAATCTGGCAT CTGGGGTCCCATCGCGGTTCAAAGGCAGTAGATCTGGGACAGAGTT CACTCTCACCATCAGCGACCTGGAGTGTGCCGATGCTGCCACTTACT ACTGTCAAGGCTATGATTATAGTACTGCTGGTGCCTATCCTTTCGGC GGAGGGACCGCGGTGGTGGTCAAAGGTGATCCAGTTGCACCTACTG TCCTCATCTTCCCACCAGCTGCTGATCAGGTGGCAACTGGAACAGTC ACCATCGTGTGTGTGGCGAATAAATACTTTCCCGATGTCACCGTCAC CTGGGAGGTGGATGGCACCACCCAAACAACTGGCATCGAGAACAG TAAAACACCGCAGAATTCTGCAGATTGTACCTACAACCTCAGCAGC ACTCTGACACTGACCAGCACACAGTACAACAGCCACAAAGAGTAC ACCTGCAAGGTGACCCAGGGCACGACCTCAGTCGTCCAGAGCTTCA ATAGGGGTGACTGTTAG
71	ATGGACACGAGGGCCCCCACTCAGCTGCTGGGGCTCCTGCTGCTCTG GCTCCCAGGTGCCACATTTGCCCAAGTGCTGACCCAGACTGCATCGC CCGTGTCTGCGGCTGTGGGAAGCACAGTCACCATCAATTGCCAGTCC AGTCAGAGCGTTTATAGTAACAAGCGCTTAGCCTGGTTTCAGCTGAA ACCAGGGCAGCCTCCCAAGCTCCTGATCTATGGTGCATCCACACTGG CATCTGGGGTCCCATCGCGATTCAAGGGCAGTGGATCTGGGACACAG TTCACTCTCACCATCAGCGACGTGCAGTGTGACGATGCTGCCACTTA CTACTGTGCAGGCGGTTATGATTGTAGTACTGGTGATTGTTGGACTTT CGGCGGAGGGACCGAGGTGGTGGTCACAGGTGATCCAGTTGCACCT ACTGTCCTCATCTTCCCACCAGCTGCTGATCAGGTGGCAACTGGAAC AGTCACCATCGTGTGTGTGGCGAATAAATACTTTCCCGATGTCACCG TCACCTGGGAGGTGGATGGCACCACCCAAACAACTGGCATCGAGAA CAGTAAAACACCGCAGAATTCTGCAGATTGTACCTACAACCTCAGCA GCACTCTGACACTGACCAGCACACAGTACAACAGCCACAAAGAGTA CACCTGCAAGGTGACCCAGGGCACGACCTCAGTCGTCCAGAGCTTC AATAGGGGTGACTGTTAG
72	ATGGACATGAGGGCCCCCACTCAGCTGCTGGGGCTCCTGCTGCTCTG GCTCCCAGGTGCCAGATGTGCTGACATCGTGATGACCCAGACTCCAT CCTCCGTGTCTGCAGCTGTGGGAGGCACAGTCACCATCAAGTGCCAG GCCAGTCAGAGCATTGGTAGTAATTTAGCCTGGTATCAGCAGAAACC

	AGGGCAGCCTCCCAAGCTCCTGATCTATGGTGCATCCACTCTGGAAT CTGGGGTCCCATCGCGGTTTAAAGGCAGTGGATCTGGGACAGAGTA CACTCTCACCATCAGCGACCTGGAGTGTGCCGATGCTGCCACTTACT ACTGTCAAAGCTATTATGAGGGTAGTGATATTGGTTATGCTTTCGGC GGAGGGACCGAGGTGGTGGTCGAAGGTGATCCAGTTGCACCTACTG TCCTCATCTTCCCACCAGCTGCTGATCAGGTGGCAACTGGAACAGTC ACCATCGTGTGTGTGGCGAATAAATACTTTCCCGATGTCACCGTCAC CTGGGAGGTGGATGGCACCACCCAAACAACTGGCATCGAGAACAGT AAAACACCGCAGAATTCTGCAGATTGTACCTACAACCTCAGCAGCA CTCTGACACTGACCAGCACACAGTACAACAGCCACAAAGAGTACAC CTGCAAGGTGACCCAGGGCACGACCTCAGTCGTCCAGAGCTTCAAT AGGGGTGACTGTTAG
73	ATGGACACGAGGGCCCCCACTCAGCTGCTGGGGCTCCTGCTGCTCTG GCTCCCAGGTGCCAGATGTGCTGACATCGTGATGACCCAGACTCCAG CCTCTGTGTCTGCAGCTGTGGGAGGCACAGTCACCATCAAGTGCCAG GCCAGTCAGAGCATTAGCAACCAACTATCCTGGTATCAGCAGAAAT CAGGGCAGCCTCCCAAGCTCCTGATCTACAGGGCATCTACTCTGGCA TCTGGGGTCCCATCGCGGTTCAAAGGCAGTGGATCTGGGACAGAGTT CACTCTCACCATCAGCGACCTGGAGTGTGCCGATGCTGCCACTTACT ACTGTCAACAGGGTTATAATAGAGATAATGTTGATAATCTTTTCGGC GGAGGGACCGAGGTGGTGGTCAAAGGTGATCCAGTTGCACCTACTG TCCTCATCTTCCCACCAGCTGCTGATCAGGTGGCAACTGGAACAGTC ACCATCGTGTGTGTGGCGAATAAATACTTTCCCGATGTCACCGTCAC CTGGGAGGTGGATGGCACCACCCAAACAACTGGCATCGAGAACAGT AAAACACCGCAGAATTCTGCAGATTGTACCTACAACCTCAGCAGCA CTCTGACACTGACCAGCACACAGTACAACAGCCACAAAGAGTACAC CTGCAAGGTGACCCAGGGCACGACCTCAGTCGTCCAGAGCTTCAAT AGGGGTGACTGTTAG

Methods of the Disclosure

[0087] Disclosed herein are methods for detecting phosphorylated tau in a sample from an individual using antibodies described herein. In some embodiments, the phosphorylated tau is selected from the group consisting of pTau-212, pTau-217, pTau-231, pTau-214, and pTau-220. In some embodiments, methods for detecting phosphorylated tau in a sample from an individual using antibodies described herein comprise improved specificity and sensitivity.

[0088] Described herein are methods for detecting phosphorylated tau in a sample from an individual comprising: performing an assay on the sample using an antibody or antibody fragment that binds to phosphorylated tau. Described herein are methods for detecting phosphorylated tau in a sample from an individual comprising: performing an immunoassay on the sample using an antibody or antibody fragment that binds to phosphorylated tau. In some embodiments, the phosphorylated tau is selected from the group consisting of pTau-212, pTau-217, pTau-231, pTau-214, pTau-220, and pTau-181. In some embodiments, the phosphorylated tau is selected from the group consisting of pTau-212, pTau-217, pTau-231, pTau-214, and pTau-220. In some embodiments, the phosphorylated tau is pTau-217. In some embodiments,

the phosphorylated tau is pTau-231. In some embodiments, the phosphorylated tau is pTau-181. In some embodiments, the phosphorylated tau is pTau-212. In some embodiments, the phosphorylated tau is pTau-217. In some embodiments, the phosphorylated tau is pTau-214. In some embodiments, the phosphorylated tau is pTau-220. In some embodiments, the phosphorylated tau is pTau-181 and pTau-217. In some embodiments, the phosphorylated tau is pTau-181 and pTau-231. In some embodiments, the phosphorylated tau is pTau-217 and pTau-231. In some embodiments, the phosphorylated tau is pTau-181, pTau-217, and pTau-231.

[0089] Further described herein are methods for detecting phosphorylated tau in a sample from an individual comprising: performing an assay on the sample using an antibody or antibody fragment that binds to multiple phosphorylated tau proteins. In some embodiments, the methods detects pTau-217 and pTau-231. In some embodiments, the methods detects pTau-212 and pTau-217. In some embodiments, the methods detects pTau-212 and pTau-231. In some embodiments, the methods detects pTau-212, pTau-217 and pTau-231.

[0090] Described herein are methods for detecting phosphorylated tau in a sample from an individual, wherein the method detects pTau-217 and pTau-231 in a sample selected from the group consisting of a plasma sample and serum sample. In some embodiments, the methods detect pTau-212 and pTau-217 in a sample selected from the group consisting of a plasma sample and serum sample. In some embodiments, the methods detect pTau-212 and pTau-231 in a sample selected from the group consisting of a plasma sample and serum sample. In some embodiments, the methods detect pTau-212, pTau-217, and pTau-231 in a sample selected from the group consisting of a plasma sample and serum sample.

[0091] Methods as described herein can comprise performing an assay on a sample, wherein the sample is selected from the group consisting of a plasma sample and serum sample. In some instances, the sample is a blood sample. In some instances, the sample is a cerebrospinal fluid sample. The sample can be a blood sample obtained by a venous blood draw. The sample can be a blood sample obtained from a finger prick blood draw. The sample can be obtained by a health care provider or by the subject. The method can comprise obtaining a sample from a subject. In some cases, the sample is obtained from the subject during a visit to the clinic or the hospital.

[0092] Further described herein, in some embodiments, are methods to determine a level of a biomarker selected from the group consisting of Aβ42, Aβ40, Aβ38, BACE1, hFABP, TREM2, YKL-40, IP-10, neurogranin, SNAP-25, synaptotagmin, alpha-synuclein, TDP-43, ferritin, VILIP-1, NfL, GFAP, and combinations thereof. In some instances, the biomarker is Aβ42. In some instances, the biomarker is Aβ40. In some instances, the biomarker is Aβ42 and Aβ40. In

some instances, the biomarker is APOE. In some instances, the biomarker is selected from the group consisting of APOE2, APOE3, and APOE4. In some instances, the biomarker is APOE4.

[0093] In some embodiments, methods for detecting phosphorylated tau in a sample comprise an immunoassay or a ligand assay using the antibodies or antibody fragments described herein. In some cases, the assay is selected from the group consisting of enzyme-linked immunosorbent assay (ELISA), a colorimetric immunoassay, a homogeneous immunoassay, a non-optical immunoassay, a fluorescence immunoassay, a chemiluminescence immunoassay, an electro-chemiluminescence immunoassay, a fluorescence resonance energy transfer (FRET) immunoassay, a time resolved fluorescence immunoassay, a lateral flow immunoassay, a microspot immunoassay, a surface plasmon resonance assay, a ligand assay, a clotting assay, a chromatography assay, and immunocapture coupled with mass spectrometry. In some cases, the assay comprises an immunoassay. In some cases, the assay is selected from the group consisting of a Western blot, enzyme-linked immunosorbent assays (ELISA), and chromatography. In some cases, the immunoassays are single-plexed. In some cases, the immunoassays are multiplexed.

[0094] Methods as described herein can comprise a plurality of immunoassays using the antibodies or antibody fragments described herein. In some cases, the plurality of immunoassays are the same immunoassay (e.g., four or more ELISA assays). When the plurality of immunoassays are the same immunoassay, each of the plurality of immunoassays can detect a different phosphorylated tau. When the plurality of immunoassays are the same immunoassay, each of the plurality of immunoassays can be performed in the same reaction chamber or a different reaction chamber. A reaction chamber can be any suitable space for performing an immunoassay. Examples of reaction chambers include, but are not limited to, a well in a microplate, an Eppendorf tube, or a droplet.

[0095] In some cases, the plurality of immunoassays are different immunoassays. When the plurality of immunoassays are different immunoassays, each of the plurality of immunoassays can detect a different phosphorylated tau. When the plurality of immunoassays are different immunoassays, each of the plurality of immunoassays can be performed in the same reaction chamber or a different reaction chamber.

[0096] In some cases, the assay comprises a non-immunoassay. In some cases, the assay is selected from the group consisting of High Performance Liquid Chromatography (HPLC), High Performance Liquid Chromatography Mass spectrometry (HPLC-MS), Gas Chromatography Mass Spectrometry (GC-MS), Liquid Chromatography Mass spectrometry (LC-MS), Liquid Chromatography Tandem Mass spectrometry (LC-MS/MS), immunohistochemistry (IHC), polymerase chain reaction (PCR), quantitative PCR (qPCR), and combinations thereof.

[0097] Methods as described herein using the antibodies described herein may be used for establishing Alzheimer's disease in the individual based on detection of phosphorylated tau. In some embodiments, Alzheimer's disease in the individual is established if pTau-212, pTau-217, pTau-231, pTau-214, pTau-220, or combinations thereof is detected in the sample from the individual.

[0098] Methods as described herein using the antibodies described herein may be used for prognosis of the individual for developing Alzheimer's disease based on detection of phosphorylated tau. In some embodiments, prognosis of the individual for developing Alzheimer's disease is determined if pTau-212, pTau-217, pTau-231, pTau-214, pTau-220, or combinations thereof is detected in the sample from the individual.

[0099] Methods as described herein using the antibodies described herein may be used accurately and specifically establish Alzheimer's disease (AD) in an individual as compared to a disease or disorder or neurologically and cognitively unimpaired condition, selected from the group consisting of a non- Alzheimer's disease(AD) neurodegenerative disease, a Aβ-negative non-AD neurodegenerative disease, a Aβ-positive non-AD neurodegenerative diseases, behavioral variant of frontotemporal dementia (BvFTD), primary progressive aphasia (PPA), vascular dementia (VaD), Parkinson's disease (PD), PD with dementia (PDD), multiple system atrophy (MSA), progressive supranuclear palsy (PSP), corticobasal syndrome (CBS), Aβ-negative cognitively impaired or unimpaired controls and combinations thereof. In some embodiments, the methods as described herein using the antibodies described herein comprise an improved accuracy or specificity of at least or about 70%, 80%, 90%, 95%, 99%, or more at establishing AD as compared to a disease or disorder or neurologically and cognitively unimpaired condition.

[00100] Methods as described herein using the antibodies described herein may be used accurately and specifically establish Alzheimer's disease (AD) in an individual as compared to a neuropathological examination or clinical diagnosis. In some embodiments, the methods as described herein using the antibodies described herein comprise an improved accuracy or specificity of at least or about 70%, 80%, 90%, 95%, 99%, or more at establishing AD as compared to a neuropathological examination or clinical diagnosis. In some embodiments, the neuropathological examination or clinical diagnosis comprises neurological tests, mental exams, or brain imaging (e.g. MRI, CT, or PET scans).

[00101] Methods as described herein using the antibodies described herein may be capable of detecting phosphorylated tau in the sample at a low limit of detection. In some embodiments, the methods as described herein using the antibodies described herein are capable of detecting

phosphorylated tau in the sample at a limit of detection of at least about 1.5 picogram per milliliter (pg/mL). In some embodiments, the methods as described herein using the antibodies described herein are capable of detecting phosphorylated tau in the sample at a limit of detection of at least about 5 picogram per milliliter (pg/mL). In some embodiments, the methods as described herein using the antibodies described herein are capable of detecting phosphorylated tau in the sample at a limit of detection in a range of about 0.5 pg/mL to about 10 pg/mL, about 1 pg/mL to about 8 pg/mL, about 1.5 pg/mL to about 7 pg/mL, about 2 pg/mL to about 6 pg/mL, or about 3 pg/mL to about 5 pg/mL.

Production of Tau Antibodies

[00102] In some embodiments, antibodies or antibody fragments described herein are produced using any method known in the art to be useful for the synthesis of antibodies or antibody fragments, in particular, by chemical synthesis or by recombinant expression, and are preferably produced by recombinant expression techniques.

[00103] In some instances, an antibody or its binding fragment thereof is expressed recombinantly, and the nucleic acid encoding the antibody or its binding fragment is assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., 1994, BioTechniques 17:242), which involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligation of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

[00104] Alternatively, a nucleic acid molecule encoding an antibody is optionally generated from a suitable source (e.g., an antibody cDNA library, or cDNA library generated from any tissue or cells expressing the immunoglobulin) by PCR amplification using synthetic primers hybridizable to the 3′ and 5′ ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence.

[00105] In some instances, an antibody or its binding is optionally generated by immunizing an animal, such as a mouse, to generate polyclonal antibodies or, more preferably, by generating monoclonal antibodies, e.g., as described by Kohler and Milstein (1975, Nature 256:495-497) or, as described by Kozbor et al. (1983, Immunology Today 4:72) or Cole et al. (1985 in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Alternatively, a clone encoding at least the Fab portion of the antibody is optionally obtained by screening Fab expression libraries (e.g., as described in Huse et al., 1989, Science 246:1275-1281) for clones of Fab fragments that bind the specific antigen or by screening antibody libraries (See, e.g., Clackson et al., 1991, Nature 352:624; Hane et al., 1997 Proc. Natl. Acad. Sci. USA 94:4937).

[00106] In some embodiments, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, Proc. Natl. Acad. Sci. 81:851-855; Neuberger et al., 1984, Nature 312:604-608; Takeda et al., 1985, Nature 314:452-454) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity are used. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region.

[00107] In some embodiments, techniques described for the production of single chain antibodies (U.S. Pat. No. 4,694,778; Bird, 1988, Science 242:423-42; Huston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; and Ward et al., 1989, Nature 334:544-54) are adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in E. coli are also optionally used (Skerra et al., 1988, Science 242:1038-1041).

[00108] In some embodiments, an expression vector comprising the nucleotide sequence of an antibody or the nucleotide sequence of an antibody is transferred to a host cell by conventional techniques (e.g., electroporation, liposomal transfection, and calcium phosphate precipitation), and the transfected cells are then cultured by conventional techniques to produce the antibody. In specific embodiments, the expression of the antibody is regulated by a constitutive, an inducible or a tissue, specific promoter.

[00109] In some embodiments, a variety of host-expression vector systems is utilized to express an antibody, or its binding fragment described herein. Such host-expression systems represent vehicles by which the coding sequences of the antibody is produced and subsequently purified, but also represent cells that are, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody or its binding fragment in situ. These include, but are not limited to, microorganisms such as bacteria (e.g., E. coli and B. subtilis) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing an antibody or its binding fragment coding sequences; yeast (e.g., Saccharomyces Pichia) transformed with recombinant yeast expression vectors containing an antibody or its binding fragment coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing an antibody or its binding fragment coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus (CaMV) and tobacco mosaic virus (TMV)) or transformed with

recombinant plasmid expression vectors (e.g., Ti plasmid) containing an antibody or its binding fragment coding sequences; or mammalian cell systems (e.g., COS, CHO, BH, 293, 293T, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g. the adenovirus late promoter; the vaccinia virus 7.5K promoter).

[00110] For long-term, high-yield production of recombinant proteins, stable expression is preferred. In some instances, cell lines that stably express an antibody are optionally engineered. Rather than using expression vectors that contain viral origins of replication, host cells are transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells are then allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci that in turn are cloned and expanded into cell lines. This method can advantageously be used to engineer cell lines which express the antibody or its binding fragments.

[00111] In some instances, a number of selection systems are used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., 1977, Cell 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, 192, Proc. Natl. Acad. Sci. USA 48:202), and adenine phosphoribosyltransferase (Lowy et al., 1980, Cell 22:817) genes are employed in tk−, hgprt− or aprt− cells, respectively. Also, antimetabolite resistance are used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., 1980, Proc. Natl. Acad. Sci. USA 77:357; O'Hare et al., 1981, Proc. Natl. Acad. Sci. USA 78:1527); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, Proc. Natl. Acad. Sci. USA 78:2072); neo, which confers resistance to the aminoglycoside G-418 (Clinical Pharmacy 12:488-505; Wu and Wu, 1991, Biotherapy 3:87-95; Tolstoshev, 1993, Ann. Rev. Pharmacol. Toxicol. 32:573-596; Mulligan, 1993, Science 260:926-932; and Morgan and Anderson, 1993, Ann. Rev. Biochem. 62:191-217; May 1993, TIB TECH 11(5):155-215) and hygro, which confers resistance to hygromycin (Santerre et al., 1984, Gene 30:147). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds., 1993, Current Protocols in Molecular Biology, John Wiley & Sons, NY; Kriegler, 1990, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY; and in Chapters 12 and 13, Dracopoli et al. (eds), 1994, Current Protocols in Human Genetics, John Wiley & Sons, NY.; Colberre-Garapin et al., 1981, J. Mol. Biol. 150:1).

[00112] In some instances, the expression levels of an antibody are increased by vector amplification (for a review, see Bebbington and Hentschel, the use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol. 3. (Academic Press, New York, 1987)). When a marker in the vector system expressing an antibody is amplifiable, an increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the nucleotide sequence of the antibody, production of the antibody will also increase (Crouse et al., 1983, Mol. Cell Biol. 3:257).

[00113] In some instances, any method known in the art for purification of an antibody is used, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins.

Expression Vectors

[00114] In some embodiments, vectors include any suitable vectors derived from either a eukaryotic or prokaryotic sources. In some cases, vectors are obtained from bacteria (e.g. E. coli), insects, yeast (e.g. Pichia pastoris), algae, or mammalian sources. Exemplary bacterial vectors include pACYC177, pASK75, pBAD vector series, pBADM vector series, pET vector series, pETM vector series, pGEX vector series, pHAT, pHAT2, pMal-c2, pMal-p2, pQE vector series, pRSET A, pRSET B, pRSET C, pTrcHis2 series, pZA31-Luc, pZE21-MCS-1, pFLAG ATS, pFLAG CTS, pFLAG MAC, pFLAG Shift-12c, pTAC-MAT-1, pFLAG CTC, or pTAC-MAT-2.

[00115] Exemplary insect vectors include pFastBac1, pFastBac DUAL, pFastBac ET, pFastBac HTa, pFastBac HTb, pFastBac HTc, pFastBac M30a, pFastBact M30b, pFastBac, M30c, pVL1392, pVL1393, pVL1393 M10, pVL1393 M11, pVL1393 M12, FLAG vectors such as pPolh-FLAG1 or pPolh-MAT 2, or MAT vectors such as pPolh-MAT1, or pPolh-MAT2.

[00116] In some cases, yeast vectors include Gateway® pDEST™ 14 vector, Gateway® pDEST™ 15 vector, Gateway® pDEST™ 17 vector, Gateway® pDEST™ 24 vector, Gateway® pYES-DEST52 vector, pBAD-DEST49 Gateway® destination vector, pAO815 Pichia vector, pFLD1 Pichi pastoris vector, pGAPZA,B, & C Pichia pastoris vector, pPIC3.5K Pichia vector, pPIC6 A, B, & C Pichia vector, pPIC9K Pichia vector, pTEF1/Zeo, pYES2 yeast vector, pYES2/CT yeast vector, pYES2/NT A, B, & C yeast vector, or pYES3/CT yeast vector.

[00117] Exemplary algae vectors include pChlamy-4 vector or MCS vector.

[00118] Examples of mammalian vectors include transient expression vectors or stable expression vectors. Mammalian transient expression vectors may include pRK5, p3xFLAG-

CMV 8, pFLAG-Myc-CMV 19, pFLAG-Myc-CMV 23, pFLAG-CMV 2, pFLAG-CMV 6a,b,c, pFLAG-CMV 5.1, pFLAG-CMV 5a,b,c, p3xFLAG-CMV 7.1, pFLAG-CMV 20, p3xFLAG-Myc-CMV 24, pCMV-FLAG-MAT1, pCMV-FLAG-MAT2, pBICEP-CMV 3, or pBICEP-CMV 4. Mammalian stable expression vector may include pFLAG-CMV 3, p3xFLAG-CMV 9, p3xFLAG-CMV 13, pFLAG-Myc-CMV 21, p3xFLAG-Myc-CMV 25, pFLAG-CMV 4, p3xFLAG-CMV 10, p3xFLAG-CMV 14, pFLAG-Myc-CMV 22, p3xFLAG-Myc-CMV 26, pBICEP-CMV 1, or pBICEP-CMV 2.

[00119] In some instances, a cell-free system is a mixture of cytoplasmic and/or nuclear components from a cell and is used for in vitro nucleic acid synthesis. In some cases, a cell-free system utilizes either prokaryotic cell components or eukaryotic cell components. Sometimes, a nucleic acid synthesis is obtained in a cell-free system based on for example Drosophila cell, Xenopus egg, or HeLa cells. Exemplary cell-free systems include, but are not limited to, E. coli S30 Extract system, E. coli T7 S30 system, or PURExpress®.

Host Cells

[00120] In some embodiments, a host cell includes any suitable cell such as a naturally derived cell or a genetically modified cell. In some instances, a host cell is a production host cell. In some instances, a host cell is a eukaryotic cell. In other instances, a host cell is a prokaryotic cell. In some cases, a eukaryotic cell includes fungi (e.g., yeast cells), animal cell or plant cell. In some cases, a prokaryotic cell is a bacterial cell. Examples of bacterial cell include gram-positive bacteria or gram-negative bacteria. Sometimes the gram-negative bacteria is anaerobic, rod-shaped, or both.

[00121] In some instances, gram-positive bacteria include Actinobacteria, Firmicutes or Tenericutes. In some cases, gram-negative bacteria include Aquificae, Deinococcus-Thermus, Fibrobacteres–Chlorobi/Bacteroidetes (FCB group), Fusobacteria, Gemmatimonadetes, Nitrospirae, Planctomycetes–Verrucomicrobia/ Chlamydiae (PVC group), Proteobacteria, Spirochaetes or Synergistetes. Other bacteria can be Acidobacteria, Chloroflexi, Chrysiogenetes, Cyanobacteria, Deferribacteres, Dictyoglomi, Thermodesulfobacteria or Thermotogae. A bacterial cell can be Escherichia coli, Clostridium botulinum, or Coli bacilli.

[00122] Exemplary prokaryotic host cells include, but are not limited to, BL21, Mach1™, DH10B™, TOP10, DH5α, DH10Bac™, OmniMax™, MegaX™, DH12S™, INV110, TOP10F', INVαF, TOP10/P3, ccdB Survival, PIR1, PIR2, Stbl2™, Stbl3™, or Stbl4™.

[00123] In some instances, animal cells include a cell from a vertebrate or from an invertebrate. In some cases, an animal cell includes a cell from a marine invertebrate, fish,

insects, amphibian, reptile, or mammal. In some cases, a fungus cell includes a yeast cell, such as brewer's yeast, baker's yeast, or wine yeast.

[00124] Fungi include ascomycetes such as yeast, mold, filamentous fungi, basidiomycetes, or zygomycetes. In some instances, yeast includes Ascomycota or Basidiomycota. In some cases, Ascomycota includes Saccharomycotina (true yeasts, e.g. Saccharomyces cerevisiae (baker's yeast)) or Taphrinomycotina (e.g. Schizosaccharomycetes (fission yeasts)). In some cases, Basidiomycota includes Agaricomycotina (e.g. Tremellomycetes) or Pucciniomycotina (e.g. Microbotryomycetes).

[00125] Exemplary yeast or filamentous fungi include, for example, the genus: Saccharomyces, Schizosaccharomyces, Candida, Pichia, Hansenula, Kluyveromyces, Zygosaccharomyces, Yarrowia, Trichosporon, Rhodosporidi, Aspergillus, Fusarium, or Trichoderma. Exemplary yeast or filamentous fungi include, for example, the species: Saccharomyces cerevisiae, Schizosaccharomyces pombe, Candida utilis, Candida boidini, Candida albicans, Candida tropicalis, Candida stellatoidea, Candida glabrata, Candida krusei, Candida parapsilosis, Candida guilliermondii, Candida viswanathii, Candida lusitaniae, Rhodotorula mucilaginosa, Pichia metanolica, Pichia angusta, Pichia pastoris, Pichia anomala, Hansenula polymorpha, Kluyveromyces lactis, Zygosaccharomyces rouxii, Yarrowia lipolytica, Trichosporon pullulans, Rhodosporidium toru-Aspergillus niger, Aspergillus nidulans, Aspergillus awamori, Aspergillus oryzae, Trichoderma reesei, Yarrowia lipolytica, Brettanomyces bruxellensis, Candida stellata, Schizosaccharomyces pombe, Torulaspora delbrueckii, Zygosaccharomyces bailii, Cryptococcus neoformans, Cryptococcus gattii, or Saccharomyces boulardii.

[00126] Exemplary yeast host cells include, but are not limited to, Pichia pastoris yeast strains such as GS115, KM71H, SMD1168, SMD1168H, and X-33; and Saccharomyces cerevisiae yeast strain such as INVSc1.

[00127] In some instances, additional animal cells include cells obtained from a mollusk, arthropod, annelid or sponge. In some cases, an additional animal cell is a mammalian cell, e.g., from a primate, ape, equine, bovine, porcine, canine, feline or rodent. In some cases, a rodent includes mouse, rat, hamster, gerbil, hamster, chinchilla, fancy rat, or guinea pig.

[00128] Exemplary mammalian host cells include, but are not limited to, 293A cell line, 293FT cell line, 293F cells , 293 H cells, CHO DG44 cells, CHO-S cells, CHO-K1 cells, FUT8 KO CHOK1, Expi293F™ cells, Flp-In™ T-REx™ 293 cell line, Flp-In™-293 cell line, Flp-In™-3T3 cell line, Flp-In™-BHK cell line, Flp-In™-CHO cell line, Flp-In™-CV-1 cell line, Flp-In™-Jurkat cell line, FreeStyle™ 293-F cells, FreeStyle™ CHO-S cells, GripTite™ 293

MSR cell line, GS-CHO cell line, HepaRG™ cells, T-REx™ Jurkat cell line, Per.C6 cells, T-REx™-293 cell line, T-REx™-CHO cell line, and T-REx™-HeLa cell line.

[00129] In some instances, a mammalian host cell is a stable cell line, or a cell line that has incorporated a genetic material of interest into its own genome and has the capability to express the product of the genetic material after many generations of cell division. In some cases, a mammalian host cell is a transient cell line, or a cell line that has not incorporated a genetic material of interest into its own genome and does not have the capability to express the product of the genetic material after many generations of cell division.

[00130] Exemplary insect host cells include, but are not limited to, Drosophila S2 cells, Sf9 cells, Sf21 cells, High Five™ cells, and expresSF+® cells.

In some instances, plant cells include a cell from algae. Exemplary insect cell lines include, but are not limited to, strains from Chlamydomonas reinhardtii 137c, or Synechococcus elongatus PPC 7942.

NUMBERED EMBODIMENTS

[00131] Numbered embodiment 1 comprises a method for detecting phosphorylated tau in a sample from an individual comprising: performing an immunoassay on the sample using an antibody or antibody fragment comprising a variable domain, heavy chain region (VH) and a variable domain, light chain region (VL), wherein the VH comprises an amino acid sequence at least about 90% identical to a sequence as set forth in any one of SEQ ID NOs: 30-34, and wherein the VL comprises an amino acid sequence at least about 90% identical to a sequence as set forth in any one of SEQ ID NOs: 35-40. Numbered embodiment 2 comprises the method of numbered embodiment 1, wherein the phosphorylated tau is selected from the group consisting of pTau-181, pTau-212, pTau-217, pTau-231, pTau-214, and pTau-220. Numbered embodiment 3 comprises the method of numbered embodiments 1-2, wherein the phosphorylated tau is pTau-217. Numbered embodiment 4 comprises the method of numbered embodiments 1-2, wherein the phosphorylated tau is pTau-231. Numbered embodiment 5 comprises the method of numbered embodiment 2, wherein the method detects pTau-217 and pTau-231. Numbered embodiment 6 comprises the method of numbered embodiment 2, wherein the method detects pTau-212 and pTau-217. Numbered embodiment 7 comprises the method of numbered embodiment 2, wherein the method detects pTau-212 and pTau-231. Numbered embodiment 8 comprises the method of numbered embodiment 2, wherein the method detects pTau-181 and pTau-217. Numbered embodiment 9 comprises the method of numbered embodiment 2, wherein the method detects pTau-181 and pTau-231. Numbered embodiment 10 comprises the method of numbered embodiment 2, wherein the method detects pTau-181, pTau-217 and pTau-231. Numbered embodiment 11 comprises the method of numbered embodiment 2, wherein the method detects pTau-212, pTau-217 and pTau-231. Numbered embodiment 12 comprises the

method of numbered embodiment 5, wherein the method detects pTau-217 and pTau-231 in a sample selected from the group consisting of a plasma sample and serum sample. Numbered embodiment 13 comprises the method of numbered embodiment 6, wherein the method detects pTau-212 and pTau-217 in a sample selected from the group consisting of a plasma sample and serum sample. Numbered embodiment 14 comprises the method of numbered embodiment 7, wherein the method detects pTau-212 and pTau-231 in a sample selected from the group consisting of a plasma sample and serum sample. Numbered embodiment 15 comprises the method of numbered embodiment 11, wherein the method detects pTau-181 and pTau-217 in a sample selected from the group consisting of a plasma sample and serum sample. Numbered embodiment 16 comprises the method of numbered embodiment 11, wherein the method detects pTau-181 and pTau-231 in a sample selected from the group consisting of a plasma sample and serum sample. Numbered embodiment 17 comprises the method of numbered embodiment 11, wherein the method detects pTau-181, pTau-217, and pTau-231 in a sample selected from the group consisting of a plasma sample and serum sample. Numbered embodiment 18 comprises the method of numbered embodiment 11, wherein the method detects pTau-212, pTau-217, and pTau-231 in a sample selected from the group consisting of a plasma sample and serum sample. Numbered embodiment 19 comprises the method of numbered embodiments 1-18, wherein the VH comprises an amino acid sequence according to any one of SEQ ID NOs: 30-34. Numbered embodiment 20 comprises the method of numbered embodiments 1-19, wherein the VL comprises an amino acid sequence according to any one of SEQ ID NOs: 35-40. Numbered embodiment 21 comprises the method of numbered embodiments 1-20, wherein the VH comprises an amino acid sequence according to any one of SEQ ID NOs: 30-34, and wherein the VL comprises an amino acid sequence according to any one of SEQ ID NOs: 35-40. Numbered embodiment 22 comprises the method of numbered embodiments 1-21, wherein the VH comprises an amino acid sequence at least about 90% identical to SEQ ID NO: 30, and wherein the VL comprises an amino acid sequence at least about 90% identical to SEQ ID NO: 35. Numbered embodiment 23 comprises the method of numbered embodiments 1-21, wherein the VH comprises an amino acid sequence at least about 90% identical to SEQ ID NO: 31, and wherein the VL comprises an amino acid sequence at least about 90% identical to SEQ ID NO: 36. Numbered embodiment 24 comprises the method of numbered embodiments 1-21, wherein the VH comprises an amino acid sequence at least about 90% identical to SEQ ID NO: 31, and wherein the VL comprises an amino acid sequence at least about 90% identical to SEQ ID NO: 37. Numbered embodiment 25 comprises the method of numbered embodiments 1-21, wherein the VH comprises an amino acid sequence at least about 90% identical to SEQ ID NO: 32, and

wherein the VL comprises an amino acid sequence at least about 90% identical to SEQ ID NO: 38. Numbered embodiment 26 comprises the method of numbered embodiments 1-21, wherein the VH comprises an amino acid sequence at least about 90% identical to SEQ ID NO: 33, and wherein the VL comprises an amino acid sequence at least about 90% identical to SEQ ID NO: 39. Numbered embodiment 27 comprises the method of numbered embodiments 1-21, wherein the VH comprises an amino acid sequence at least about 90% identical to SEQ ID NO: 34, and wherein the VL comprises an amino acid sequence at least about 90% identical to SEQ ID NO: 40. Numbered embodiment 28 comprises the method of numbered embodiments 1-27, wherein the antibody or antibody fragment comprises an amino acid sequence at least about 90% identical to any one of SEQ ID NOs: 41-51. Numbered embodiment 29 comprises the method of numbered embodiments 1-28, further comprising performing an assay on the sample to determine a level of a biomarker selected from the group consisting of Aβ42, Aβ40, Aβ38, BACE1, hFABP, TREM2, YKL-40, IP-10, neurogranin, SNAP-25, synaptotagmin, alpha-synuclein, TDP-43, ferritin, VILIP-1, NfL, GFAP, and combinations thereof. Numbered embodiment 30 comprises the method of numbered embodiments 1-29, wherein the sample is selected from the group consisting of a blood sample, a plasma sample, a serum sample, and a cerebrospinal fluid (CSF) sample. Numbered embodiment 31 comprises the method of numbered embodiments 1-30, further comprising establishing Alzheimer's disease in the individual based on detection of phosphorylated tau. Numbered embodiment 32 comprises the method of numbered embodiments 1-31, further comprising establishing prognosis of the individual for developing Alzheimer's disease based on detection of phosphorylated tau. Numbered embodiment 33 comprises the method of numbered embodiment 32, further determining the individual's age, genotype, or expression of a biomarker. Numbered embodiment 34 comprises the method of numbered embodiment 33, wherein the biomarker is selected from the group consisting of Aβ42, Aβ40, Aβ38, BACE1, hFABP, TREM2, YKL-40, IP-10, neurogranin, SNAP-25, synaptotagmin, alpha-synuclein, TDP-43, ferritin, VILIP-1, NfL, GFAP, and combinations thereof. Numbered embodiment 35 comprises the method of numbered embodiments 1-34, wherein the method has a specificity of at least about 80% for detecting phosphorylated tau. Numbered embodiment 36 comprises the method of numbered embodiments 1-34, wherein the method has a specificity of at least about 85% for detecting phosphorylated tau. Numbered embodiment 37 comprises the method of numbered embodiments 1-34, wherein the method has a specificity of at least about 90% for detecting phosphorylated tau. Numbered embodiment 38 comprises the method of numbered embodiments 1-37, wherein the method has a sensitivity of at least about 80% for detecting phosphorylated tau. Numbered

embodiment 39 comprises the method of numbered embodiments 1-37, wherein the method has a sensitivity of at least about 85% for detecting phosphorylated tau. Numbered embodiment 40 comprises the method of numbered embodiments 1-37, wherein the method has a sensitivity of at least about 90% for detecting phosphorylated tau. Numbered embodiment 41 comprises the method of numbered embodiments 1-40, wherein the method is capable of detecting phosphorylated tau in the sample at a limit of detection of at least about 1.0 picogram per milliliter (pg/mL). Numbered embodiment 42 comprises the method of numbered embodiments 1-40, wherein the method is capable of detecting phosphorylated tau in the sample at a limit of detection of at least about 1.5 picogram per milliliter (pg/mL). Numbered embodiment 43 comprises the method of numbered embodiments 1-40, wherein the method is capable of detecting phosphorylated tau in the sample at a limit of detection of at least about 5 picogram per milliliter (pg/mL).

[00132] Numbered embodiment 44 comprises an anti-tau antibody comprising i) a heavy chain comprising variable heavy chain (VH) domain and ii) a light chain comprising a variable light chain (VL) domain, wherein the VH domain comprises HCDR1 sequence comprising a sequence selected from SEQ ID NOs: 1-5, HCDR2 sequence comprising a sequence selected from SEQ ID NOs: 6-9, and HCDR3 sequence comprising a sequence selected from SEQ ID NOs: 10-13, and VL domain comprises LCDR1 sequence comprising a sequence selected from SEQ ID NOs: 14-19, LCDR2 sequence comprising a sequence selected from SEQ ID NOs: 20-23, and LCDR3 sequence comprising a sequence selected from SEQ ID NOs: 24-29. Numbered embodiment 45 comprises the anti-tau antibody of numbered embodiment 44, wherein the HCDR1 sequence comprises SEQ ID NO: 1, HCDR2 sequence comprises SEQ ID NO: 6, HCDR3 sequence comprises SEQ ID NO: 10, LCDR1 sequence comprises SEQ ID NO: 14, LCDR2 sequence comprises SEQ ID NO: 20, and LCDR3 sequence comprises SEQ ID NO: 24. Numbered embodiment 46 comprises the anti-tau antibody of numbered embodiment 44, wherein the HCDR1 sequence comprises SEQ ID NO: 2, HCDR2 sequence comprises SEQ ID NO: 7, HCDR3 sequence comprises SEQ ID NO: 11, LCDR1 sequence comprises SEQ ID NO: 15, LCDR2 sequence comprises SEQ ID NO: 21, and LCDR3 sequence comprises SEQ ID NO: 25. Numbered embodiment 47 comprises the anti-tau antibody of numbered embodiment 44, wherein the HCDR1 sequence comprises SEQ ID NO: 2, HCDR2 sequence comprises SEQ ID NO: 7, HCDR3 sequence comprises SEQ ID NO: 11, LCDR1 sequence comprises SEQ ID NO: 16, LCDR2 sequence comprises SEQ ID NO: 22, and LCDR3 sequence comprises SEQ ID NO: 26. Numbered embodiment 48 comprises the anti-tau antibody of numbered embodiment 44, wherein the HCDR1 sequence comprises SEQ ID NO: 3, HCDR2 sequence comprises SEQ ID

NO: 8, HCDR3 sequence comprises SEQ ID NO: 10, LCDR1 sequence comprises SEQ ID NO: 17, LCDR2 sequence comprises SEQ ID NO: 20, and LCDR3 sequence comprises SEQ ID NO: 27. Numbered embodiment 49 comprises the anti-tau antibody of numbered embodiment 44, wherein the HCDR1 sequence comprises SEQ ID NO: 4, HCDR2 sequence comprises SEQ ID NO: 7, HCDR3 sequence comprises SEQ ID NO: 12, LCDR1 sequence comprises SEQ ID NO: 18, LCDR2 sequence comprises SEQ ID NO: 23, and LCDR3 sequence comprises SEQ ID NO: 28. Numbered embodiment 50 comprises the anti-tau antibody of numbered embodiment 44, wherein the HCDR1 sequence comprises SEQ ID NO: 5, HCDR2 sequence comprises SEQ ID NO: 9, HCDR3 sequence comprises SEQ ID NO: 13, LCDR1 sequence comprises SEQ ID NO: 19, LCDR2 sequence comprises SEQ ID NO: 21, and LCDR3 sequence comprises SEQ ID NO: 29. Numbered embodiment 51 comprises the anti-tau antibody of numbered embodiment 44, wherein the VH domain comprises at least 80%, at least 85%, at least 90%, at least 95% sequence identity to a sequence selected from SEQ ID NOs: 30-34. Numbered embodiment 52 comprises the anti-tau antibody of numbered embodiment 44, wherein the VL domain comprises at least 80%, at least 85%, at least 90%, at least 95% sequence identity to a sequence selected from SEQ ID NOs: 35-40.

[00133] Numbered embodiment 53 comprises the anti-tau antibody of numbered embodiments 44-52, wherein the anti-tau antibody is a chimeric antibody or antigen binding fragment thereof. Numbered embodiment 54 comprises the anti-tau antibody of numbered embodiments 44-53, wherein the anti-tau antibody comprises an IgG-scFv, nanobody, BiTE, diabody, DART, TandAb, scDiabody, scDiabody-CH3, triple body, mini-antibody, minibody, TriBi minibody, scFv-CH3 KIH, Fab-scFv-Fc KIH, Fab-scFv, scFv-CH-CL-scFv, Fab', F(ab')2, F(ab')3, F(ab')2-scFv2, scFv, scFv-KIH, Fab-scFv-Fc, tetravalent HCAb, scDiabody-Fc, diabody-Fc, tandem scFv-Fc, or intrabody. Numbered embodiment 55 comprises the anti-tau antibody of numbered embodiments 44-54, wherein the anti-tau antibody is an IgG1 antibody. Numbered embodiment 56 comprises the anti-tau antibody of numbered embodiments 44-55, wherein the anti-tau antibody is an IgG2 antibody. Numbered embodiment 57 comprises the anti-tau antibody of numbered embodiments 44-56, wherein the anti-tau antibody is an IgG4 antibody. Numbered embodiment 58 comprises the anti-tau antibody of numbered embodiments 44-57, wherein the light chain is a kappa chain. Numbered embodiment 59 comprises the anti-tau antibody of numbered embodiments 44-58, wherein the anti-tau antibody has a binding affinity to human tau of about 100 pM to about 3 nM. Numbered embodiment 60 comprises the anti-tau antibody of numbered embodiments 44-59, wherein the anti-tau antibody comprises a VH domain that is encoded by a nucleic acid comprising at least 80%, at least 85%, at least 90%,

at least 95% sequence identity to a sequence selected from SEQ ID NOs: 52-56. Numbered embodiment 61 comprises the anti-tau antibody of numbered embodiments 44-60, wherein the anti-tau antibody comprises a VL domain that is encoded by a nucleic acid comprising at least 80%, at least 85%, at least 90%, at least 95% sequence identity to a sequence selected from SEQ ID NOs: 57-62. Numbered embodiment 62 comprises the anti-tau antibody of numbered embodiments 44-61, wherein the anti-tau antibody comprises a VH domain that is encoded by a nucleic acid comprising at least 80%, at least 85%, at least 90%, at least 95% sequence identity to a sequence selected from SEQ ID NOs: 52-56 and a VL domain that is encoded by a nucleic acid comprising at least 80%, at least 85%, at least 90%, at least 95% sequence identity to a sequence selected from SEQ ID NOs: 57-62. Numbered embodiment 63 comprises the anti-tau antibody of numbered embodiments 44-62, wherein the anti-tau antibody comprises a VH domain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NOs: 52-56. Numbered embodiment 64 comprises the anti-tau antibody of numbered embodiments 44-63, wherein the anti-tau antibody comprises a VL domain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NOs: 57-62. Numbered embodiment 65 comprises the anti-tau antibody of numbered embodiments 44-64 wherein the anti-tau antibody comprises a VH domain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NOs: 52-56 and a VL domain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NOs: 57-62.

EXAMPLES

[00134] The following examples are given for the purpose of illustrating various embodiments of the invention and are not meant to limit the present invention in any fashion. The present examples, along with the methods described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Changes therein and other uses which are encompassed within the spirit of the invention as defined by the scope of the claims will occur to those skilled in the art.

[00135] **Example 1: Tau Antibody Screening**

[00136] Tau antibodies that detect phosphorylated Tau were assayed in the Simoa® bead assay using a 2-step or 3-step protocol assay according to manufacturer's instructions. *See* **Fig. 1**.

[00137] The antibodies tested were Antibody 1, Antibody 2, Antibody 3, Antibody 4, Antibody 5, and Antibody 6. The capture results can be seen in **Figs. 2A-2D**. **Fig. 2A** is data

from an 2-step protocol assay in which all capture antibodies were tested against Tau-12 detector (detects Tau at the N-terminus). The data demonstrates all captures had similar results with improved sensitivity seen with Antibody 6. **Fig. 2B** is data from an 2-step protocol assay in which all capture antibodies were tested against HT7-BT2 detectors (detects Tau in the mid-domain region). Both biotinylated antibodies were used. Data shows about a 10-fold increase in background compared to Tau-12 detector. No signal at 1000 pg/mL for any capture was detected. **Fig. 2C** is data from an 3-step protocol assay in which all capture antibodies were tested against Tau-12 detector. The data demonstrates reduced sensitivity compared to the 2-step protocol and improved sensitivity seen with Antibody 6. **Fig. 2D** is data from an 3-step protocol assay in which all capture antibodies were tested against HT7-BT2 detectors. Both biotinylated antibodies were used. Data shows about a 10-fold increase in background compared to Tau-12 detector. Based on the results, the 2-step protocol was then further optimized for sensitivity.

[00138] Example 2. Pharmacokinetics of Tau Antibodies

[00139] Antibodies were tested for pharmacokinetic profile.

[00140] The antigen information for the antibodies is seen in **Table 10**.

Table 10.

Description	Antigen Name	SEQ ID NO:	Sequence
pT217a	WZN-1A	74	RSRTPSLP(pT)PPTREPKC
pT217b	WZN-1B	75	TPSLP(pT)PPTREPKKVAC
T217	WZN-1C	76	RSRTPSLPTPPTREPKKVAC
pT212	WZN-1D	77	RSR(pT)PSLPTPPTREPKC
pS214	WZN-1E	78	RSRTP(pS)LPTPPTREPKC
pT220	WZN-1F	79	RSRTPSLPTPP(pT)REPKKVAC
pT231	WZN-1G	80	KVAVVR(pT)PPKSPSSAC
T231	WZN-1H	81	KVAVVRTPPKSPSSAC

[00141] Antibodies were generated and purified. The antibodies were assayed using a standard indirect ELISA protocol. Briefly, peptide antigens corresponding to SEQ ID NOs: 74-81 were diluted to 1 ug/ml in PBS and plated onto a Greiner Bio One Microlon 96 well plate. Peptide antigens were produced by the manufacturer Abcam. WZN-1A and WZN-1B served as targets. WZN-1C, WZN-1D, WZN-1E, WZN-1F, WZN-1G, and WZN-1H served as negative controls. In the peptide antigen sequences, a phosphorylated residue is indicated by (pT) for phosphorylated threonine or (pS) for phosphorylated serine. After blocking with 1% BSA in PBS

pH 7.4, antibodies were serially diluted 1 to 4 with an initial concentration of 1 ug/ml. After incubation, unbound antibodies were washed off with 1X TBST and HRP labeled goat anti-rabbit secondary antibody was applied according to the manufacturer's instructions. Subsequently, unbound secondary antibody was washed off with 1X TBST and 3,3'5,5'-tetramethylbenzidine (TMB) was applied for 5 minutes at room temperature and plates were read at 650nm. Data is seen in **Fig. 3**. **Fig. 3** shows screening data of the different monoclonal antibodies to varying peptide concentrations.

[00142] Example 3. Tau Antibodies for Immunohistochemistry

[00143] Tau antibodies described herein were tested in immunohistochemistry assays.

[00144] Briefly, all antibodies were optimised using a range of concentrations (0.01 – 3.00 ug/ml) and stained using a Leica Bond RX automated IHC platform: ER1 antigen retrieval (sodium citrate, pH 6) 20 mins at 100 °C; primary antibody 15 minutes at RT; IVD grade Leica Polymer Refine HRP detection 8 minutes at room temperature; DAB chromogen 10 minutes at room temperature, and finally hematoxylin counterstain 5 minutes at room temperature. Antibodies that passed basic IHC staining went on to undergo IHC staining following alkaline phosphatase (AP) treatment (200 U/ml, 37 °C for 60 minutes). A vehicle-only control (buffer containing no AP) was also employed. Positive antigen control tissues were FFPE normal human cerebral cortex and cerebral cortex from an Alzheimer patient. Negative antigen control tissues were FFPE normal human liver, skeletal muscle and heart muscle. All tissues were collated into a tissue micro array to streamline the IHC staining process. Negative reagent (detection system only) controls were employed and shown to be negative. Benchmark antibodies stained alongside the test antibodies were rabbit monoclonal [EPR22524-95] to Tau (ab254256, Abcam plc) and rabbit monoclonal [EPR1884(2)] to Tau (phospho S214) (ab170892, Abcam plc).

[00145] The benchmark antibodies demonstrated that the antibodies exhibited negative staining in the negative control tissue and positive staining in the positive control tissue (data not shown). Data for the Tau antibodies is seen in **Figs. 4A-4G** (Antibody 6), **Figs. 5A-5G** (Antibody 5), and **Figs. 6A-6G** (Antibody 2). Antibody 5 and Antibody 6 demonstrated similar data as benchmark antibodies in that negative staining was observed in negative control tissues that included normal heart, normal liver, and normal skeletal muscle, and normal cerebral cortex and positive staining was observed in positive control tissues that included Alzheimer cerebral cortex. Antibodies 1-4 did not exhibit similar data as benchmark antibodies.

[00146] Example 4. Detection of Tau peptides using Tau antibodies

[00147] Tau antibodies described herein that detect phosphorylated Tau were tested in ELISA assays. Tau antibodies were first tested for pTau 217 reactivity by indirect ELISA. **Fig.** 7 displays a diagram depicting the indirect ELISA assay format utilized. Briefly, streptavidin beads were bound to a plate and biotinylated peptide was added to the plates under conditions allowing for biotin-streptavidin binding. The biotinylated peptide was a synthetic peptide comprising a portion of Tau and possessing a phosphorylated threonine residue at position 217 (pT217). This was the target peptide. After binding of the synthetic peptide to the plate by the formation of the biotin-streptavidin complex, Tau antibodies were added to the plates under conditions allowing for antibody-target peptide binding. After binding, plates were washed to remove any unbound antibody and plates then had a secondary antibody, or a tracer antibody, added (either goat anti-mouse antibody conjugated to peroxidase or donkey anti-rabbit antibody conjugated to peroxidase) directed to the species from which the Tau antibodies were derived. After binding, plates were washed to remove any unbound tracer antibody and plates next had TMB ELISA Peroxidase chromogenic substrate (3, 3', 5, 5' - Tetramethylbenzidine) added to visualize antibody reactivity in indirect ELISA experiments. Antibody sample binding was quantitated using an ELISA microplate reader.

[00148] As shown in **Fig.** 7, five antibodies were tested for an ability to detect phosphorylated Tau using this indirect ELISA technique. IBA493 mAb corresponds to a rabbit anti-Tau antibody capable of binding to Tau phosphorylated at threonine residue 217 (pTau 217) (Eli Lilly and Company). PT3 corresponds to a mouse anti-phospho (T212/T217) Tau selective antibody (Janssen Biotech Inc.). 30H10L2 corresponds to Antibody 2 described herein. 71H1L2 corresponds to Antibody 6 described herein. 62H10L7 corresponds to Antibody 5 described herein. All five antibodies were assayed for reactivity with pTau 217 in two separate ELISA instruments at the following concentrations: 10^{-2}, 10^{-1}, 10^{-0}, 10^{1}, 10^{2}, 10^{3}, and 10^{4} ng/mL per plate. As can been seen in the Bio-pt655 (phospho T217) and Bio-pt660 (phospho T217) graphs, both IBA493 mAb and PT3 demonstrated a robust, concentration-dependent level reactivity to pTau 217. Antibody 2 demonstrated a more modest concentration-dependent level reactivity to pTau 217 revealed at 10^{4} ng/mL per plate. Antibody 5 and Antibody 6 did not demonstrate reactivity to pTau 217 in these assays. A graph in **Fig.** 7 showing the results of this ELISA assay using the five test antibodies on phosphatase-treated pTau demonstrated the specificity of antibodies IBA493 mAb, PT3, and Antibody 2 in detecting phosphorylated Tau.

[00149] Tau antibodies described herein that detect phosphorylated Tau were tested in Simoa®-based assays. **Figs. 8-24** display results Simoa® assays designed to sensitive tests for

Tau reactivity to antibodies described herein. In some aspects, Simoa®-based assays can be approximately 1000X more sensitivity at detecting a given analyte when compared to detection of the same analyte in an indirect ELISA assay. This elevated sensitivity of Simoa®-based assays allows for the development and use of biomarkers that previously could not have generated a detectable signal using a traditional assay such as indirect ELISA. The elevated sensitivity of Simoa®-based assays when compared to conventional immunoassays such as indirect ELISA is due to the fact that the Simoa® method is capable of detecting single target molecules whereas conventional immunoassays typically require large reaction volume and millions of fluorophores, or millions of antibody-conjugated enzymes reacted to a color-producing substrate, before an optical signal can be detected. For Simoa®-based assays, average enzyme per bead (AEB) denotes raw signal output.

[00150] In **Fig. 8**, Antibody 2, as described herein and is capable of detecting pTau 217, was used in a Simoa®-based assay to detect a level of an analyte per plasma sample derived from an individual. Signal-to-noise (S/N) ratio was determined by Simoa®for each sample and plotted in a graph. 120 plasma samples were taken from individuals and assayed. The graphed S/N ratio indicated that all tested samples apart from one yielded a signal within the expected concentration range. When plasma samples were diluted 1:3 and then assayed again, only 3 of the 120 samples yielded a result below measurement of a blank control and only 5 samples registered a measurement of S/N 1.5, which was determined to be the limit of detection (LOD). In **Fig. 8**, with each of the 120 plasma samples assayed, a calculation was made to determine the coefficient of variation (CV%) for each sample and the results were graphed against measured concentration. 10 of the 120 samples yielded a CV% greater than 20 and from this analysis, the estimated analytical lower limit of quantitation (LLOQ) was determined to be 0.08 pg/mL. This LLOQ value represents the lowest amount of an analyte (Tau phosphorylated at T217) that can be quantitatively determined with an acceptable level of precision. These results in **Fig. 8** indicated the sensitivity of the Simoa® method to detect Tau phosphorylated at T217 using Antibody 2.

[00151] In **Fig. 9**, calibration curves were generated for the Simoa® pTau-217 assay and graphed [AEB vs log(CAL) pg/mL] using 68 CSF samples and 120 plasma samples separated into 4 plates using Antibody 2 and 4 plates using ADx Neuroscience antibody ADx204. In another graph from this assay performed on a separate instrument [AEB vs log(CAL) pg/mL] with AEB plotted on a log scale demonstrated the fit of the data can enable accurate analyte quantitation calculations when measuring samples.

[00152] In **Fig. 10**, 38 paired CSF and EDTA plasma samples were measured with the Simoa® pTau-217 assay using Antibody 2. This assay is also named as ALZpath Dx. Results were graphed and samples were indicated with their clinical diagnosis (non-AD, uncertain, or AD). These results, and the statistical analysis thereof, indicated a strong correlation between CSF and plasma pTau levels as measured with the Simoa® pTau-217 assay using Antibody 2 (R value ~ 0.7 and P value for two-tailed T test < 0.0001 between non-AD and AD).

[00153] In **Fig. 11**, 42 CSF samples were measured with the Simoa® pTau-217 assay using Antibody 2 and a Simoa® pTau-181 assay using a pTau-181 antibody from Quanterix® (Quanterix® Corp., Item number 103714) and plotted against each other. This demonstrated that the Simoa® pTau-217 assay using Antibody 2 showed the expected relationship with an analyte implicated in AD detected in CSF (pTau-181). Statistical analysis indicated an R value ~ 0.8 and P value for two-tailed T test < 0.0001.

[00154] In **Fig. 12**, 42 CSF samples were measured with the Simoa® pTau-217 assay using Antibody 2 and a Simoa® pTau assay using Innotest pTau-181 antibody and plotted against each other. This demonstrated that the Simoa® pTau-217 assay using Antibody 2 showed the expected relationship with an analyte implicated in AD detected in CSF (pTau). Statistical analysis indicated an R value ~ 0.77 and P value for two-tailed T test < 0.0001.

[00155] In **Fig. 13**, 42 CSF samples were measured with a Simoa® HD-X assay using Antibody 2 as a capture antibody, ADx204 antibody as a detector, and a peptide as calibrator. This demonstrated that the Simoa® assays using known AD biomarkers showed the expected relationship. Statistical analysis indicated an R value ~ 0.9 and P value for two-tailed T test < 0.0001.

[00156] In **Fig. 14**, CSF samples and plasma samples were measured with the Simoa® pTau-217 assay using Antibody 2 and graphed in separate graphs. Clinical diagnosis of AD, or control with no AD diagnosis, was used as the classifier for each sample. Analysis of the graphed results indicated a significant difference between samples derived from individuals with a clinical AD diagnosis vs controls for both CSF samples and plasma samples. Area under the curve (AUC) calculation was 0.94 for CSF samples and 0.86 for plasma samples. These results indicated that the Simoa® pTau-217 assay using Antibody 2 was able to differentiate AD cases in CSF and plasma.

[00157] In **Fig. 15**, 4 EDTA plasma samples with high pTau levels serve as quality controls (labelled QC_L1, QC_L2, QC_M, and QC_H) were measured with the Simoa® pTau-217 assay using Antibody 2 in duplicate test and pTau levels were calculated. Plotting the results from

repeated testing demonstrated the precision and reproducibility of the Simoa® pTau-217 assay using Antibody 2.

[00158] In **Fig. 16**, control samples from Fig. 15 and additional measured samples were measured with the Simoa® pTau-217 assay using Antibody 2 and plotted in two separate experiments to generate precision profiles. The precision profiles are based on measured sample concentration and inter-run CV% of the 4 QC samples. From this experiment, a functional LLOQ of pTau-217 in this assay was determined to be 0.26 pg/mL.

[00159] In **Fig. 17**, parallelism was assessed in the Simoa® pTau-217 assay using Antibody 2. A determination of parallelism is also important in that it shows if a signal is specific. Parallelism determines whether actual samples containing high endogenous analyte concentrations provide the same degree of detection in the assay in a standard curve after dilutions. This can represent differences in antibody binding affinity to endogenous analyte and a standard or calibration analyte. This can ensure that recombinant standards parallel native recognition of the endogenous analyte. In this experiment, 4 plasma samples, each from different donors, with relatively high concentration of detected pTau-217 and a spiked dilution buffer (sample 5) were diluted with a factor of 2 in 5 steps, starting at a dilution of 3X. The concentrations dropped below LLOD from dilution factor 12X onwards for all 4 plasma samples. In a graph of log (measured pg/mL) vs log [dilution factor(DF)] plasma measurements over spike measurements demonstrated linearity in detection along the various dilutions. 3 out of the 4 plasma samples were determined to fall within the accepted range of parallelism, with Sample 4 falling just outside of the accepted range. The accepted range of parallelism is < 15%. These results demonstrated that the Simoa® pTau-217 assay using Antibody 2 on plasma samples yielded consistent and precise calculations of pTau-217 levels across various concentrations thus demonstrated its utility as a biomarker assay.

[00160] In **Figs 18-19**, dilution linearity using the Simoa® pTau-217 assay using Antibody 2 was performed to demonstrate that a sample with a spike concentration about the upper limit of quantification (ULOQ) can be diluted to a concentration within the working range while still yielding a reliable assay result. In **Fig. 18**, three spiked samples (s1, s2, and s3) and the Calibration sample were assayed and plotted as log(measured pg/mL) vs log (DF) to determine dilution linearity. In **Fig. 19**, three spiked samples (s1, s2, and s3) and the Calibration sample were assayed and plotted as log(measured pg/mL) vs log (DF) to determine dilution linearity with the highest spike point omitted from s1, s2, and s3 since it was out of the calibration range of 50 pg/mL.

[00161] In **Fig. 20**, the Simoa® pTau-217 assay using Antibody 2 was used to assess samples taken from a memory clinic cohort. Plasma samples were measured and graphed for calculated pTau217 concentrations. Clinical diagnosis of AD was used as the classifier. AUC was calculated at 0.916 indicating success in distinguishing AD+ from AD- within this cohort with the Simoa® pTau-217 assay using Antibody 2. Also in **Fig. 20**, a receiver operating characteristic (ROC) curve was plotted to illustrate the diagnostic ability of this binary classifier (AD+ or AD-) system as it is possible that the discrimination threshold between classifiers is varied.

[00162] In **Fig. 21-22**, clinical performance of the Simoa® pTau-217 assay using Antibody 2 was compared to a Simoa® pTau-181 assays using antibody P-tau181 – Quanterix®. In **Fig. 21**, Antibody 2 was able to distinguish assayed plasma samples from taken from AD dementia individuals vs Controls (P value $1.3e^{-12}$ for Antibody 2). In **Fig. 22**, the commercially available P-tau181 – Quanterix® Simoa® assay (Quanterix® Corp., Item number 103714) was also able to distinguish assayed plasma samples from taken from AD dementia individuals vs Controls (P value $9.6e^{-08}$). Data from the individuals from which the samples were derived for data from **Figs. 21-22** is listed in **Fig. 22**.

[00163] In **Fig. 23** precision plots were generated for the Simoa® assay using P-tau217 Antibody 2 and P-tau181 – Quanterix® antibody (Quanterix® Corp., Item number 103714). Calculated LLODs were 0.55 pg/mL and 0.24 pg/mL, respectively. Concentrations were not back calculated and LLOD values are accordingly not back calculated.

[00164] In **Fig. 24** ROC curves were plotted for the P-tau181 – Quanterix® antibody (Quanterix® Corp., Item number 103714) and P-tau217 Antibody 2. Analysis of the data indicated that P-tau217 Antibody 2 exhibited superior sensitivity and specificity when comparing to the Simoa® assay using the P-tau181 – Quanterix® antibody in differentiating AD-dementia from controls. The diagnostic accuracy of Antibody 2 for AD dementia in this Simoa® method is 92.5% when tested on plasma samples. The diagnostic specificity of Antibody 2 for AD dementia in this Simoa® method is 85% when tested on plasma samples.

[00165] In **Fig. 25** a schematic diagram of Tau polypeptide indicating the relative location of various protein domains and the locations of threonine residues which can be assayed for phosphorylation status using methods disclosed herein is depicted. The location of pT217 within the P2 domain of Tau is indicated. pT181 resides within the P1 domain and pT231 resides near the border between the P2 and R1 domains.

[00166] In **Fig. 26** various Tau antibodies were assayed using indirect ELISA and extent of reactivity to a Tau fragment with non-phosphorylated T217 (Bio-pt654) and full length Tau

(Tau441) were graphed. IBA493 mAB and PT3 displayed concentration dependent reactivity to both Bio-pt654 and Tau441. Antibody 2, 5, and 6 described herein do not display any reactivity to either Bio-pt654 or Tau441 in this assaying demonstrating precision and specificity in pTau-217 detection for Antibodies 2, 5, and 6.

[00167] In **Fig. 27** various Tau antibodies were assayed using indirect ELISA and extent of reactivity to a Tau fragment with phosphorylated T181 (Bio-pt126) and phosphorylated T231 (Bio-pt146) were graphed. IBA493 mAB and Antibody 2 described herein displayed concentration dependent reactivity to Bio-pt126. IBA493 mAB was the only antibody of those tested displaying concentration dependent reactivity to Bio-pt146. This demonstrates that IBA493 mAB, PT3, and Antibody 2 described herein were each distinguishable based on which analytes each antibody interacted with via indirect ELISA. IBA493 mAB interacts with pTau-217, non-phospho T217, full length Tau, pTau-181, and pTau-231. PT3 interacts with pTau-217, non-phospho T217, and full length Tau. Antibody 2 interacts with pTau-217 and pTau-181. IBA493 mAB's interaction with non-phospho T217 was also shown to be considerably less than PT3's interaction with non-phospho T217.

[00168] In **Fig. 28**, a diagram of an assay using Antibody 2 to detect capture of particular Tau peptides is depicted. In this assay, Antibody 2 is bound to a plate and sample wells from the plate are subjected to various biotinylated peptides under conditions conducive to forming specific antibody-ligand interactions. Samples are then washes to remove excess unbound biotinylated peptide. Streptavidin beads conjugated to peroxidase and then added to the samples to allow biotin-streptavidin complexes to form on peptide bound antibodies. TMB_{red} substrate is then added and samples are measured for colorimetric development using an ELISA plate reader. The results were graphed and out of various pTau-217, pTau-231, and pTau-181 peptides tested, Adx-pt655 yielded specific dose-dependent reactivity. These results illustrate the specificity of Antibody 2 to specific features of pTau (namely Tau phosphorylated at threonine 217). Antibody 5 and Antibody 6 tested by indirect ELISA under the same conditions using the same Tau peptides yielded no specific dose-dependent reactivity toward the Tau peptides tested.

[00169] Various pTau-217 antibodies corresponding to Antibody 1, Antibody 2, Antibody 3, Antibody 4, Antibody 5, and Antibody 5 described here were also evaluated as capture antibodies either directly coated onto or onto streptavidin-coated plates on the Mesoscale Discovery technology platform. This system uses non-radioactive, electrochemiluminescent labels, thereby conferring significant advantages over traditional ELISA assays. These advantages include lower background signal, improved sensitivity, and a dynamic range of detection.

[00170] In **Fig. 29**, Western blots were used to assess binding of various antibodies to brain lysate samples from AD patients and Control subjects. In the five Western blots shown, samples are loaded according to the same sample key shown in **Fig. 29**. Protein ladders were run in lanes 1 and 10. Phosphatase treated pTau loaded in an amount of 0.05 ug was run in lane 2. Full-length Tau (Tau411) loaded in an amount of 0.05 ug was run in lane 3. Lanes 4-6 contain samples from different Control subjects with a dilution factor of 5. Lanes 7-9 contain samples from different AD subjects with a dilution factor of 5. The results indicated that IBA394 mAb and PT3 both bound to and immunoprecipitated different length isoforms of Tau in control samples and AD samples and immunoprecipitated significantly more Tau in AD samples while showing no interaction with synthetic full length Tau or phosphatase treated pTau. Antibody 2 (30H2L10) bound to and immunoprecipitated different length isoforms of Tau in AD samples but did not immunoprecipitate a significant amount to of Tau in samples from Control individuals. Antibody 5 and Antibody 6 did not yield detectable Western blot signals in this assay.

[00171] While preferred embodiments of the present disclosure have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the disclosure. It should be understood that various alternatives to the embodiments of the disclosure described herein may be employed in practicing the disclosure. It is intended that the following claims define the scope of the disclosure and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT WE CLAIM IS:

1. A method for detecting phosphorylated tau in a sample from an individual comprising: performing an immunoassay on the sample using an antibody or antibody fragment comprising a variable domain, heavy chain region (VH) and a variable domain, light chain region (VL), wherein the VH comprises an amino acid sequence at least about 90% identical to a sequence as set forth in any one of SEQ ID NOs: 30-34, and wherein the VL comprises an amino acid sequence at least about 90% identical to a sequence as set forth in any one of SEQ ID NOs: 35-40.

2. The method of claim 1, wherein the phosphorylated tau is selected from the group consisting of pTau-181, pTau-212, pTau-217, pTau-231, pTau-214, and pTau-220.

3. The method of any one of claims 1-2, wherein the phosphorylated tau is pTau-217.

4. The method of any one of claims 1-2, wherein the phosphorylated tau is pTau-231.

5. The method of claim 2, wherein the method detects pTau-217 and pTau-231.

6. The method of claim 2, wherein the method detects pTau-212 and pTau-217.

7. The method of claim 2, wherein the method detects pTau-212 and pTau-231.

8. The method of claim 2, wherein the method detects pTau-181 and pTau-217.

9. The method of claim 2, wherein the method detects pTau-181 and pTau-231.

10. The method of claim 2, wherein the method detects pTau-181, pTau-217, and pTau-231.

11. The method of claim 2, wherein the method detects pTau-212, pTau-217 and pTau-231.

12. The method of claim 5, wherein the method detects pTau-217 and pTau-231 in a sample selected from the group consisting of a plasma sample and serum sample.

13. The method of claim 6, wherein the method detects pTau-212 and pTau-217 in a sample selected from the group consisting of a plasma sample and serum sample.

14. The method of claim 7, wherein the method detects pTau-212 and pTau-231 in a sample selected from the group consisting of a plasma sample and serum sample.

15. The method of claim 8, wherein the method detects pTau-181 and pTau-217 in a sample selected from the group consisting of a plasma sample and serum sample.

16. The method of claim 9, wherein the method detects pTau-181 and pTau-231 in a sample selected from the group consisting of a plasma sample and serum sample.

17. The method of claim 10, wherein the method detects pTau-181, pTau-217, and pTau-231 in a sample selected from the group consisting of a plasma sample and serum sample.

18. The method of claim 11, wherein the method detects pTau-212, pTau-217, and pTau-231 in a sample selected from the group consisting of a plasma sample and serum sample.

19. The method of any one of claims 1-18, wherein the VH comprises an amino acid sequence according to any one of SEQ ID NOs: 30-34.

20. The method of any one of claims 1-19, wherein the VL comprises an amino acid sequence according to any one of SEQ ID NOs: 35-40.

21. The method of any one of claims 1-20, wherein the VH comprises an amino acid sequence according to any one of SEQ ID NOs: 30-34, and wherein the VL comprises an amino acid sequence according to any one of SEQ ID NOs: 35-40.

22. The method of any one of claims 1-21, wherein the VH comprises an amino acid sequence at least about 90% identical to SEQ ID NO: 30, and wherein the VL comprises an amino acid sequence at least about 90% identical to SEQ ID NO: 35.

23. The method of any one of claims 1-21, wherein the VH comprises an amino acid sequence at least about 90% identical to SEQ ID NO: 31, and wherein the VL comprises an amino acid sequence at least about 90% identical to SEQ ID NO: 36.

24. The method of any one of claims 1-21, wherein the VH comprises an amino acid sequence at least about 90% identical to SEQ ID NO: 31, and wherein the VL comprises an amino acid sequence at least about 90% identical to SEQ ID NO: 37.

25. The method of any one of claims 1-21, wherein the VH comprises an amino acid sequence at least about 90% identical to SEQ ID NO: 32, and wherein the VL comprises an amino acid sequence at least about 90% identical to SEQ ID NO: 38.

26. The method of any one of claims 1-21, wherein the VH comprises an amino acid sequence at least about 90% identical to SEQ ID NO: 33, and wherein the VL comprises an amino acid sequence at least about 90% identical to SEQ ID NO: 39.

27. The method of any one of claims 1-21, wherein the VH comprises an amino acid sequence at least about 90% identical to SEQ ID NO: 34, and wherein the VL comprises an amino acid sequence at least about 90% identical to SEQ ID NO: 40.

28. The method of any one of claims 1-27, wherein the antibody or antibody fragment comprises an amino acid sequence at least about 90% identical to any one of SEQ ID NOs: 41-51.

29. The method of any one of claims 1-28, further comprising performing an assay on the sample to determine a level of a biomarker selected from the group consisting of Aβ42, Aβ40,

Aβ38, BACE1, hFABP, TREM2, YKL-40, IP-10, neurogranin, SNAP-25, synaptotagmin, alpha-synuclein, TDP-43, ferritin, VILIP-1, NfL, GFAP, and combinations thereof.

30. The method of any one of claims 1-29, wherein the sample is selected from the group consisting of a blood sample, a plasma sample, a serum sample, and a cerebrospinal fluid (CSF) sample.

31. The method of any one of claims 1-30, further comprising establishing Alzheimer's disease in the individual based on detection of phosphorylated tau.

32. The method of any of the claims 1-31, further comprising establishing prognosis of the individual for developing Alzheimer's disease based on detection of phosphorylated tau.

33. The method of claim 32, further determining the individual's age, genotype, or expression of a biomarker.

34. The method of claim 33, wherein the biomarker is selected from the group consisting of Aβ42, Aβ40, Aβ38, BACE1, hFABP, TREM2, YKL-40, IP-10, neurogranin, SNAP-25, synaptotagmin, alpha-synuclein, TDP-43, ferritin, VILIP-1, NfL, GFAP, and combinations thereof.

35. The method of any one of claims 1-34, wherein the method has a specificity of at least about 80% for detecting phosphorylated tau.

36. The method of any one of claims 1-34, wherein the method has a specificity of at least about 85% for detecting phosphorylated tau.

37. The method of any one of claims 1-34, wherein the method has a specificity of at least about 90% for detecting phosphorylated tau.

38. The method of any one of claims 1-37, wherein the method has a sensitivity of at least about 80% for detecting phosphorylated tau.

39. The method of any one of claims 1-37, wherein the method has a sensitivity of at least about 85% for detecting phosphorylated tau.

40. The method of any one of claims 1-37, wherein the method has a sensitivity of at least about 90% for detecting phosphorylated tau.

41. The method of any one of claims 1-40, wherein the method is capable of detecting phosphorylated tau in the sample at a limit of detection of at least about 1.0 picogram per milliliter (pg/mL).

42. The method of any one of claims 1-40, wherein the method is capable of detecting phosphorylated tau in the sample at a limit of detection of at least about 1.5 picogram per milliliter (pg/mL).

43. The method of any one of claims 1-40, wherein the method is capable of detecting phosphorylated tau in the sample at a limit of detection of at least about 5 picogram per milliliter (pg/mL).

44. An anti-tau antibody comprising i) a heavy chain comprising variable heavy chain (VH) domain and ii) a light chain comprising a variable light chain (VL) domain, wherein the VH domain comprises HCDR1 sequence comprising a sequence selected from SEQ ID NOs: 1-5, HCDR2 sequence comprising a sequence selected from SEQ ID NOs: 6-9, and HCDR3 sequence comprising a sequence selected from SEQ ID NOs: 10-13, and VL domain comprises LCDR1 sequence comprising a sequence selected from SEQ ID NOs: 14-19, LCDR2 sequence comprising a sequence selected from SEQ ID NOs: 20-23, and LCDR3 sequence comprising a sequence selected from SEQ ID NOs: 24-29.

45. The anti-tau antibody of claim 44, wherein the HCDR1 sequence comprises SEQ ID NO: 1, HCDR2 sequence comprises SEQ ID NO: 6, HCDR3 sequence comprises SEQ ID NO: 10, LCDR1 sequence comprises SEQ ID NO: 14, LCDR2 sequence comprises SEQ ID NO: 20, and LCDR3 sequence comprises SEQ ID NO: 24.

46. The anti-tau antibody of claim 44, wherein the HCDR1 sequence comprises SEQ ID NO: 2, HCDR2 sequence comprises SEQ ID NO: 7, HCDR3 sequence comprises SEQ ID NO: 11, LCDR1 sequence comprises SEQ ID NO: 15, LCDR2 sequence comprises SEQ ID NO: 21, and LCDR3 sequence comprises SEQ ID NO: 25.

47. The anti-tau antibody of claim 44, wherein the HCDR1 sequence comprises SEQ ID NO: 2, HCDR2 sequence comprises SEQ ID NO: 7, HCDR3 sequence comprises SEQ ID NO: 11, LCDR1 sequence comprises SEQ ID NO: 16, LCDR2 sequence comprises SEQ ID NO: 22, and LCDR3 sequence comprises SEQ ID NO: 26.

48. The anti-tau antibody of claim 44, wherein the HCDR1 sequence comprises SEQ ID NO: 3, HCDR2 sequence comprises SEQ ID NO: 8, HCDR3 sequence comprises SEQ ID NO: 10, LCDR1 sequence comprises SEQ ID NO: 17, LCDR2 sequence comprises SEQ ID NO: 20, and LCDR3 sequence comprises SEQ ID NO: 27.

49. The anti-tau antibody of claim 44, wherein the HCDR1 sequence comprises SEQ ID NO: 4, HCDR2 sequence comprises SEQ ID NO: 7, HCDR3 sequence comprises SEQ ID NO: 12, LCDR1 sequence comprises SEQ ID NO: 18, LCDR2 sequence comprises SEQ ID NO: 23, and LCDR3 sequence comprises SEQ ID NO: 28.

50. The anti-tau antibody of claim 44, wherein the HCDR1 sequence comprises SEQ ID NO: 5, HCDR2 sequence comprises SEQ ID NO: 9, HCDR3 sequence comprises SEQ ID NO: 13,

LCDR1 sequence comprises SEQ ID NO: 19, LCDR2 sequence comprises SEQ ID NO: 21, and LCDR3 sequence comprises SEQ ID NO: 29.

51. The anti-tau antibody of claim 44, wherein the VH domain comprises at least 80%, at least 85%, at least 90%, at least 95% sequence identity to a sequence selected from SEQ ID NOs: 30-34.

52. The anti-tau antibody of claim 44, wherein the VL domain comprises at least 80%, at least 85%, at least 90%, at least 95% sequence identity to a sequence selected from SEQ ID NOs: 35-40.

53. The anti-tau antibody of any one of claims 44-52, wherein the anti-tau antibody is a chimeric antibody or antigen binding fragment thereof.

54. The anti-tau antibody of any one of claims 44-53, wherein the anti-tau antibody comprises an IgG-scFv, nanobody, BiTE, diabody, DART, TandAb, scDiabody, scDiabody-CH3, triple body, mini-antibody, minibody, TriBi minibody, scFv-CH3 KIH, Fab-scFv-Fc KIH, Fab-scFv, scFv-CH-CL-scFv, Fab', F(ab')2, F(ab')3, F(ab')2-scFv2, scFv, scFv-KIH, Fab-scFv-Fc, tetravalent HCAb, scDiabody-Fc, diabody-Fc, tandem scFv-Fc, or intrabody.

55. The anti-tau antibody of any one of claims 44-54, wherein the anti-tau antibody is an IgG1 antibody.

56. The anti-tau antibody of any one of claims 44-55, wherein the anti-tau antibody is an IgG2 antibody.

57. The anti-tau antibody of any one of claims 44-56, wherein the anti-tau antibody is an IgG4 antibody.

58. The anti-tau antibody of any one of claims 44-57, wherein the light chain is a kappa chain.

59. The anti-tau antibody of any one of claims 44-58, wherein the anti-tau antibody has a binding affinity to human tau of about 100 pM to about 3 nM.

60. The anti-tau antibody of any one of claims 44-59, wherein the anti-tau antibody comprises a VH domain that is encoded by a nucleic acid comprising at least 80%, at least 85%, at least 90%, at least 95% sequence identity to a sequence selected from SEQ ID NOs: 52-56.

61. The anti-tau antibody of any one of claims 44-60, wherein the anti-tau antibody comprises a VL domain that is encoded by a nucleic acid comprising at least 80%, at least 85%, at least 90%, at least 95% sequence identity to a sequence selected from SEQ ID NOs: 57-62.

62. The anti-tau antibody of any one of claims 44-61, wherein the anti-tau antibody comprises a VH domain that is encoded by a nucleic acid comprising at least 80%, at least 85%, at least 90%, at least 95% sequence identity to a sequence selected from SEQ ID NOs: 52-56 and

a VL domain that is encoded by a nucleic acid comprising at least 80%, at least 85%, at least 90%, at least 95% sequence identity to a sequence selected from SEQ ID NOs: 57-62.

63. The anti-tau antibody of any one of claims 44-62, wherein the anti-tau antibody comprises a VH domain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NOs: 52-56.

64. The anti-tau antibody of any one of claims 44-63, wherein the anti-tau antibody comprises a VL domain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NOs: 57-62.

65. The anti-tau antibody of any one of claims 44-64, wherein the anti-tau antibody comprises a VH domain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NOs: 52-56 and a VL domain that is encoded by a nucleic acid comprising a sequence identical to SEQ ID NOs: 57-62.

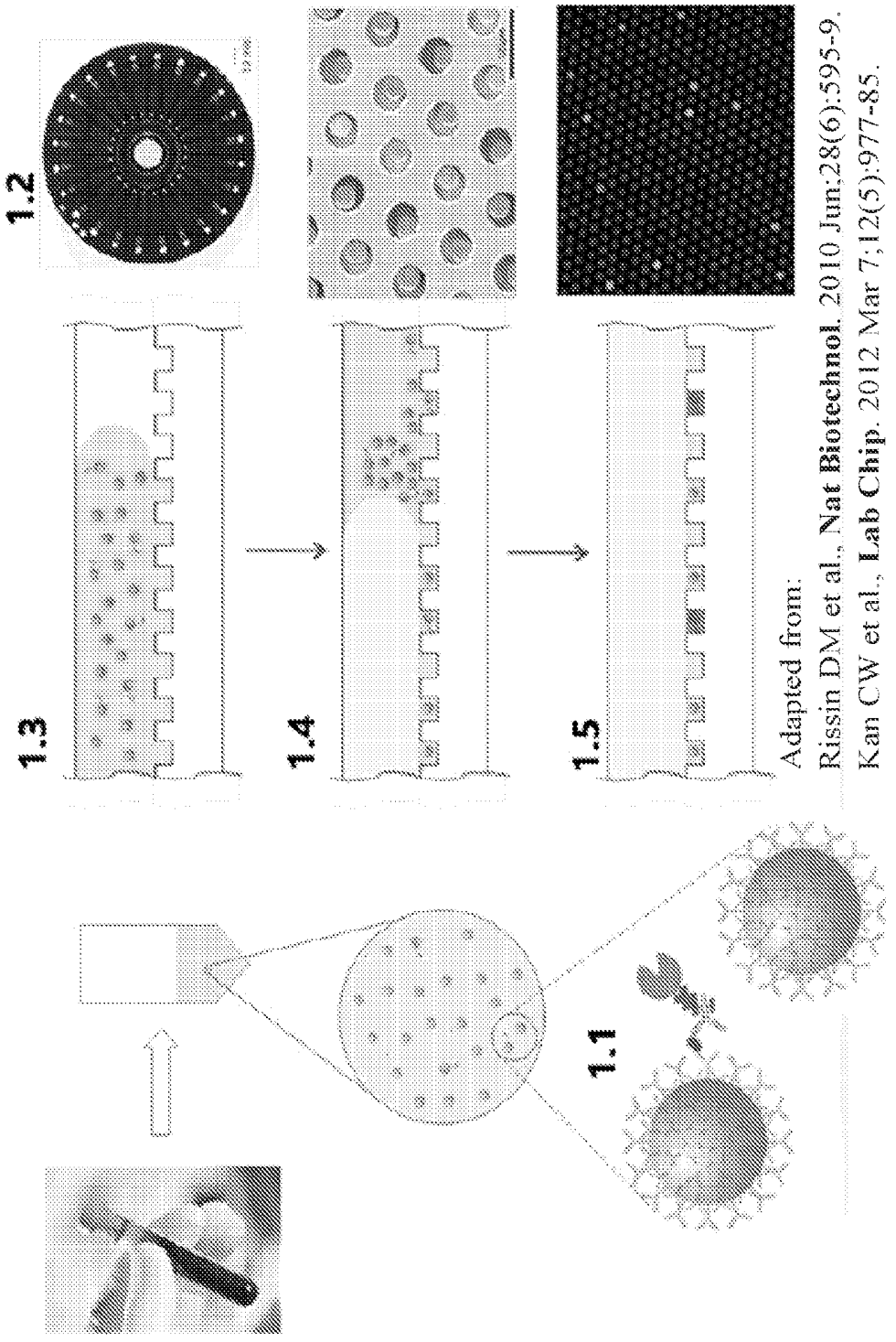

1.1
1.2
1.3
1.4
1.5
Adapted from:
Rissin DM et al., Nat Biotechnol. 2010 Jun;28(6):595-9.
Kan CW et al., Lab Chip. 2012 Mar 7;12(5):977-85.

FIG. 1

Capture	pTau (pg/mL)	Ave AEB	S/B	LOD (pg/mL)
H7L4	0	0.0059	-	6.8
	1	0.0051	0.9	
	10	0.0087	1.5	
	100	0.0357	6.0	
	1000	0.2782	47	
H3L12	0	0.0065	-	5.0
	1	0.0078	1.2	
	10	0.0092	1.4	
	100	0.0369	5.7	
	1000	0.2622	41	
H1L2	0	0.0034	-	2.6
	1	0.0036	1.1	
	10	0.0069	2.0	
	100	0.0326	9.5	
	1000	0.2832	83	

FIG. 2A

Capture	pTau (pg/mL)	Ave AEB	S/B
H7L4	0	0.0491	-
	1	0.0487	1.0
	10	0.0433	0.9
	100	0.0436	0.9
	1000	0.0441	0.9
H3L12	0	0.0660	-
	1	0.0669	1.0
	10	0.0640	1.0
	100	0.0660	1.0
	1000	0.0595	0.9
H1L2	0	0.0364	-
	1	0.0344	0.9
	10	0.0318	0.9
	100	0.0302	0.8
	1000	0.0323	0.9

FIG. 2B

Capture	pTau (pg/mL)	Ave AEB	S/B	LOD (pg/mL)
H7L4	0	0.0034	-	14.7
	1	0.0035	1.0	
	10	0.0045	1.3	
	100	0.0078	2.3	
	1000	0.0447	13	
H3L12	0	0.0056	-	88.1
	1	0.0052	0.9	
	10	0.0046	0.8	
	100	0.0106	1.9	
	1000	0.0474	8.4	
H1L2	0	0.0026	-	9.1
	1	0.0029	1.1	
	10	0.0036	1.4	
	100	0.0069	2.7	
	1000	0.0331	13	

FIG. 2C

Capture	pTau (pg/mL)	Ave AEB	S/B
H7L4	0	0.0204	-
	1	0.0218	1.1
	10	0.0210	1.0
	100	0.0227	1.1
	1000	0.0247	1.2
H3L12	0	0.0348	-
	1	0.0356	1.0
	10	0.0352	1.0
	100	0.0366	1.1
	1000	0.0347	1.0
H1L2	0	0.0123	-
	1	0.0125	1.0
	10	0.0157	1.3
	100	0.0141	1.2
	1000	0.0157	1.3

FIG. 2D

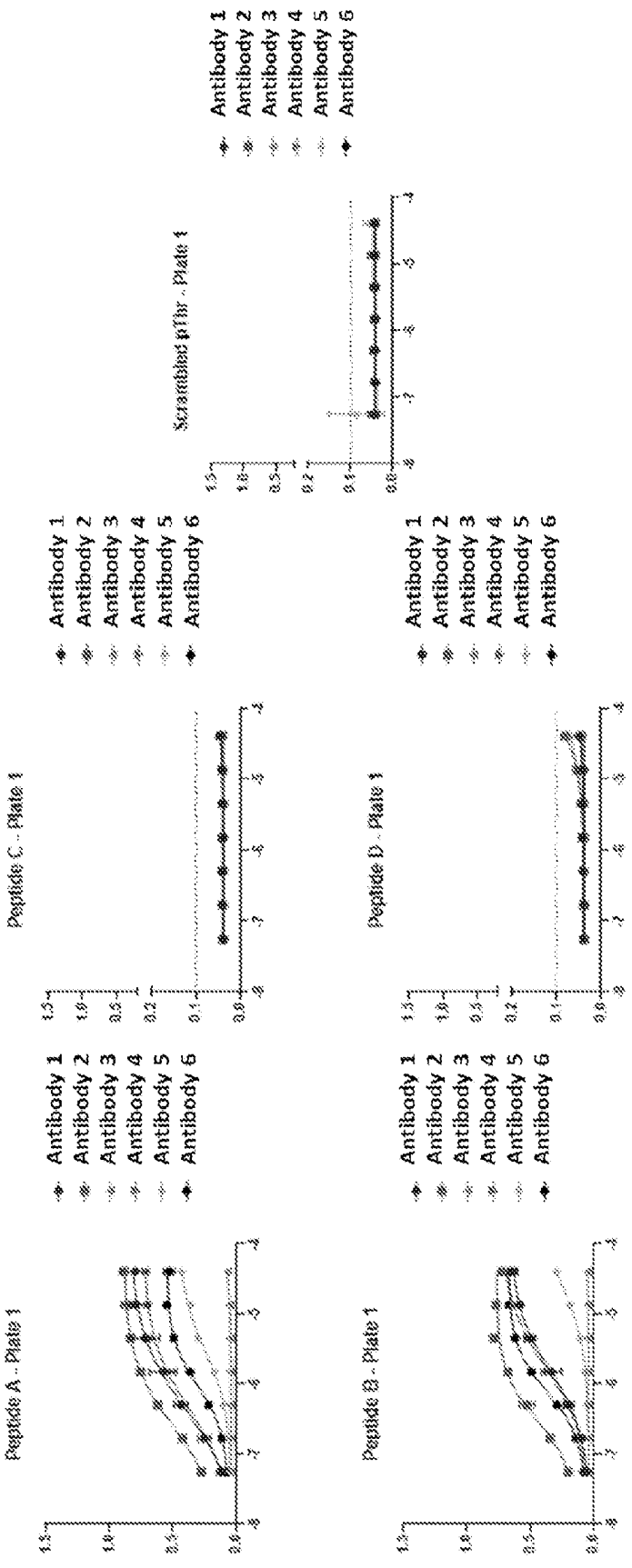
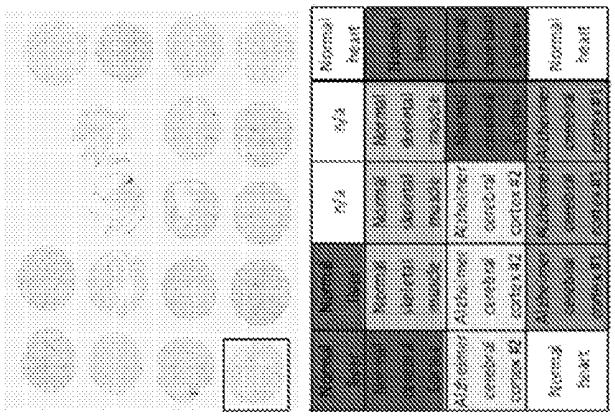

Peptide A - Plate 1
Peptide B - Plate 1
Peptide C - Plate 1
Peptide D - Plate 1
Scrambled pTau - Plate 1
Antibody 1
Antibody 2
Antibody 3
Antibody 4
Antibody 5
Antibody 6

FIG. 3



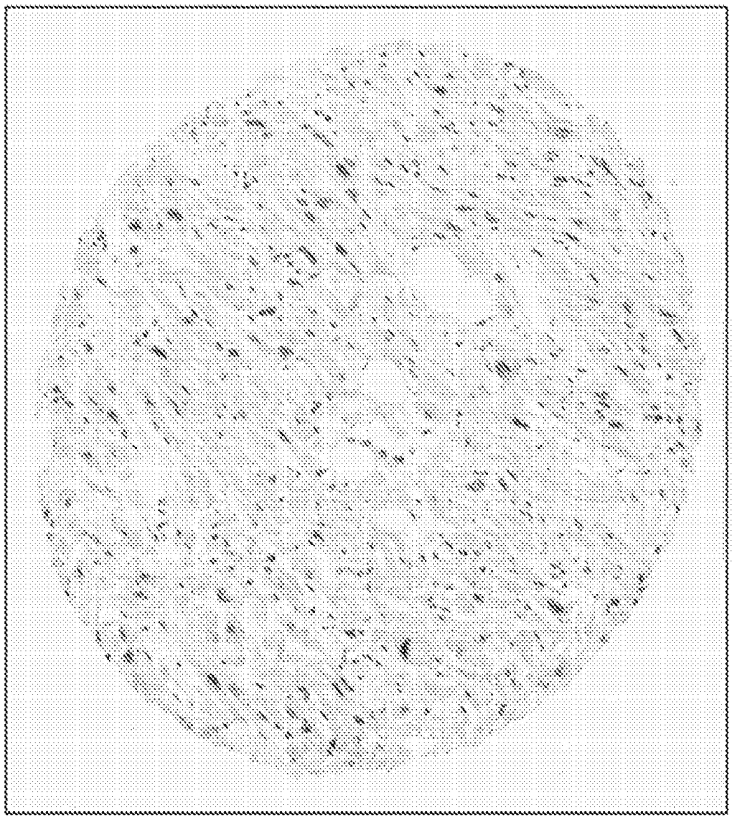
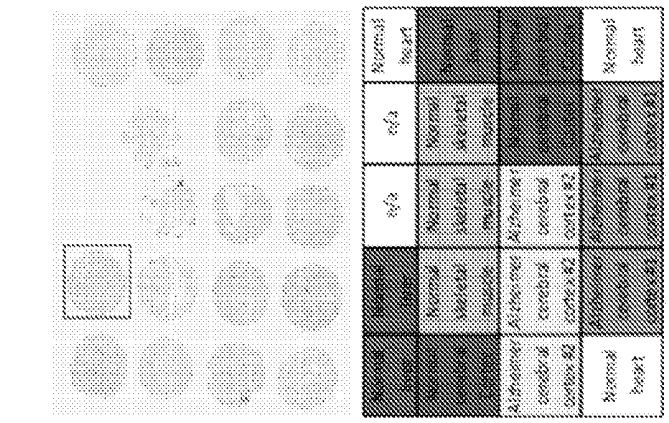


FIG. 4A



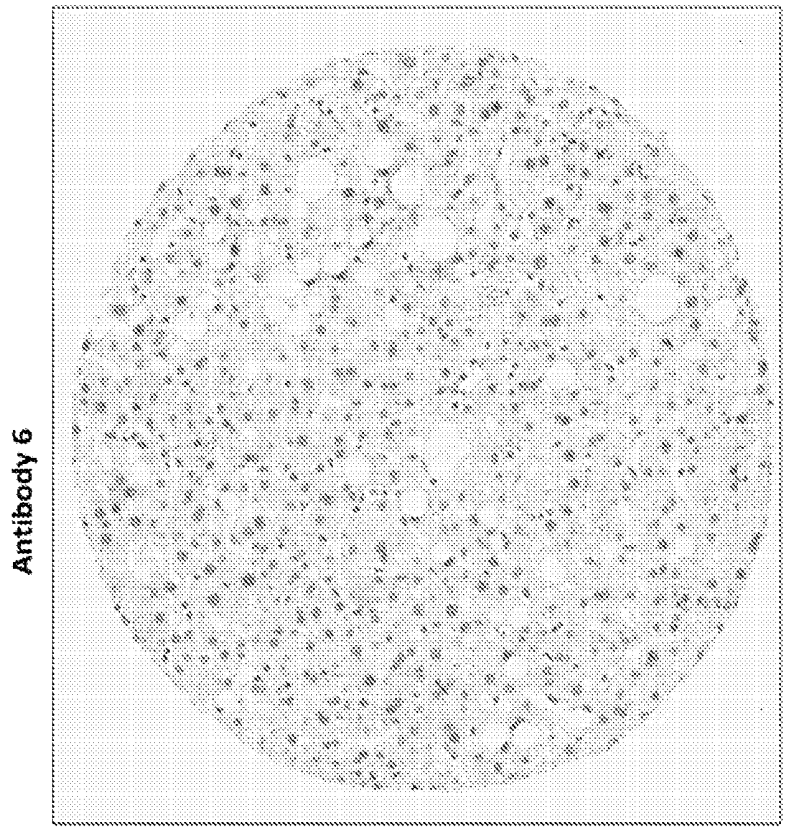
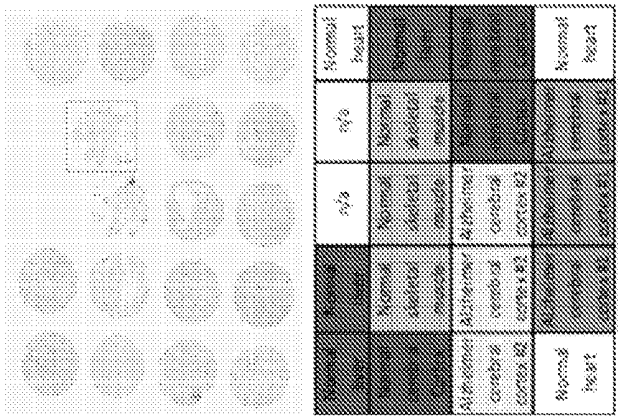


FIG. 4B



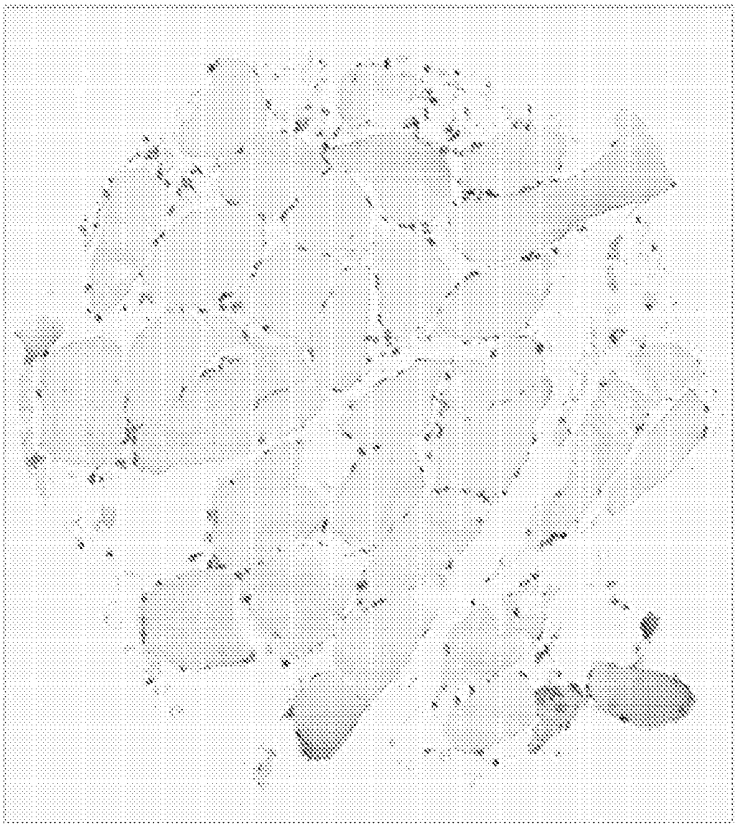
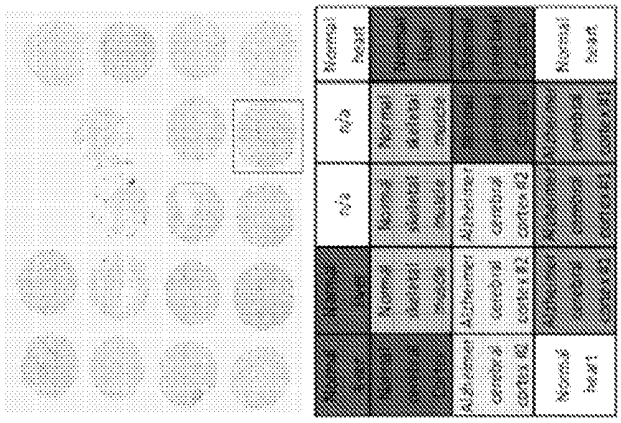


FIG. 4C



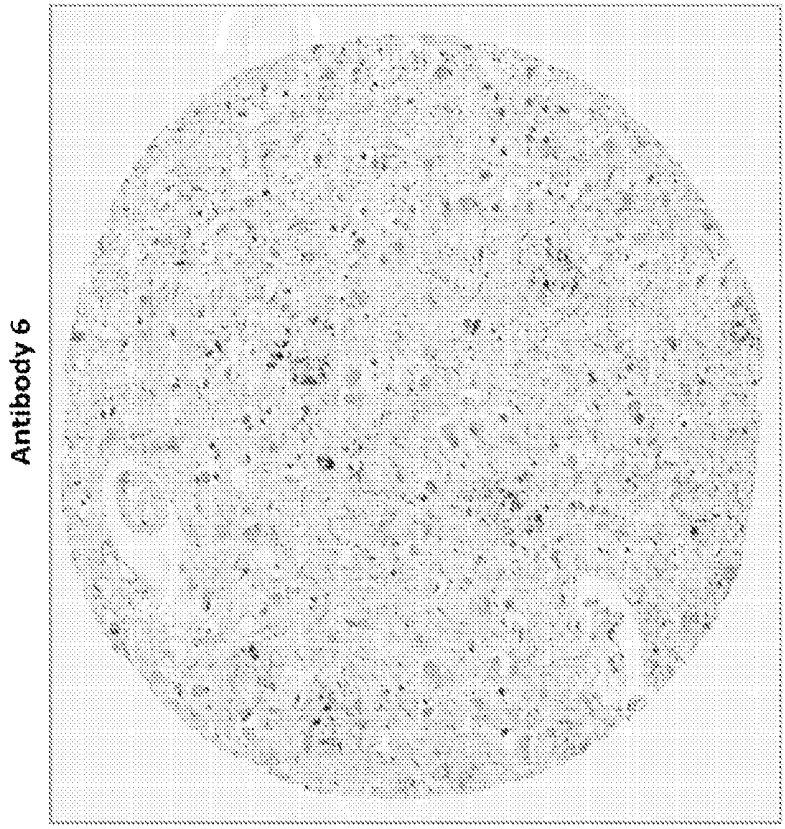
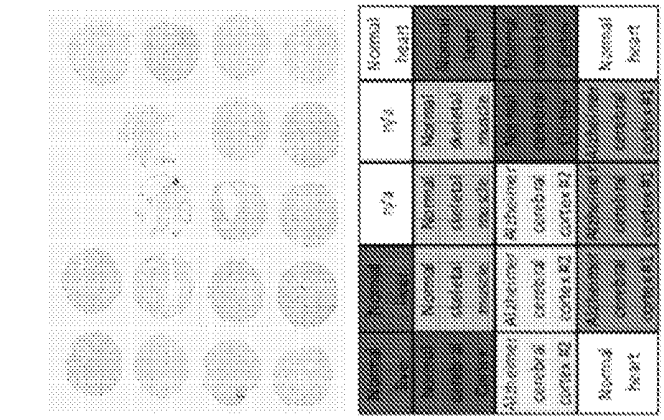


FIG. 4D



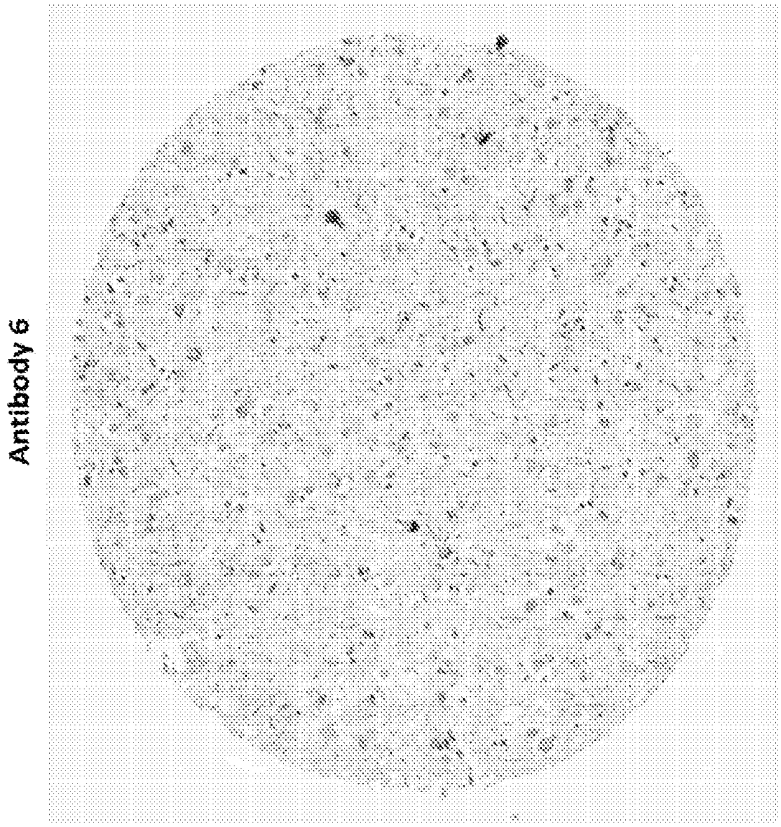
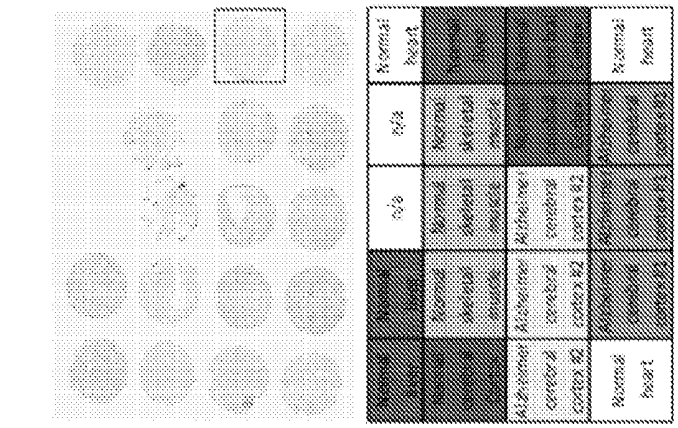


FIG. 4E



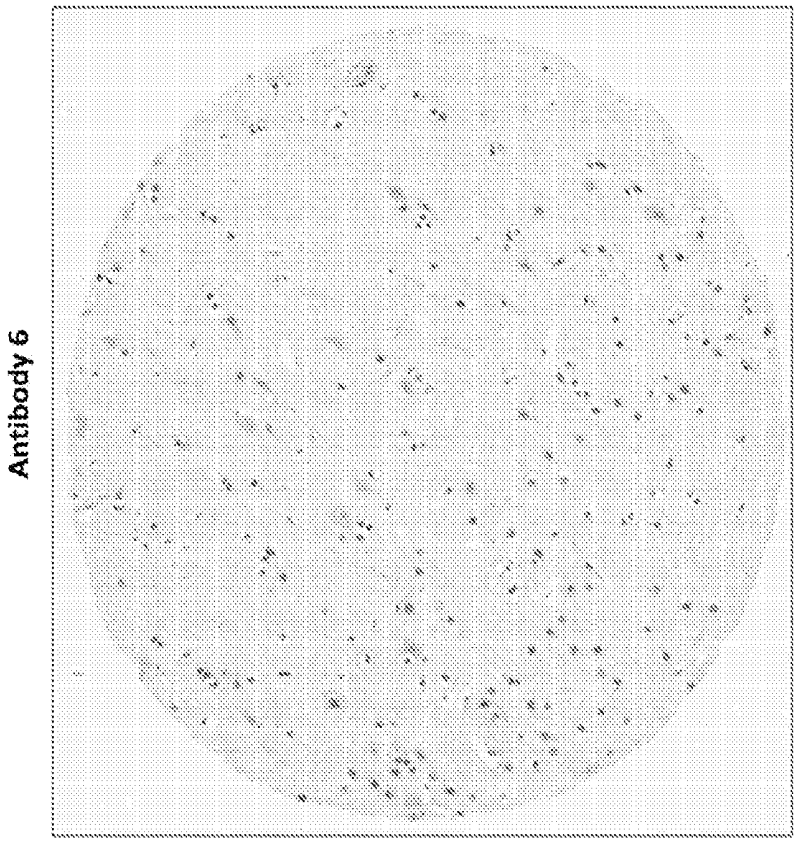
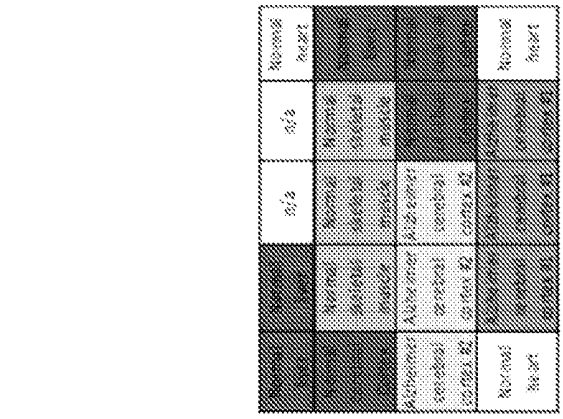


FIG. 4F



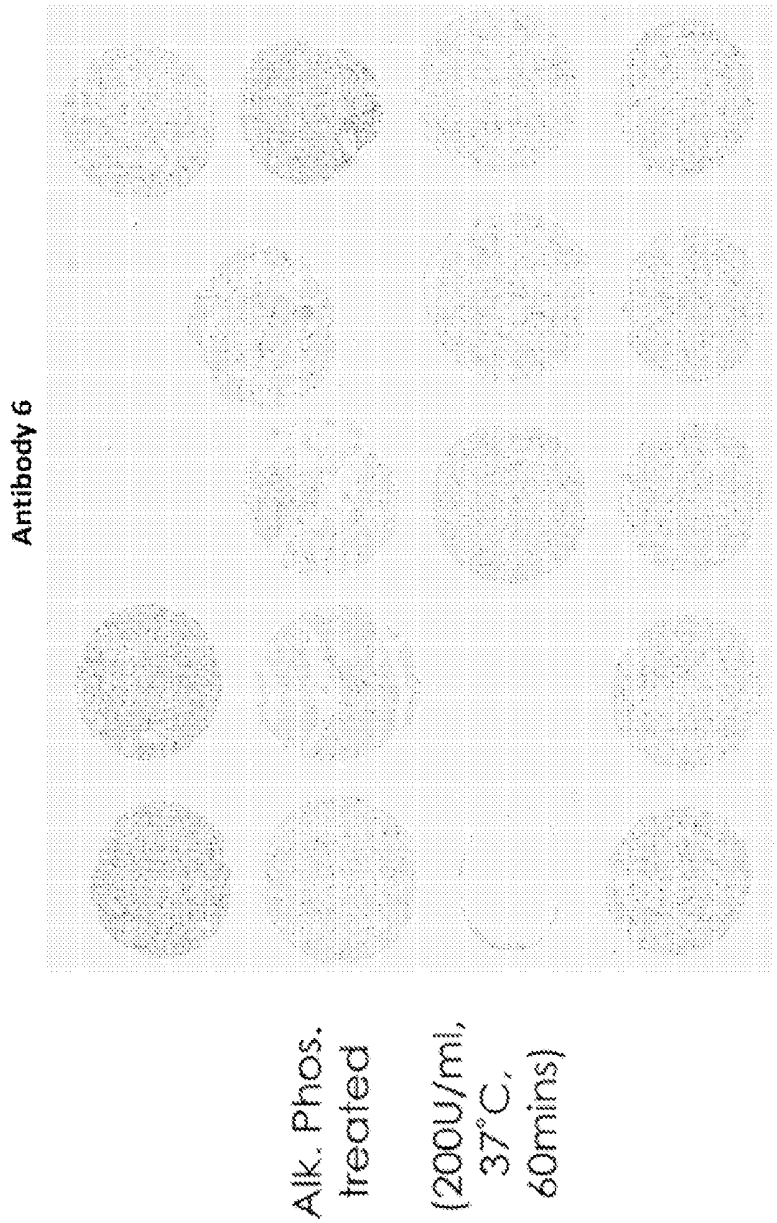


FIG. 4G

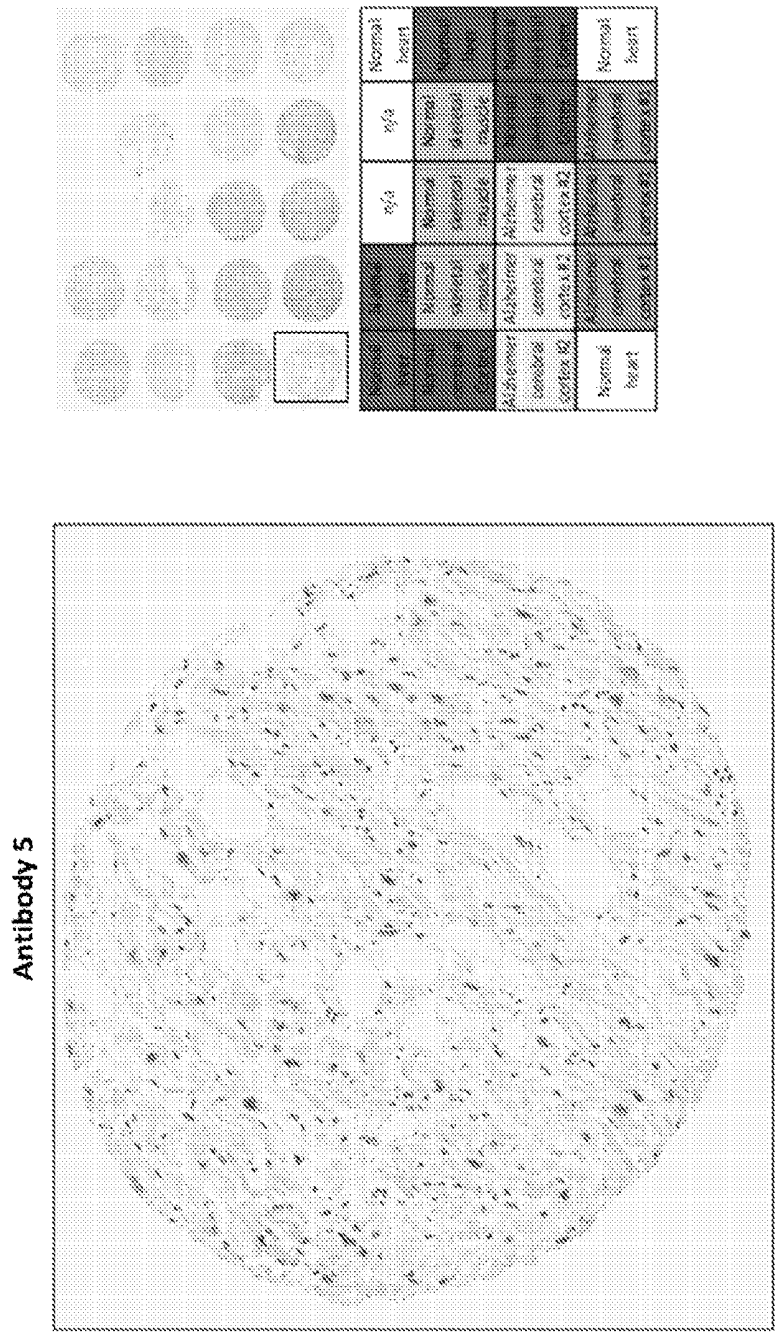
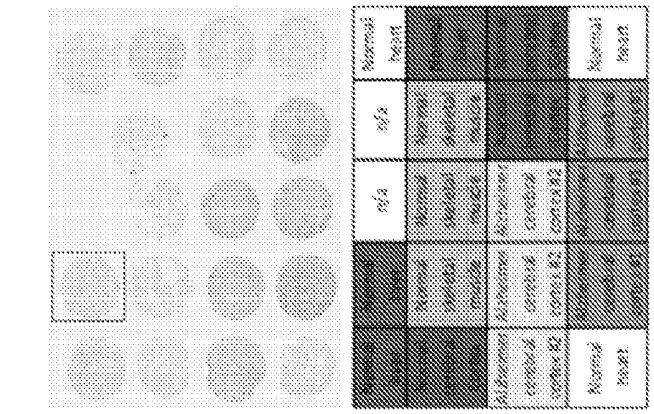


FIG. 5A



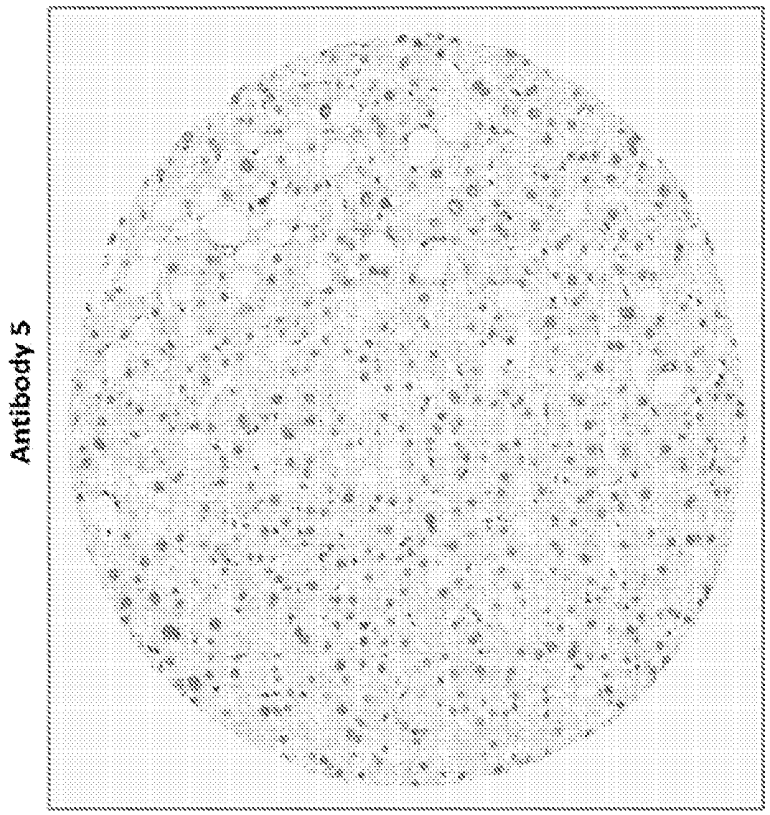
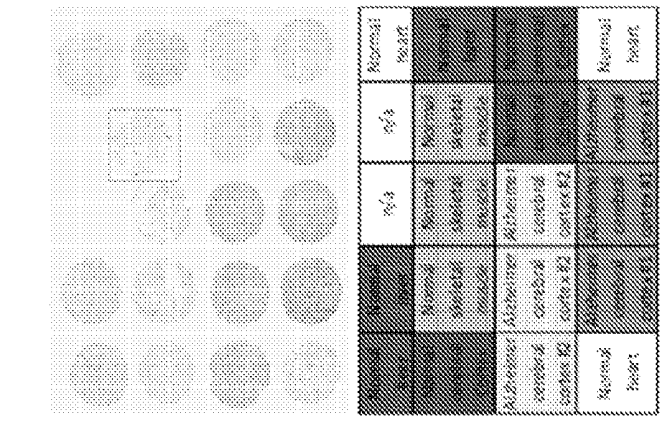


FIG. 5B



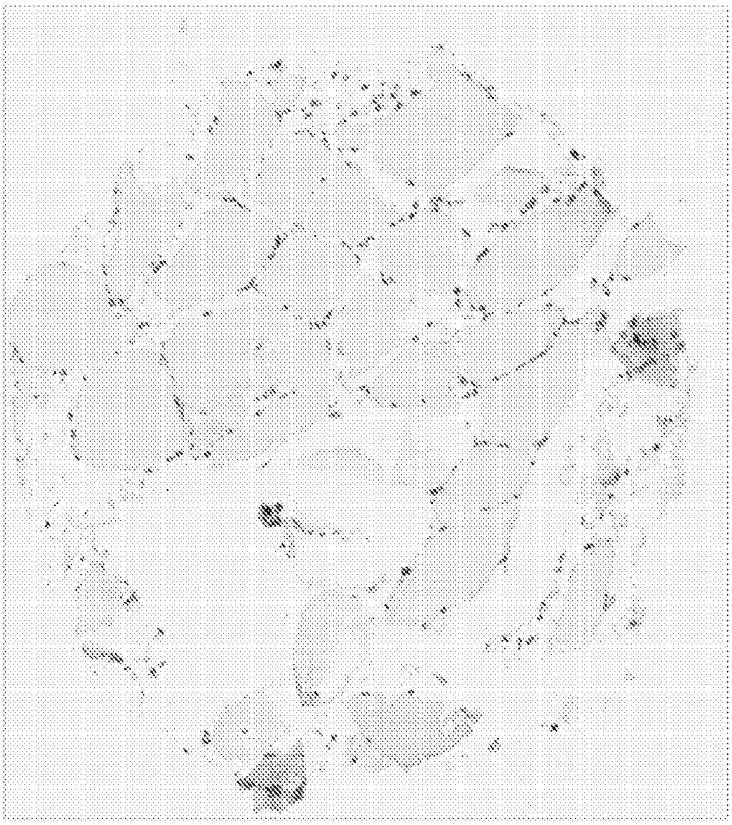
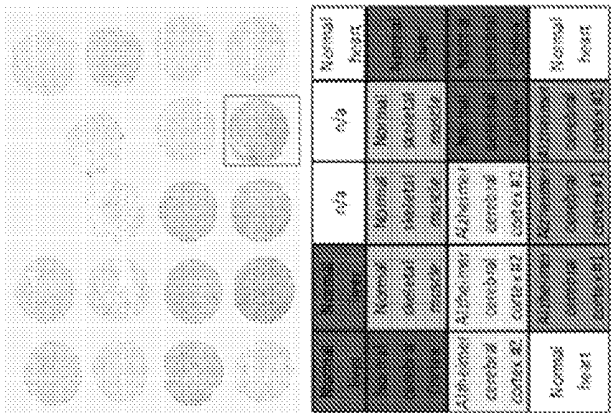


FIG. 5C



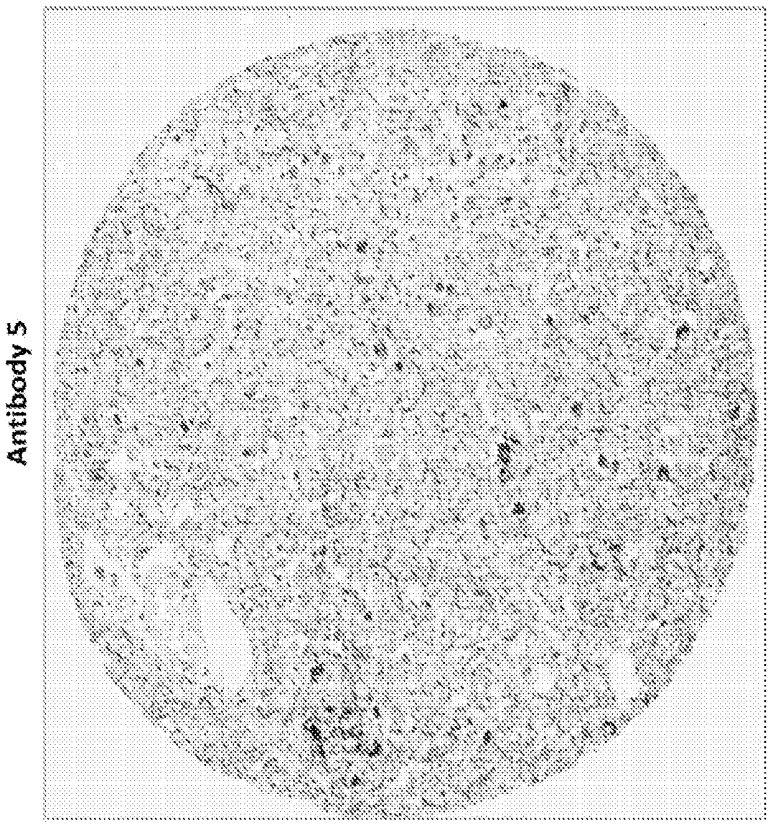
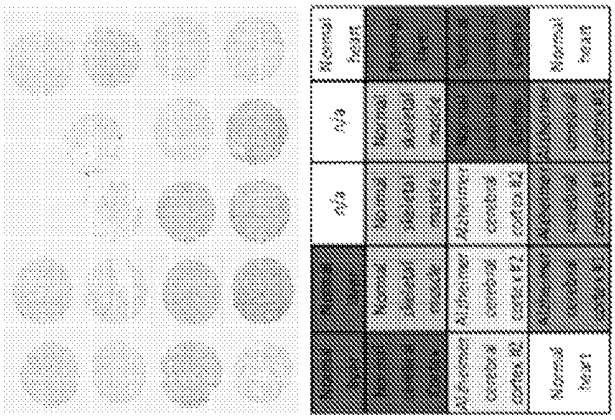


FIG. 5D



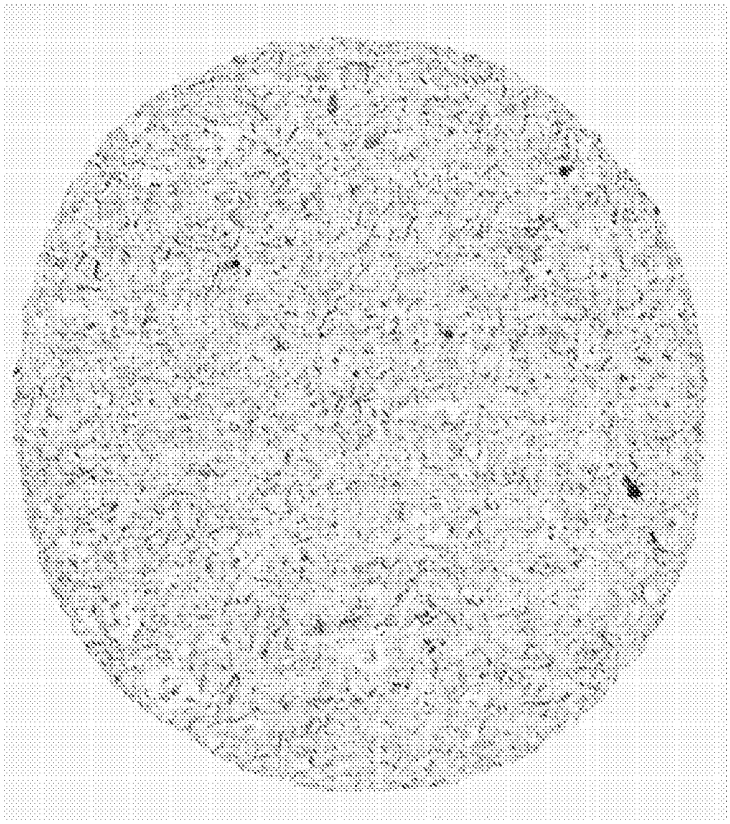
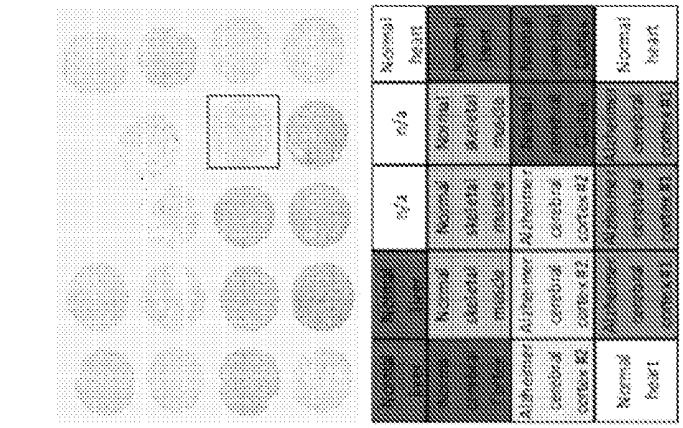


FIG. 5E



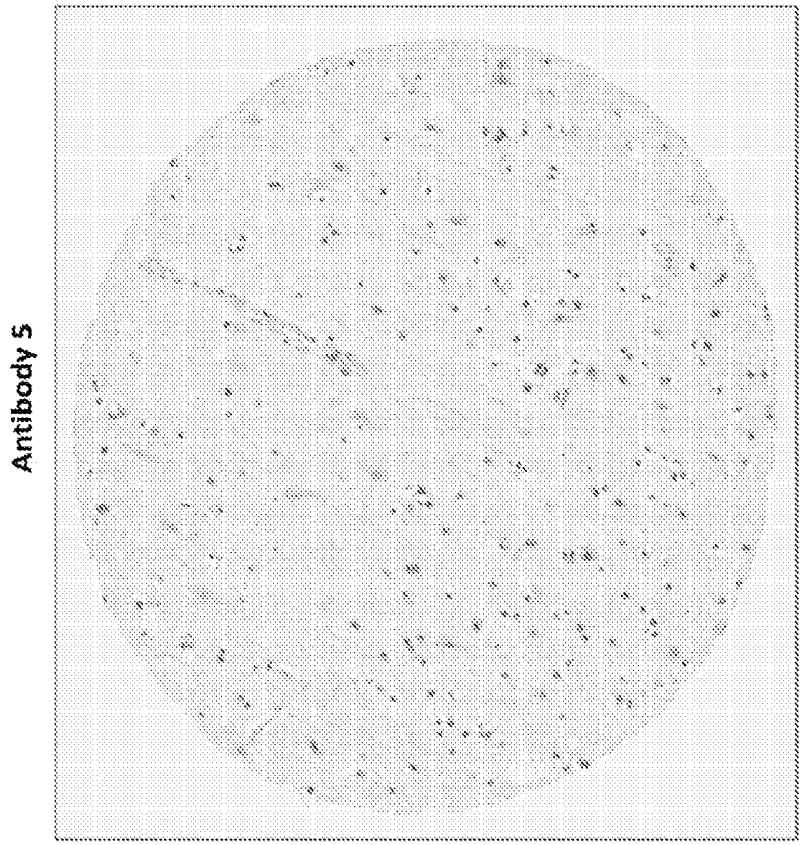
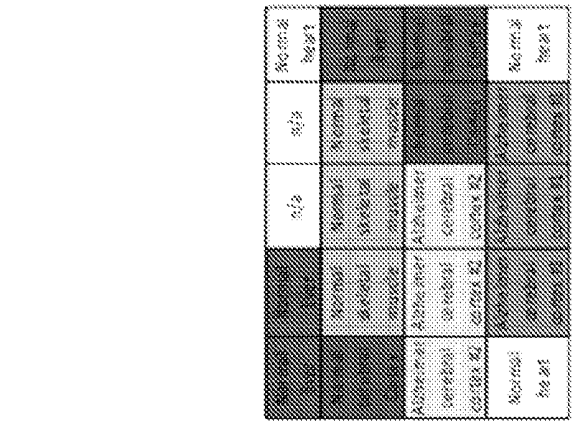


FIG. 5F



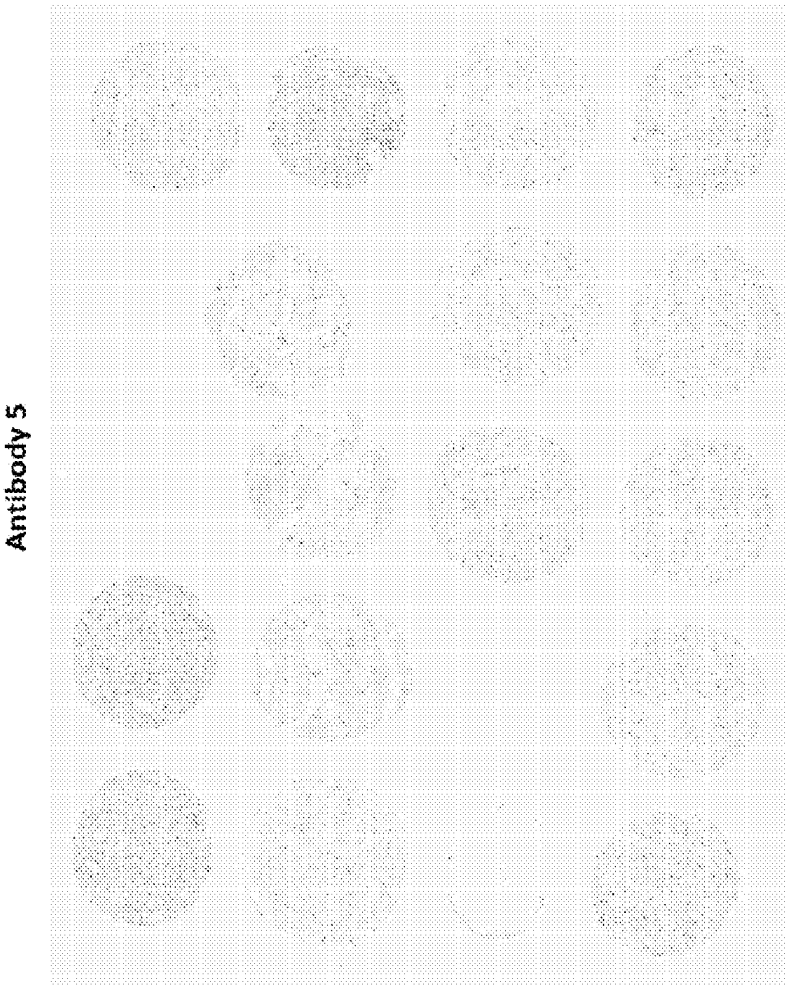
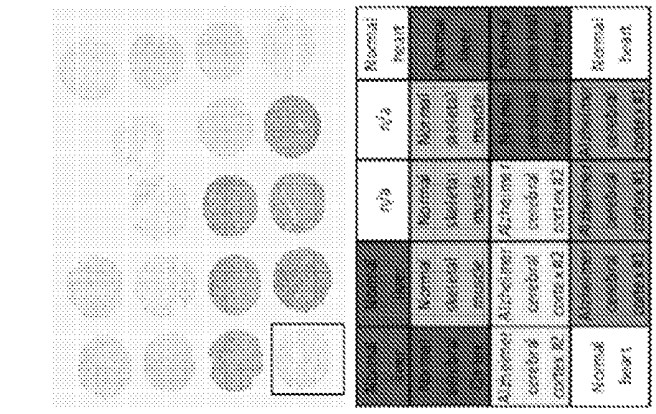


FIG. 5G



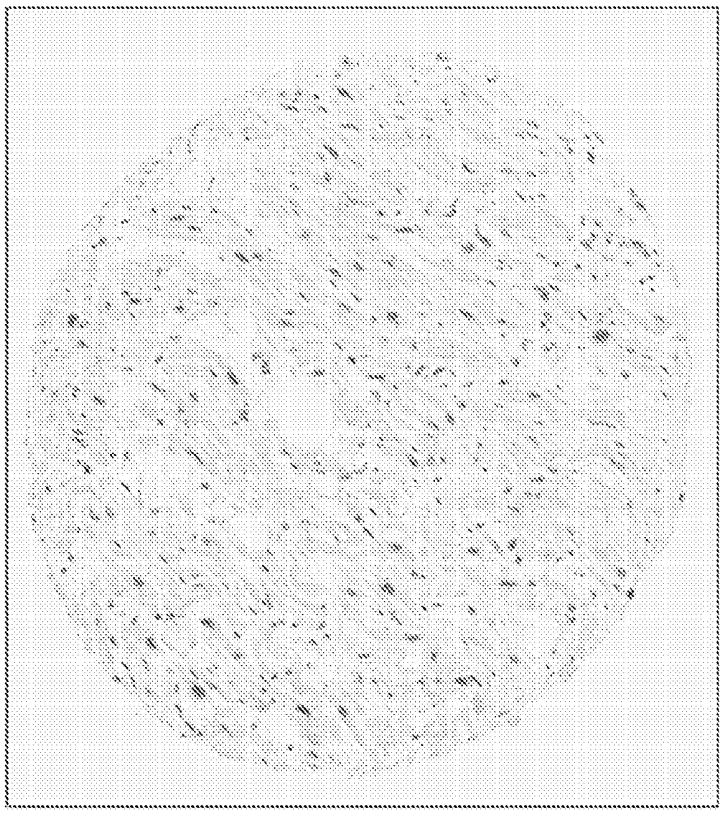
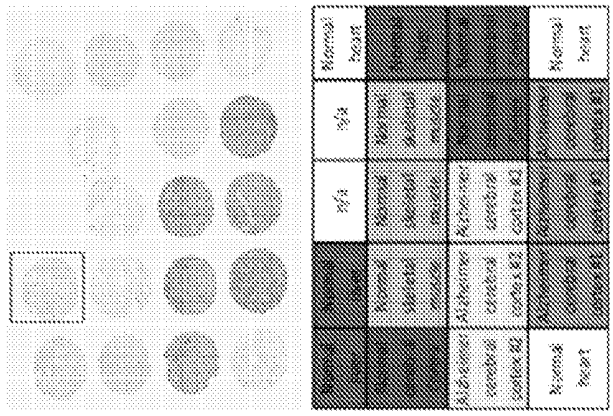


FIG. 6A



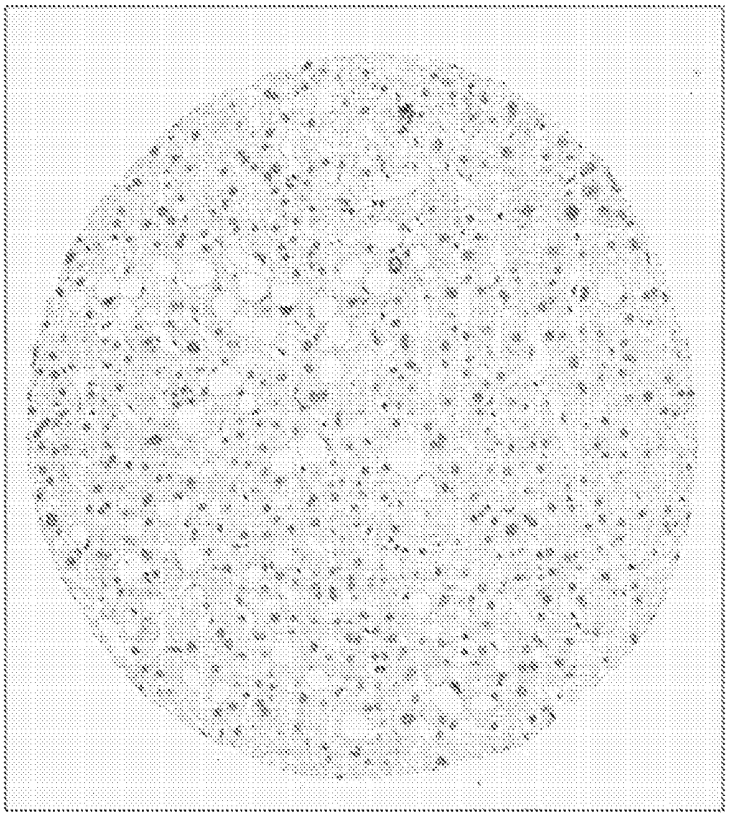
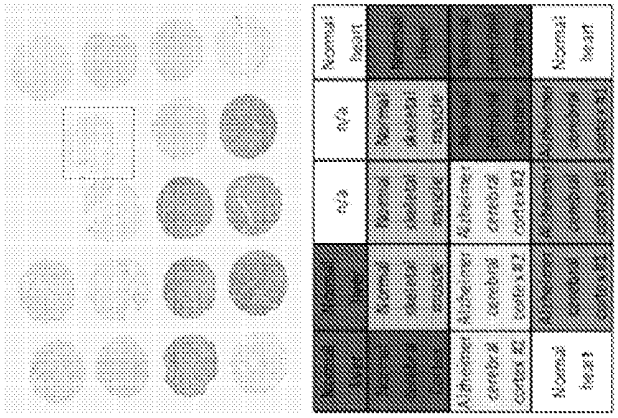


FIG. 6B



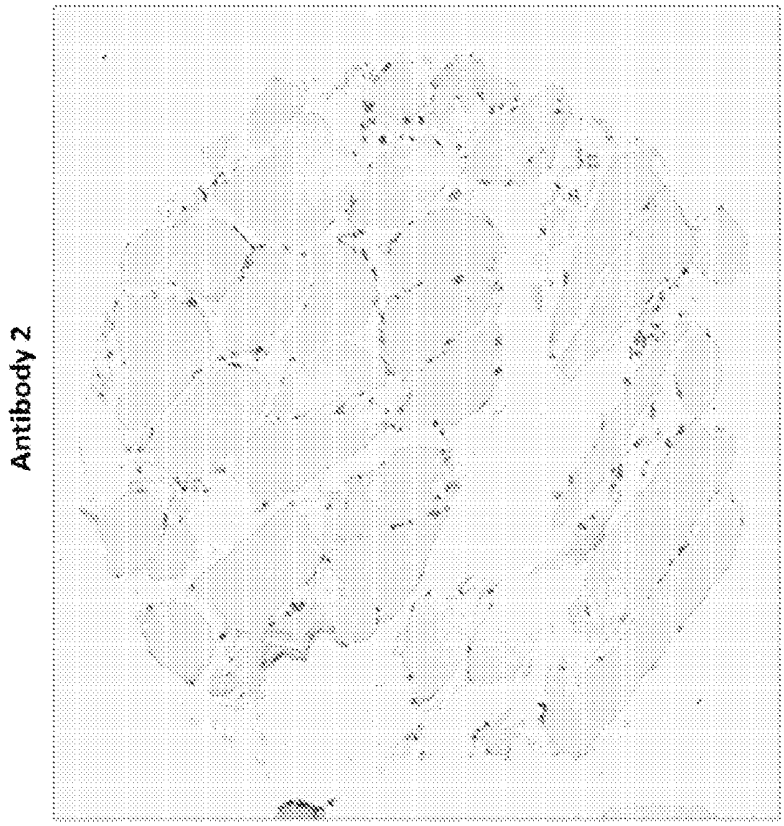
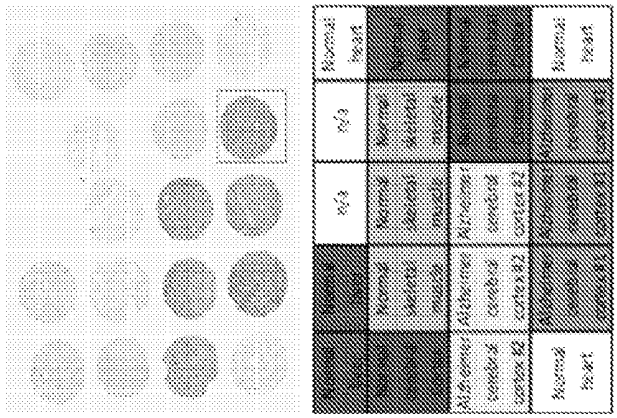


FIG. 6C



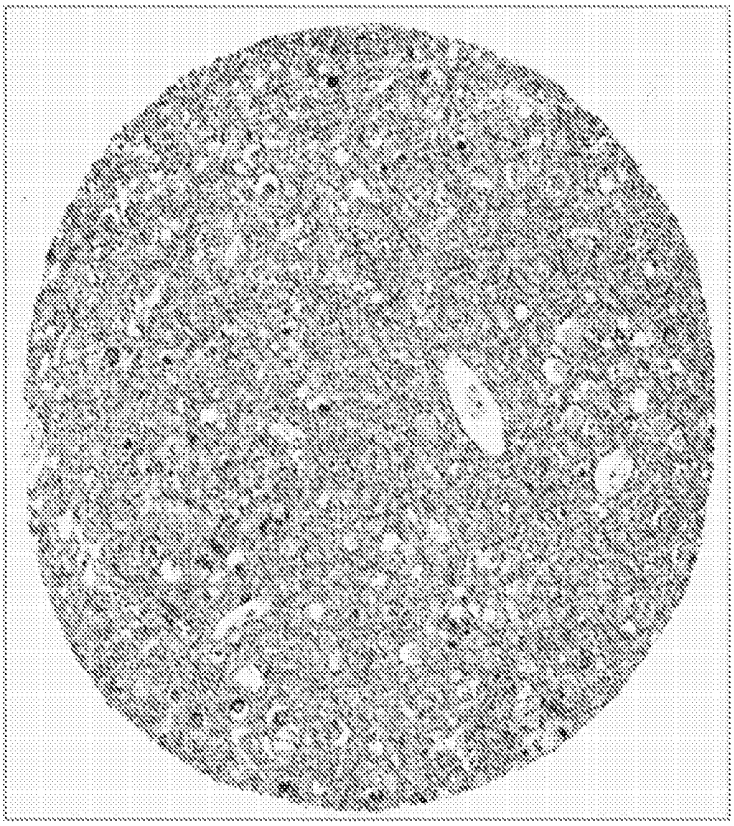
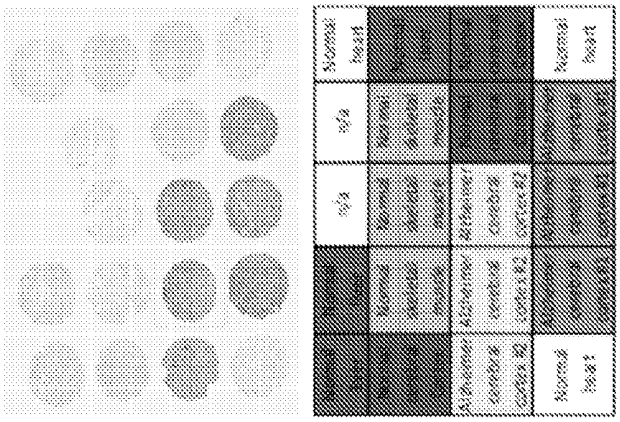


FIG. 6D



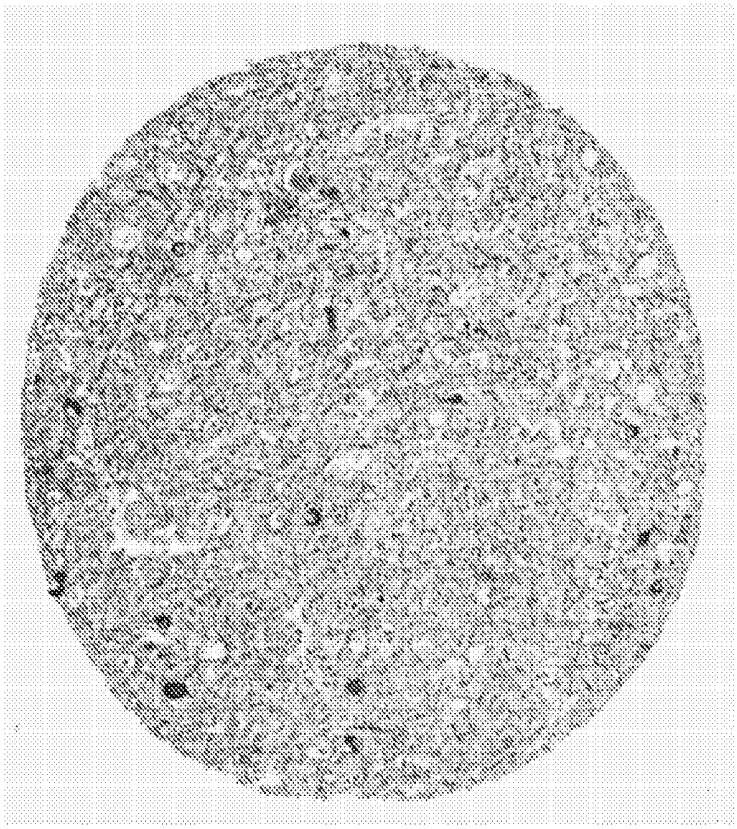
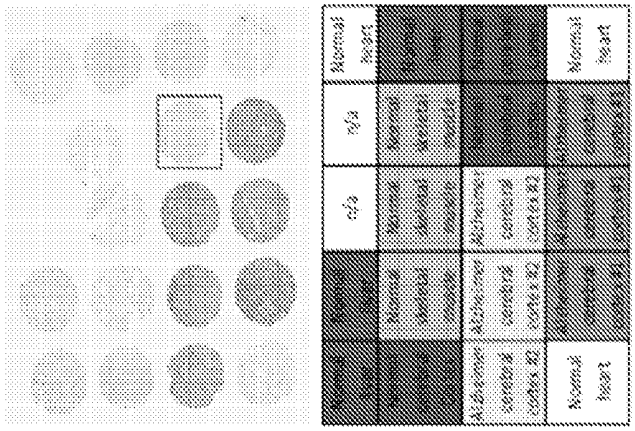


FIG. 6E



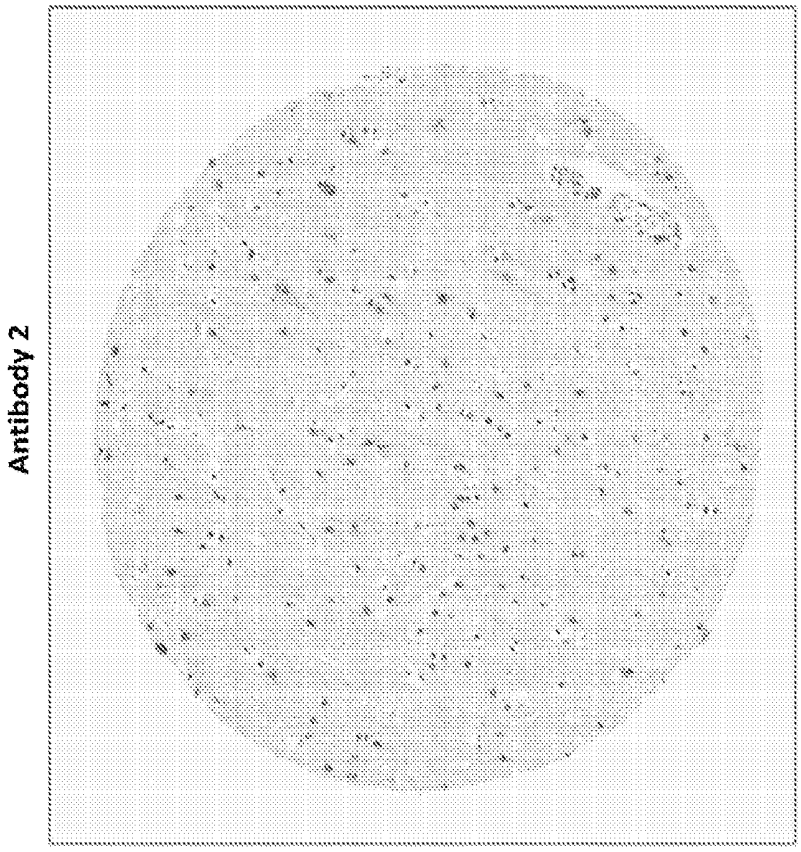


FIG. 6F

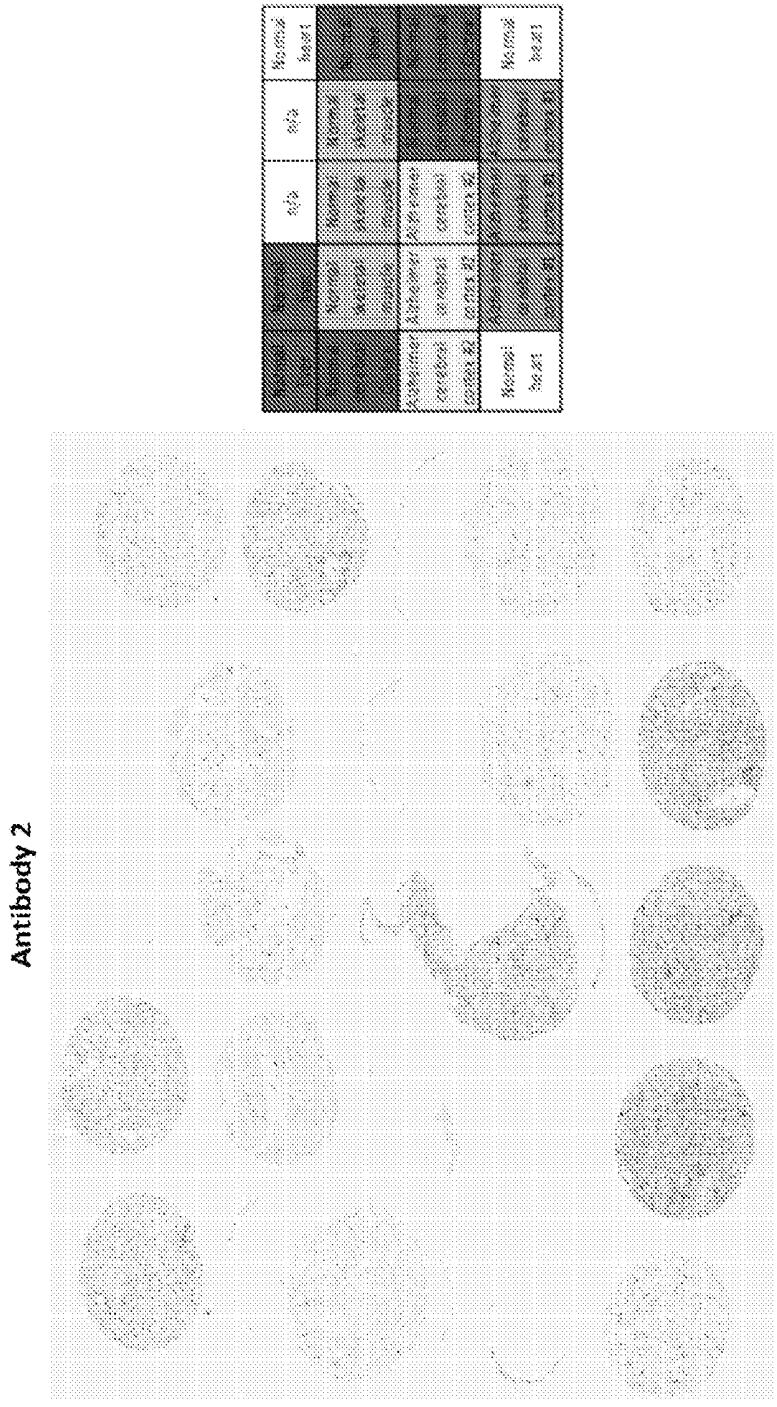


FIG. 6G

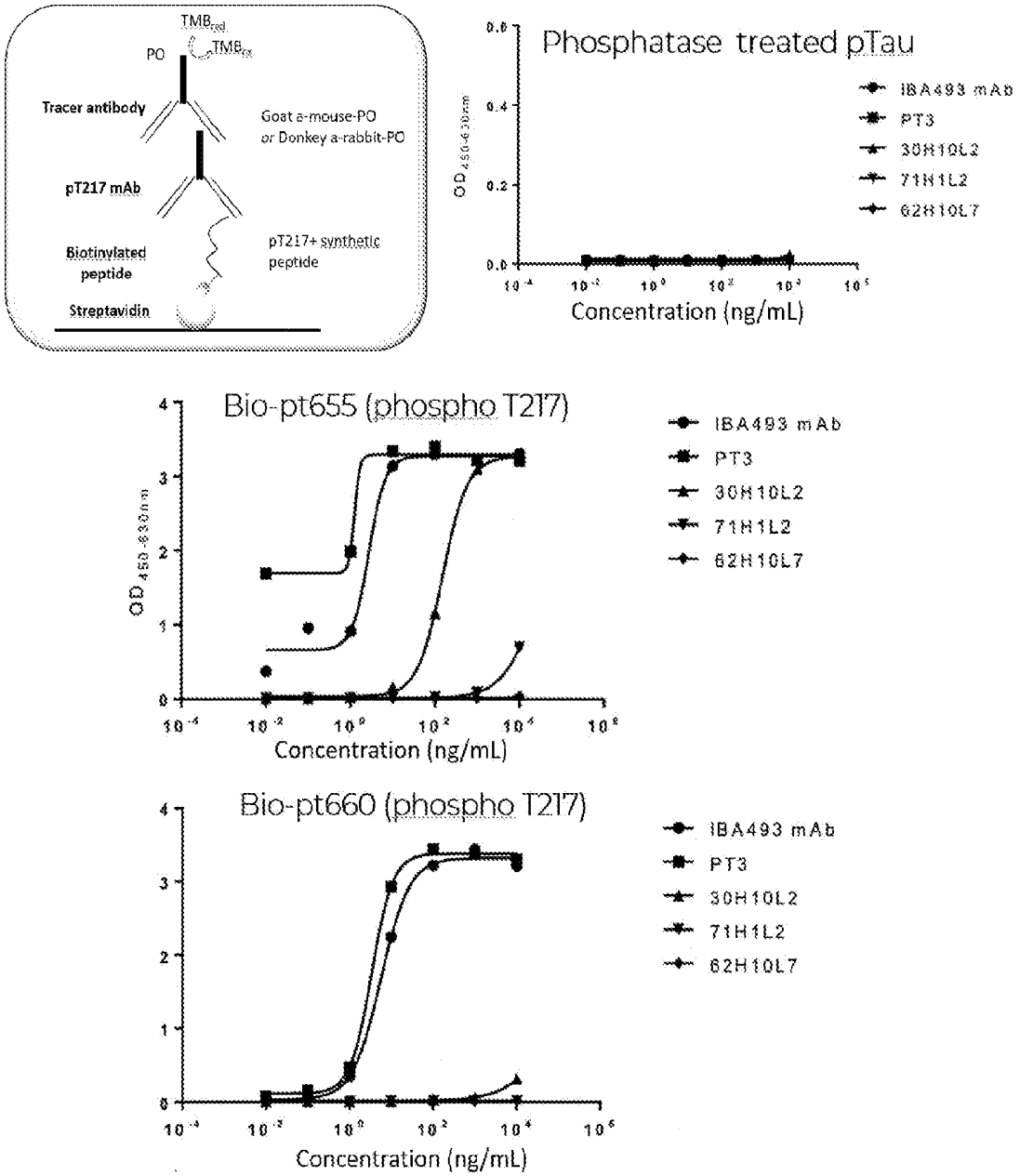
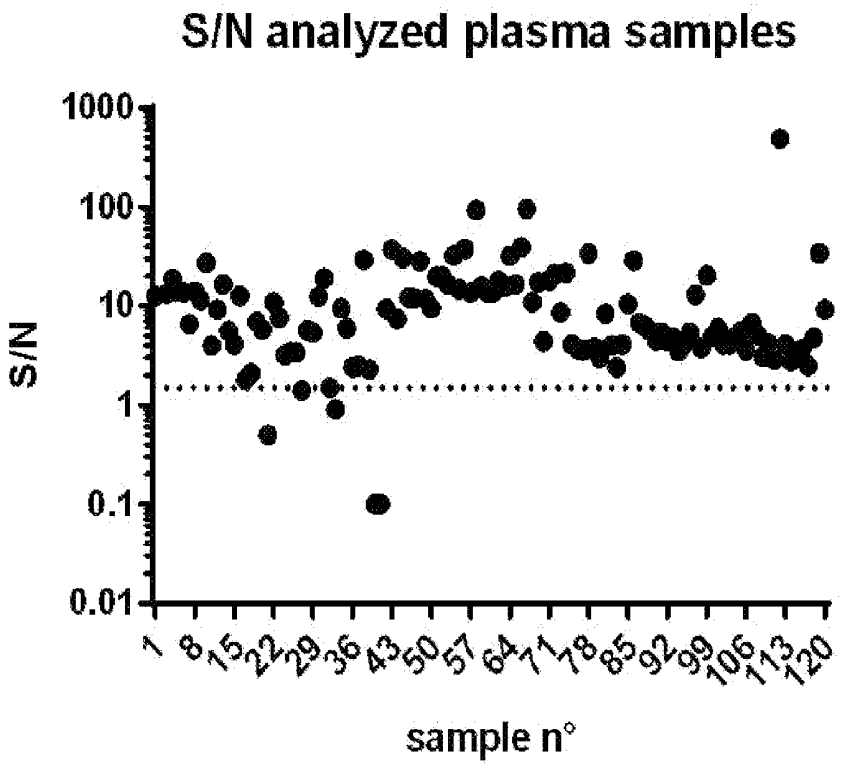

TMB red
TMB ox
PO
Tracer antibody
Goat a-mouse-PO
or Donkey a-rabbit-PO
pT217 mAb
Biotinylated peptide
pT217+ synthetic peptide
Streptavidin
Phosphatase treated pTau
IBA493 mAb
PT3
30H10L2
71H1L2
62H10L7
Concentration (ng/mL)
Bio-pt655 (phospho T217)
Concentration (ng/mL)
Bio-pt660 (phospho T217)
Concentration (ng/mL)

FIG. 7


S/N analyzed plasma samples
1000
100
10
1
0.1
0.01
S/N
1
8
15
22
29
36
43
50
57
64
71
78
85
92
99
106
113
120
sample n°

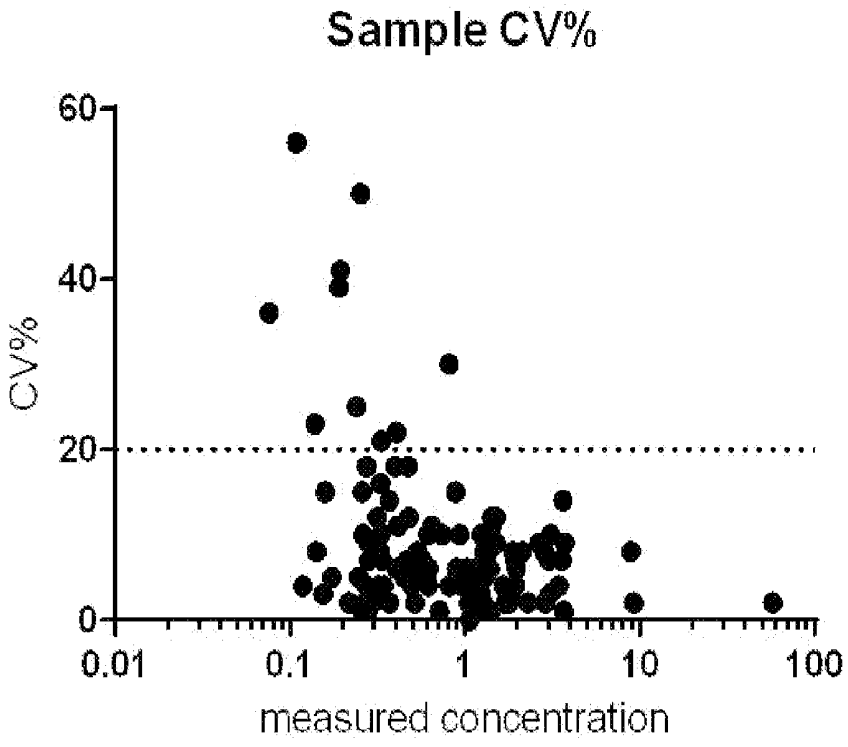

Sample CV%
60
40
20
0
CV%
0.01
0.1
1
10
100
measured concentration

FIG. 8

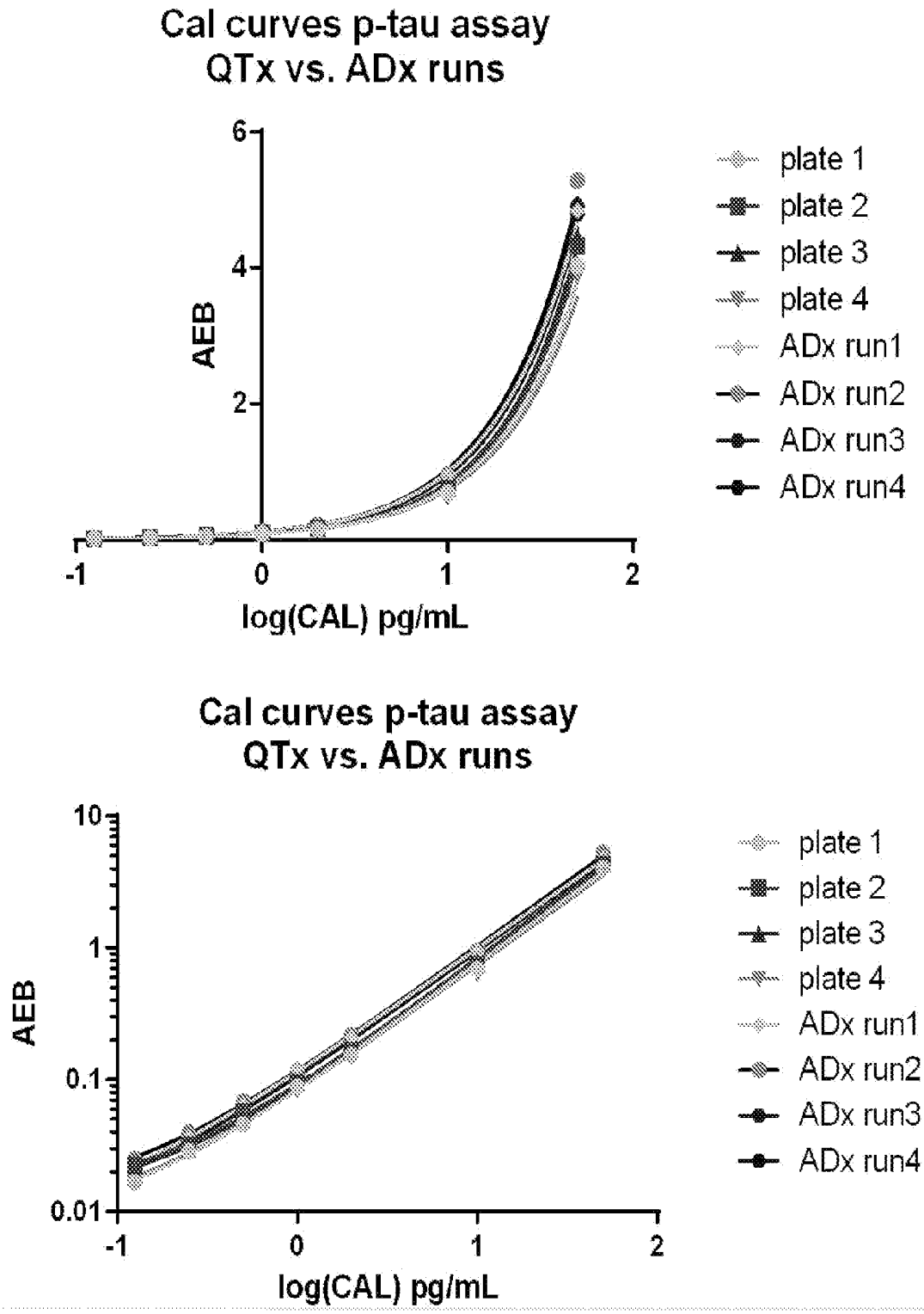
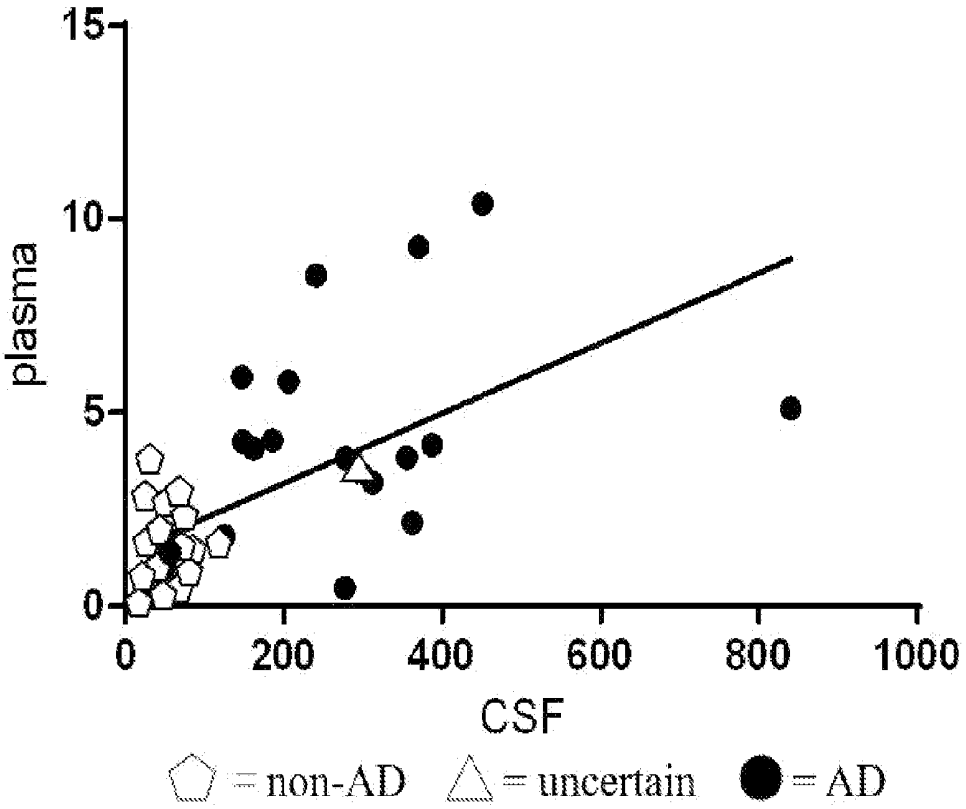
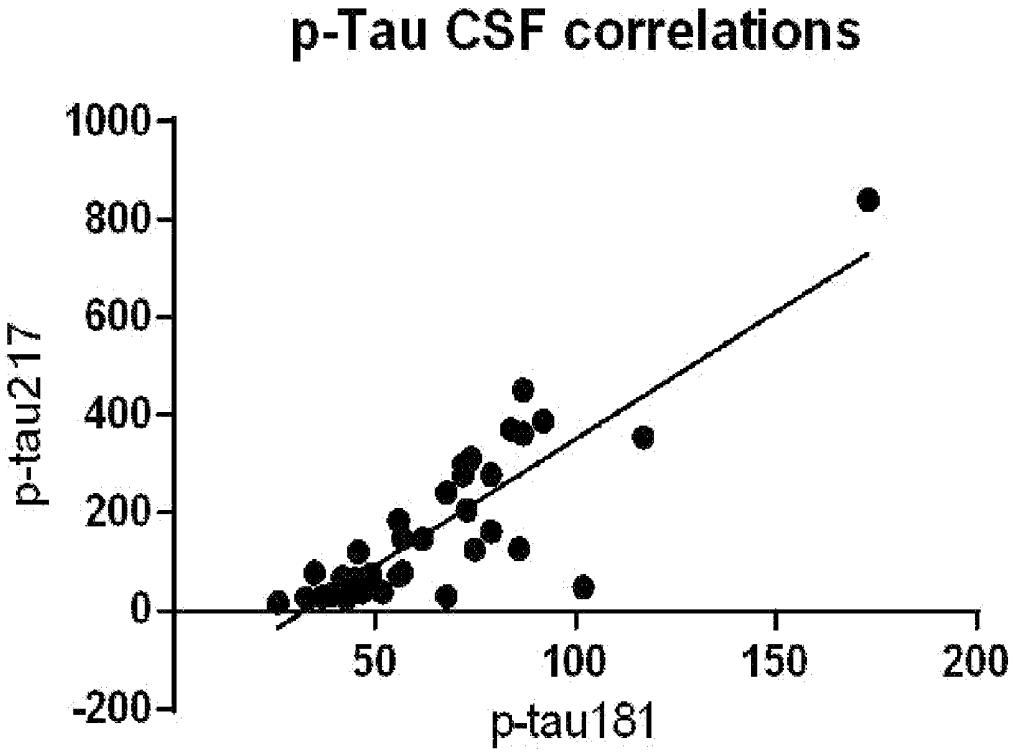

Cal curves p-tau assay
QTx vs. ADx runs
AEB
log(CAL) pg/mL
plate 1
plate 2
plate 3
plate 4
ADx run1
ADx run2
ADx run3
ADx run4
Cal curves p-tau assay
QTx vs. ADx runs
AEB
log(CAL) pg/mL
plate 1
plate 2
plate 3
plate 4
ADx run1
ADx run2
ADx run3
ADx run4

FIG. 9



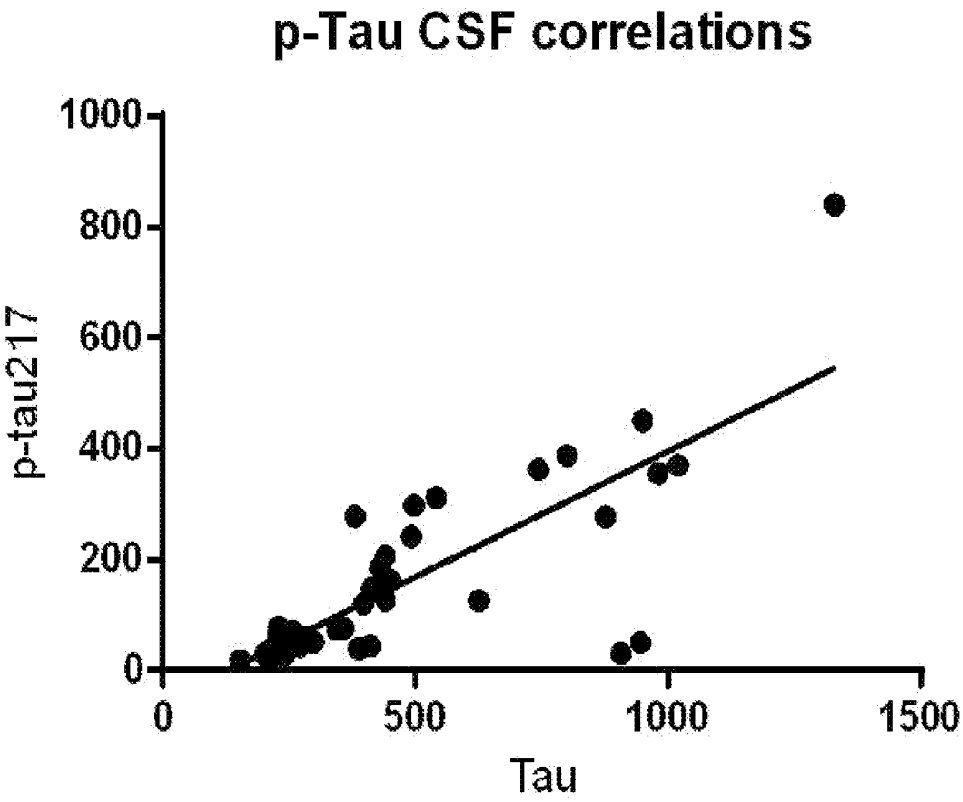
	CSF vs. plasma
Spearman r	
r	0.6965
95% confidence interval	0.4772 to 0.8341
P value	
P (two-tailed)	< 0.0001
P value summary	****
Exact or approximate P value?	Approximate
Significant? (alpha = 0.05)	Yes
Number of XY Pairs	38

FIG. 10



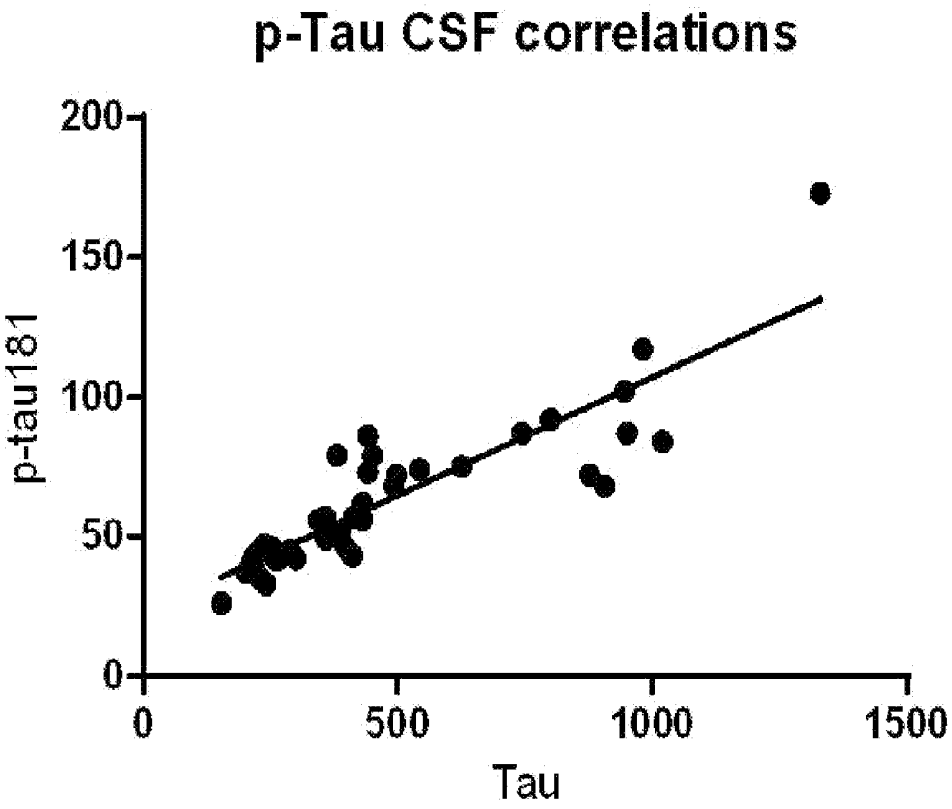
	p-tau181 vs. p-tau217
Spearman r	
r	0.7952
95% confidence interval	0.6424 to 0.8872
P value	
P (two-tailed)	< 0.0001
P value summary	****
Exact or approximate P value?	Approximate
Significant? (alpha = 0.05)	Yes
Number of XY Pairs	42

FIG. 11



	Tau vs. p-tau217
Spearman r	
r	0.7729
95% confidence interval	0.6071 to 0.8742
P value	
P (two-tailed)	< 0.0001
P value summary	****
Exact or approximate P value?	Approximate
Significant? (alpha = 0.05)	Yes
Number of XY Pairs	42

FIG. 12



	Tau vs. p-tau181
Spearman r	
r	0.8978
95% confidence interval	0.8136 to 0.9451
P value	
P (two-tailed)	< 0.0001
P value summary	****
Exact or approximate P value?	Approximate
Significant? (alpha = 0.05)	Yes
Number of XY Pairs	42

FIG. 13

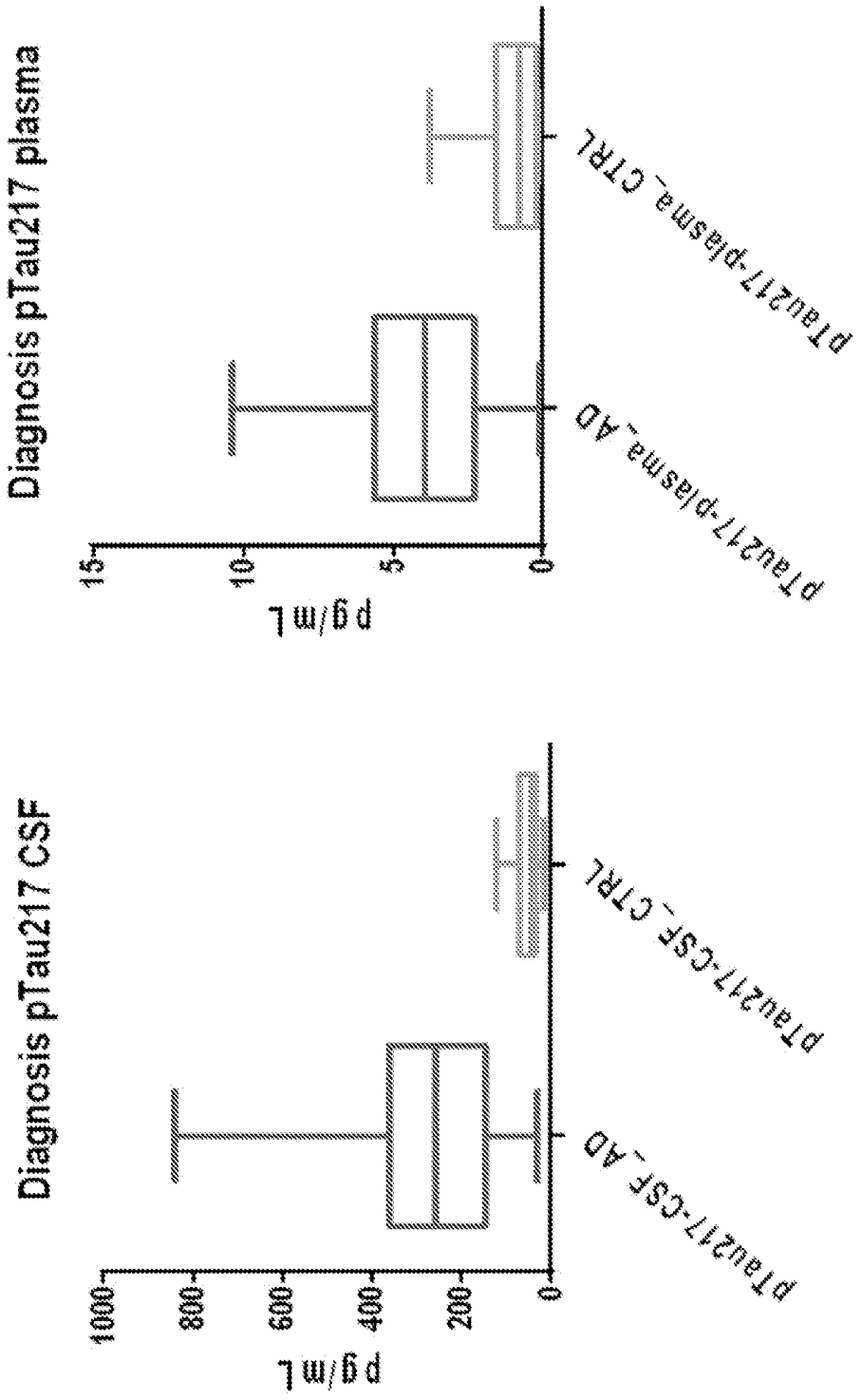

Diagnosis pTau217 plasma
pg/mL
0
5
10
15
pTau217-plasma_AD
pTau217-plasma_CTRL
Diagnosis pTau217 CSF
pg/mL
0
200
400
600
800
1000
pTau217-CSF_AD
pTau217-CSF_CTRL

FIG. 14

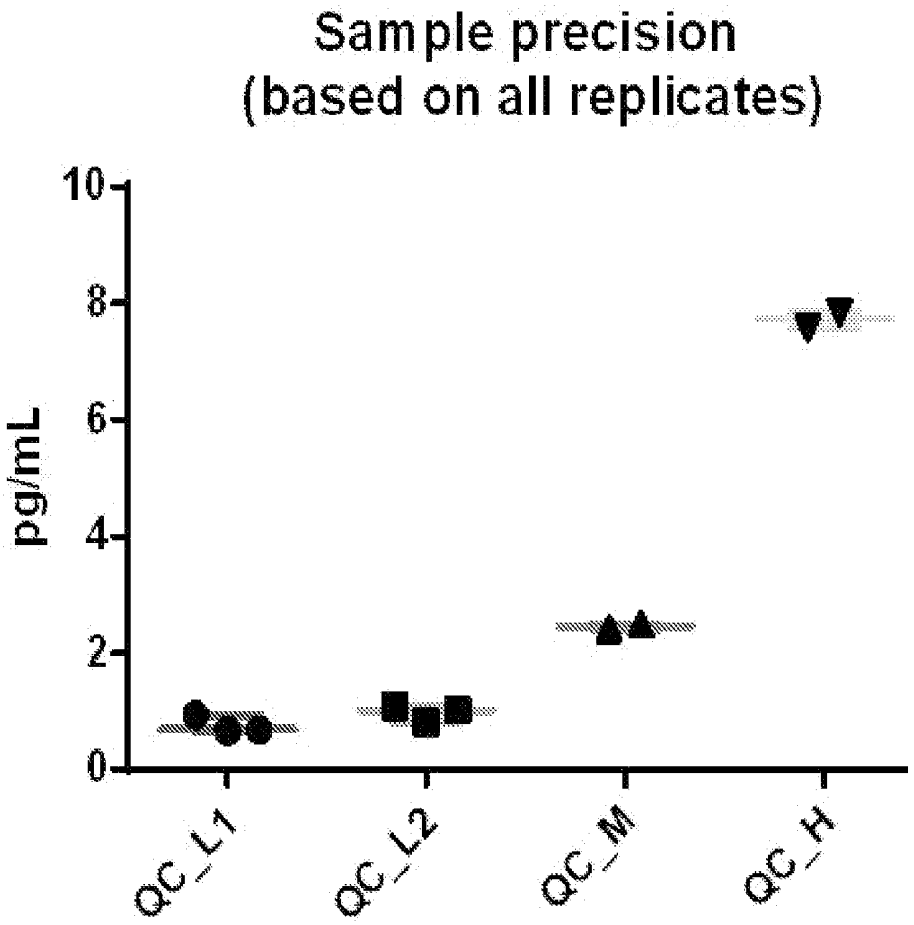
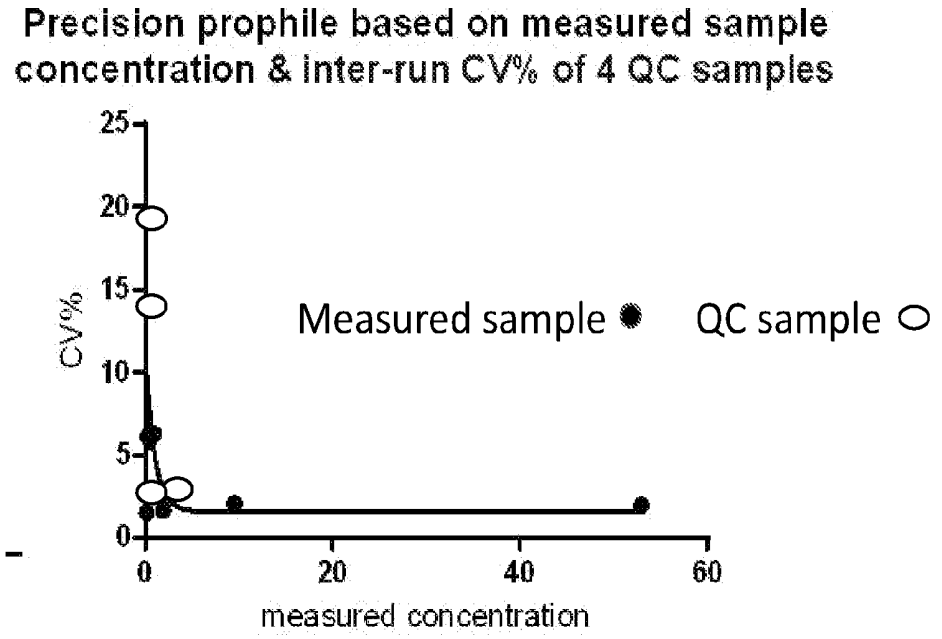

Sample precision
(based on all replicates)
pg/mL
10
8
6
4
2
0
QC_L1
QC_L2
QC_M
QC_H

FIG. 15



	QC_L1	QC_L2	QC_M	QC_H
Number of values	3	3	2	2
Coefficient of variation	19.42%	14.00%	2.70%	2.36%

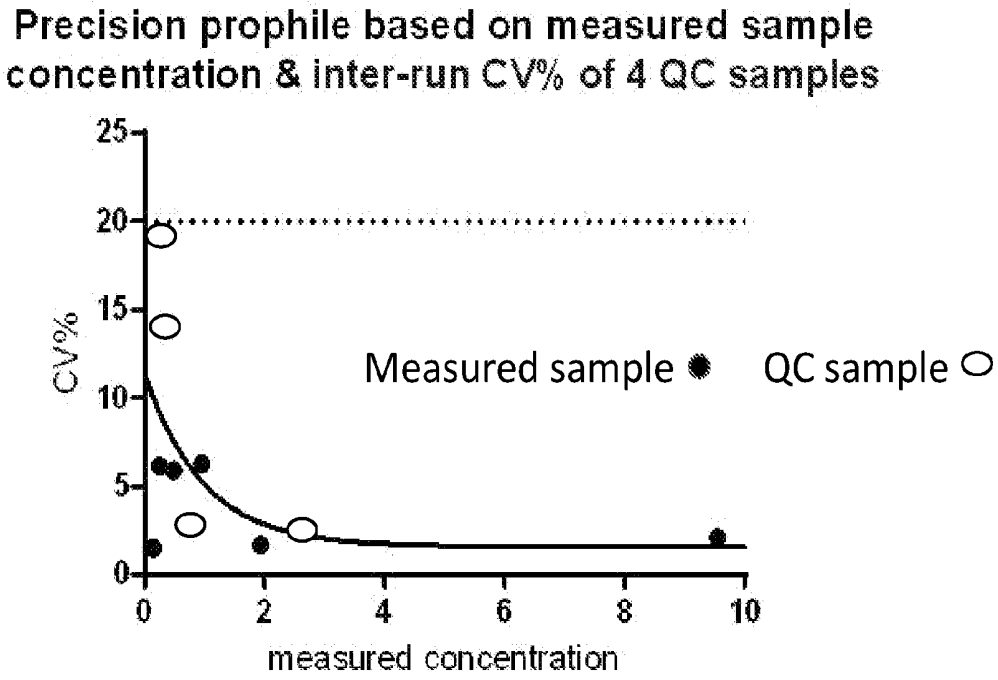
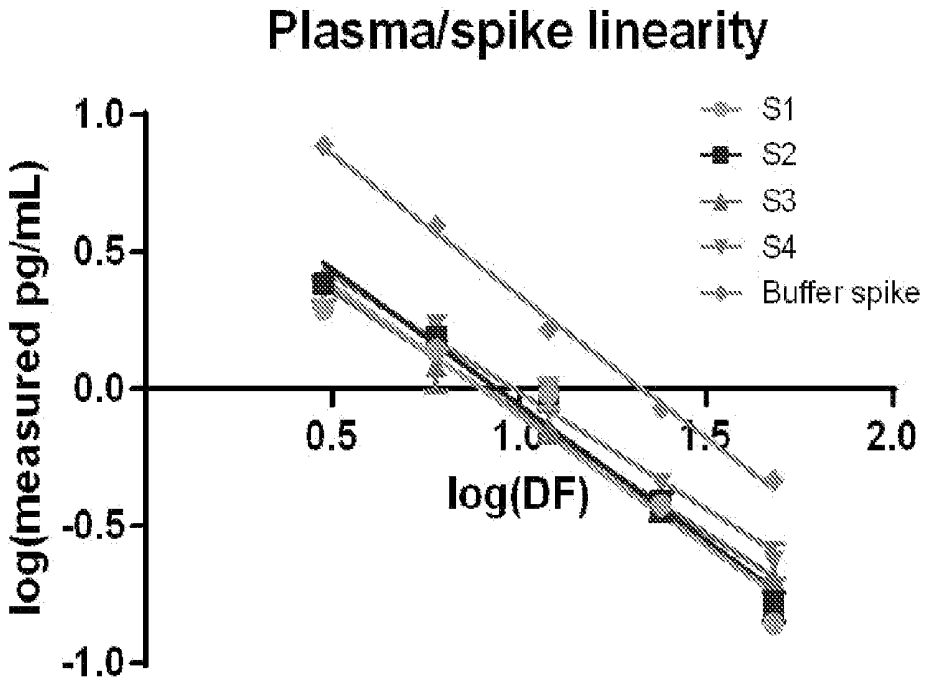


FIG. 16

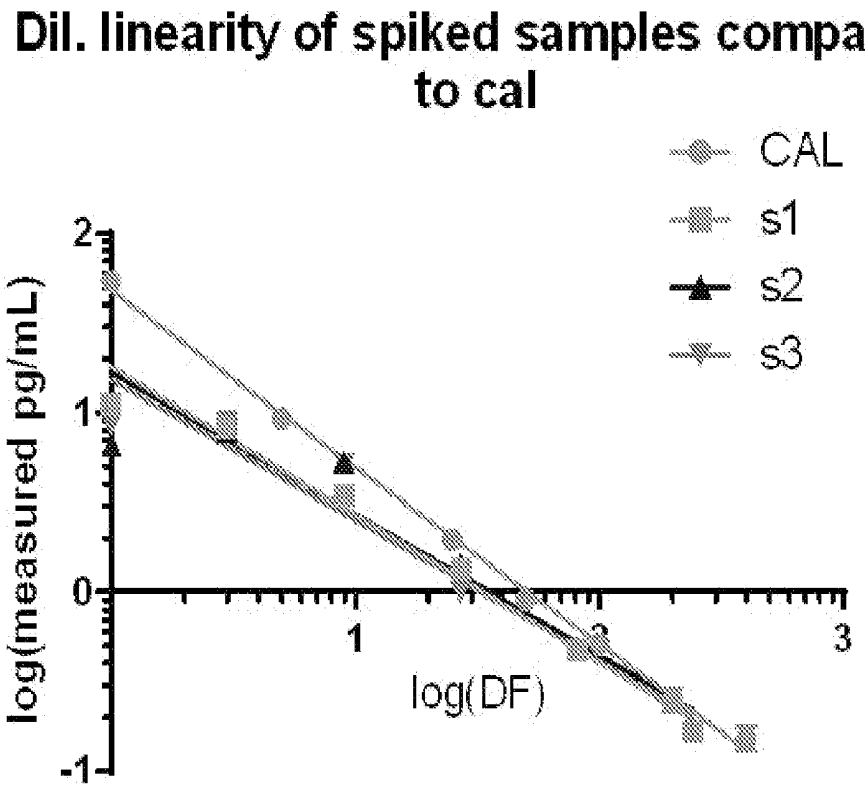


	S1	S2	S3	S4	Buffer spike
Y Intercept	0.86	0.93	0.80	0.84	1.38
Slope	-0.95	-0.99	-0.88	-0.85	-1.03
% Difference	92%	96%	85%	82%	100%

Name Marker	Df (x)	Df (x)	Df (x)	Df (x)	Df (x)	Df (x)
c7AU217	1	3	3	3	3	3
	5	6	6	6	6	6
	25	12	12	12	12	12
	50	24	24	24	24	24
	100	48	48	48	48	48
	200					
Slope (a)	0.8669	0.7865	0.8273	0.7459	0.7239	0.9167
in range (%)	85-115	91	95	86	84	106

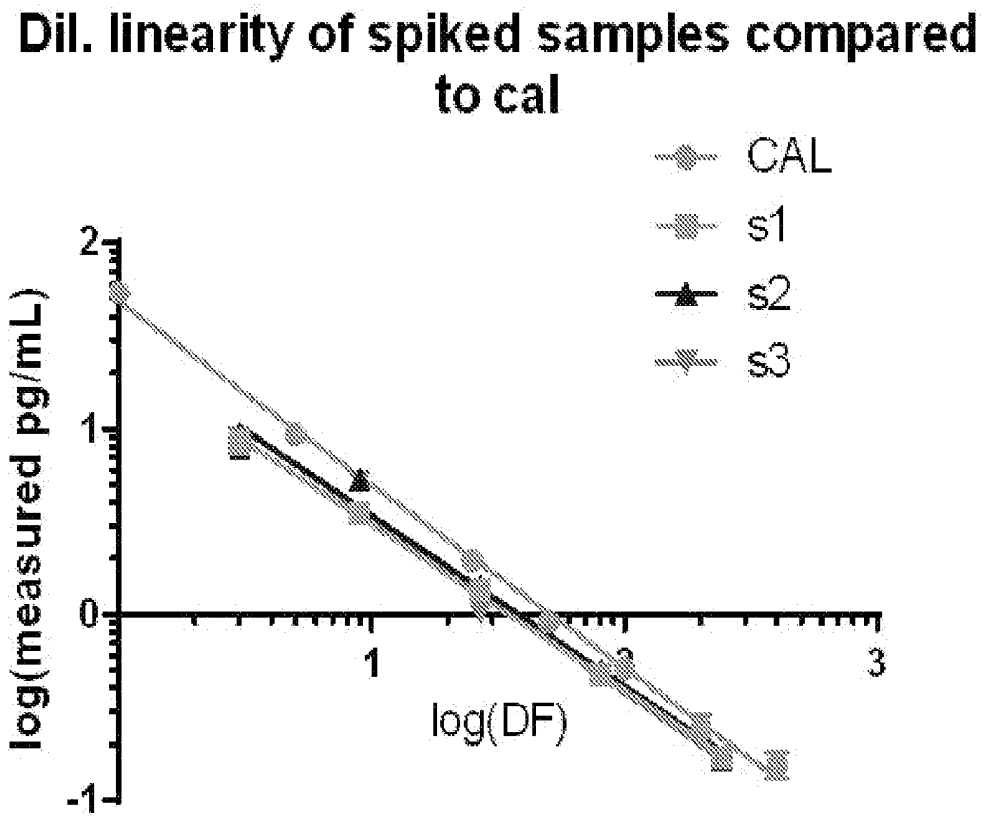
Df = Dilution factor of samples used in assay

FIG. 17



	CAL	s1	s2	s3
Y Intercept	1.68	1.26	1.23	1.19
Slope	-0.99	-0.83	-0.79	-0.78
% Difference		84%	80%	79%

FIG. 18



	CAL	s1	s2	s3
Y Intercept	1.68	1.38	1.45	1.46
Slope	-0.99	-0.89	-0.92	-0.94
% Difference		90%	93%	95%

FIG. 19

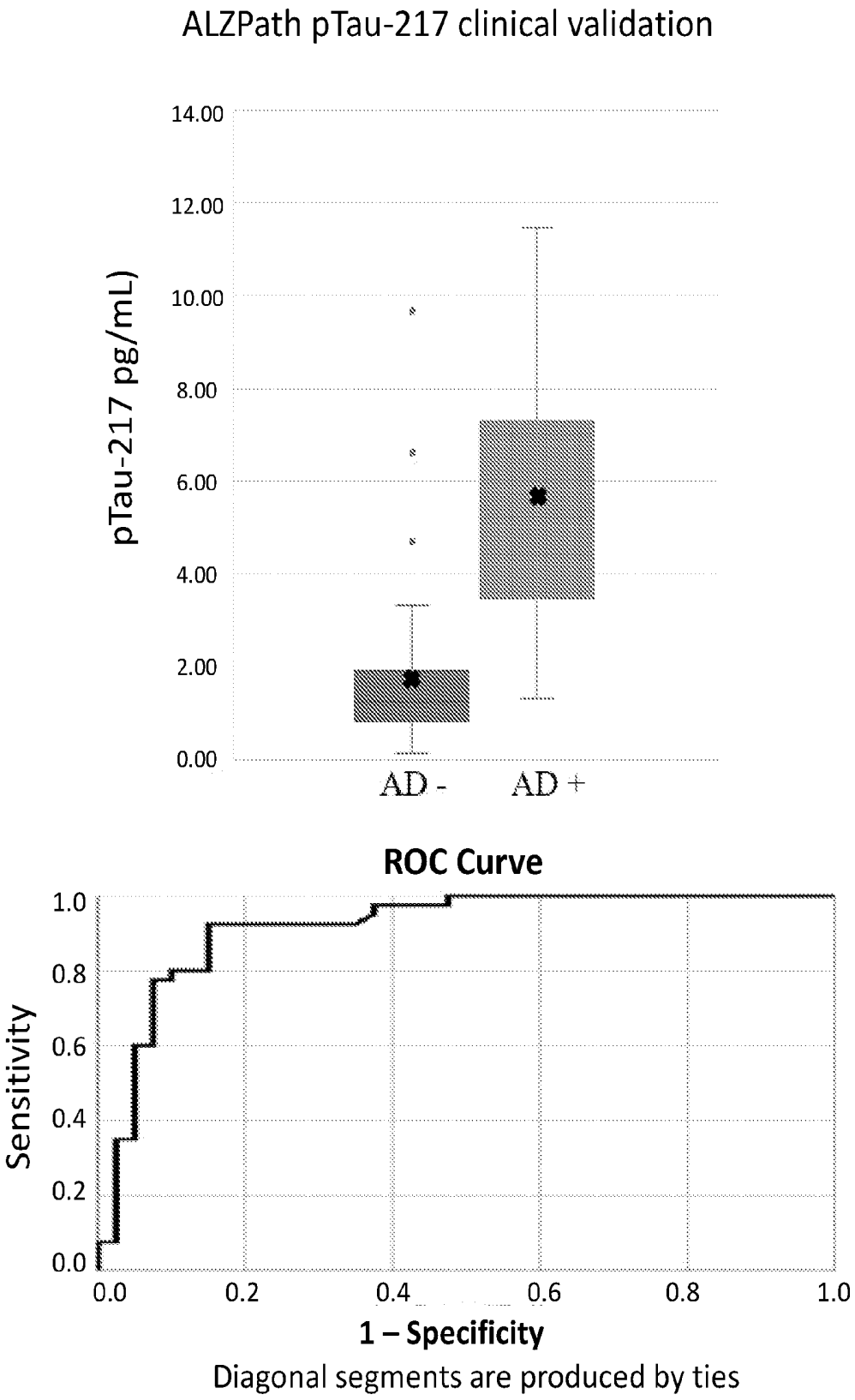

ALZPath pTau-217 clinical validation
pTau-217 pg/mL)
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
AD -
AD +
ROC Curve
Sensitivity
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.2
0.4
0.6
0.8
1.0
1 – Specificity
Diagonal segments are produced by ties

FIG. 20

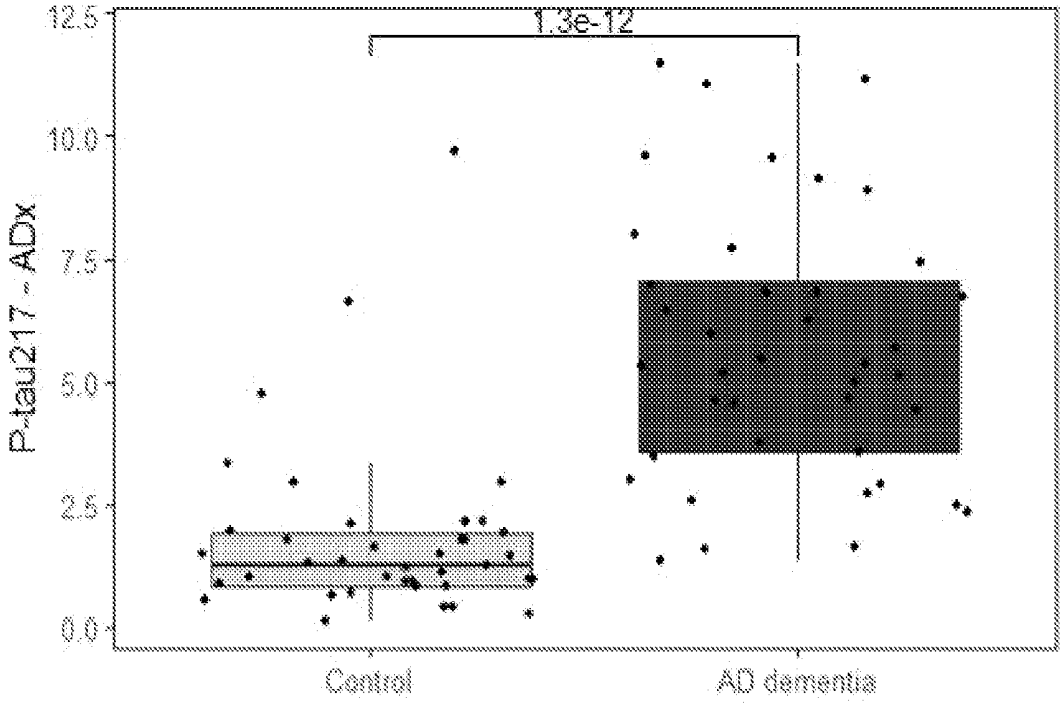
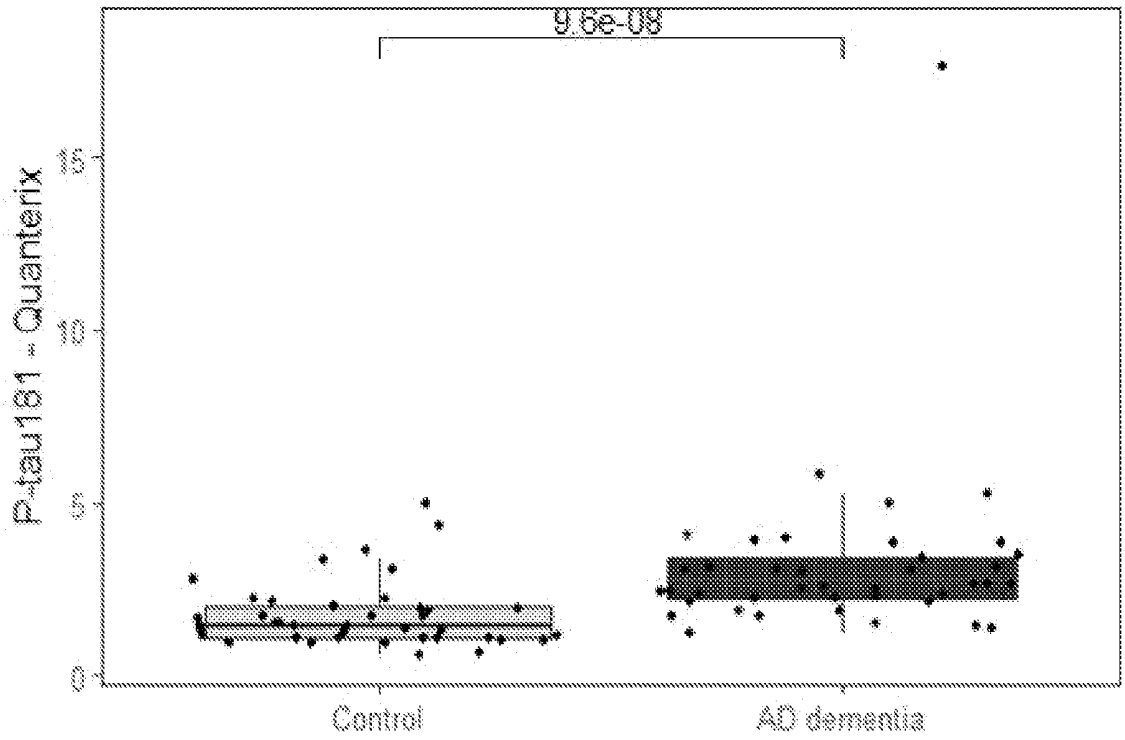
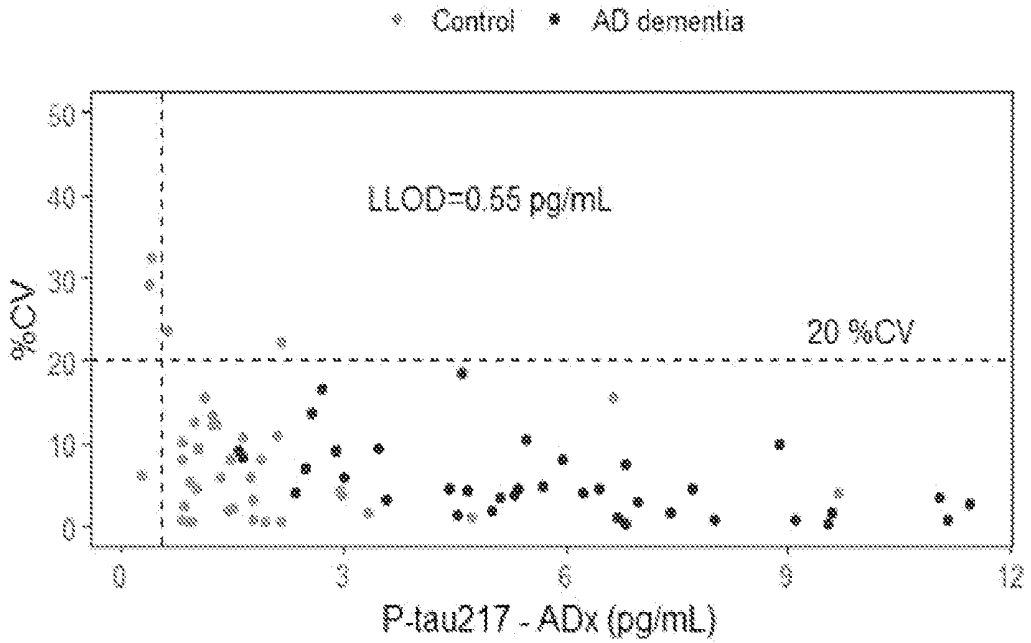


FIG. 21



		Stratified for diagnosis			
		Control		AD dementia	
	Total	Female	Male	Female	Male
Total, n	80	28	12	28	12
Sex female (%)	28 (70%)	28 (70%)	12 (30%)	28 (70%)	12 (30%)
Age. year (median [range])	64 [54-83]	63 [54-81]	66 [58-83]	65 [57-71]	66.5 [61-72]

FIG. 22


Control
AD dementia
LLOD=0.55 pg/mL
20 %CV
%CV
P-tau217 - ADx (pg/mL)

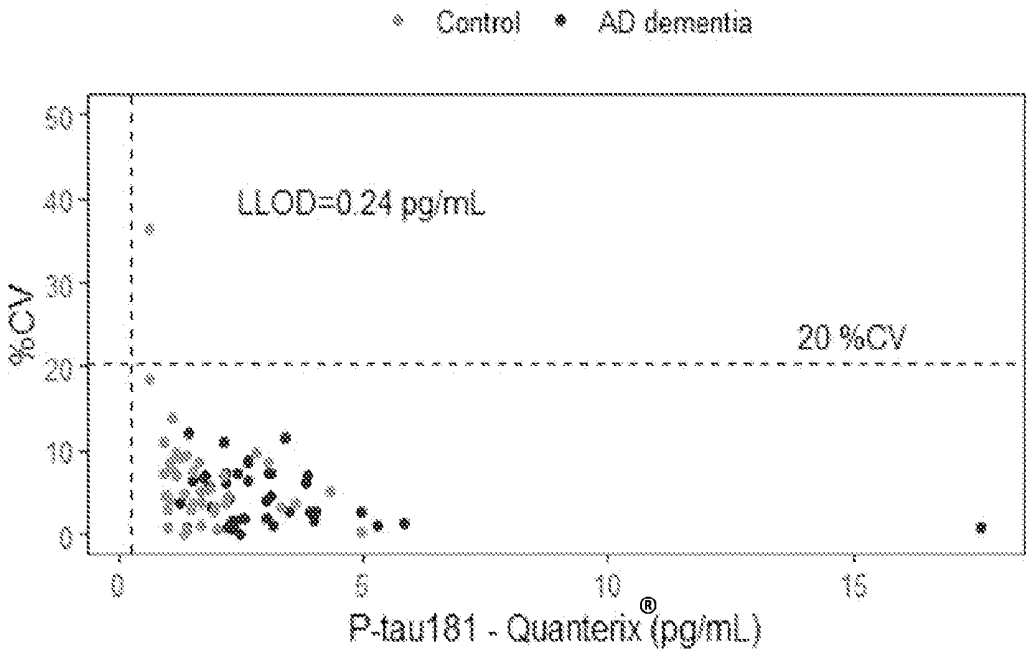
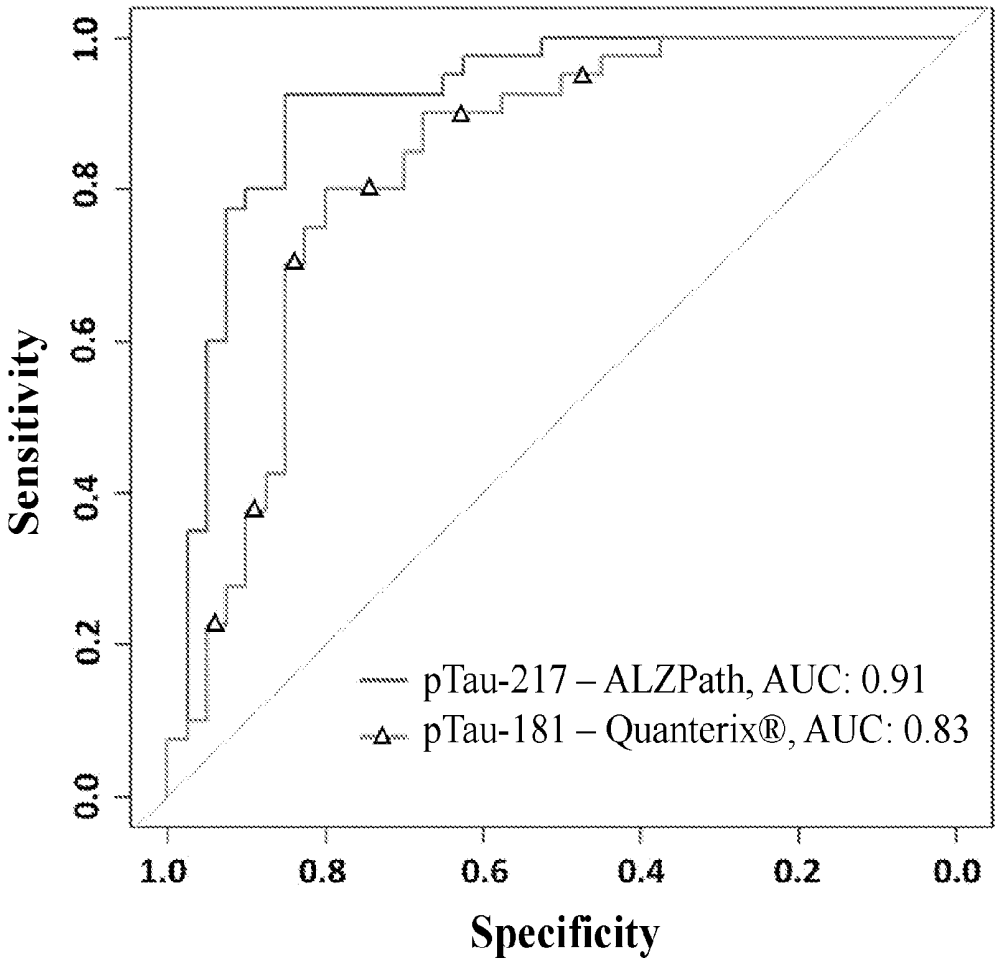

Control
AD dementia
LLOD=0.24 pg/mL
20 %CV
%CV
P-tau181 - Quanterix® (pg/mL)

FIG. 23



	AD-dementia	Controls		Differentiation	AD-dementia vs Controls		
	Median (IQR)	Median	Fold change	AUC (95% CI)	Cut off	% Sensitivity	% Specificity
pTau-217 ALZPath	5.2 (3.5 - 6.8)	1.3 (0.8 – 1.9)	4.2	0.91 (0.85 – 0.98)	2.26	92.5	85
pTau-181 Quanterix®	2.5 (2.2 - 3.3)	1.4 (1.1 – 1.9)	1.8	0.83 (0.74 – 0.920	2.14	80	80

FIG. 24

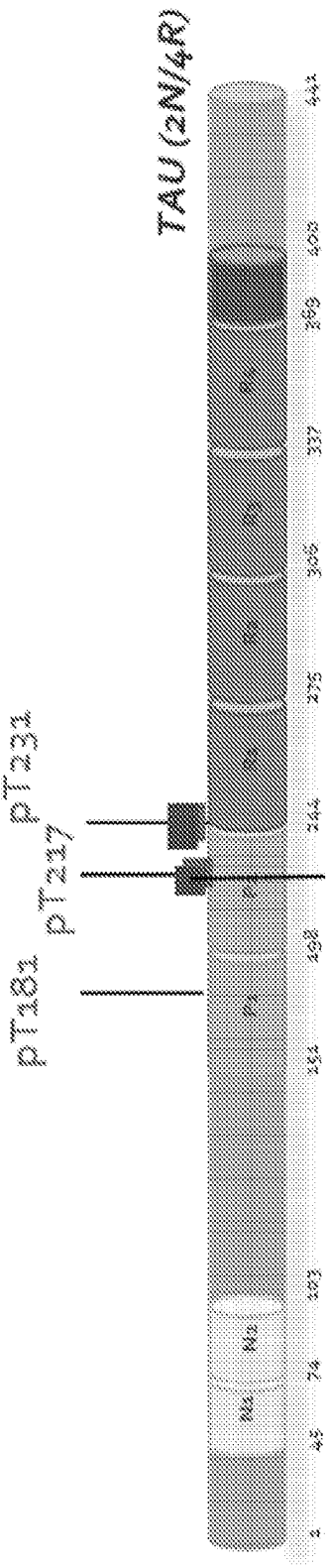

TAU (2N/4R)
pT181
pT217
pT231
1
45
74
103
151
198
244
275
305
337
369
402
441

FIG. 25

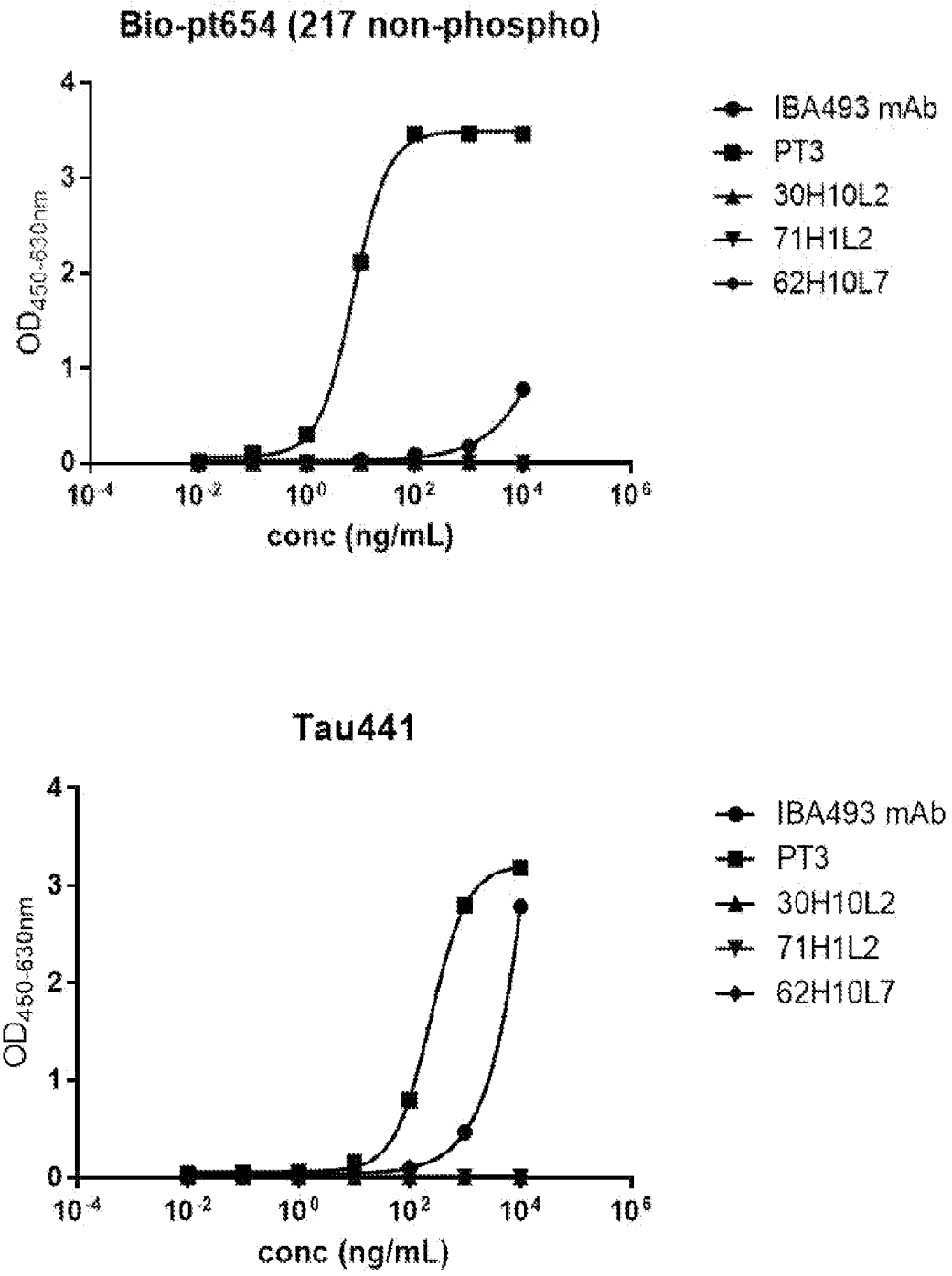

Bio-pt654 (217 non-phospho)
OD450-630nm
conc (ng/mL)
IBA493 mAb
PT3
30H10L2
71H1L2
62H10L7
Tau441
OD450-630nm
conc (ng/mL)
IBA493 mAb
PT3
30H10L2
71H1L2
62H10L7

FIG. 26

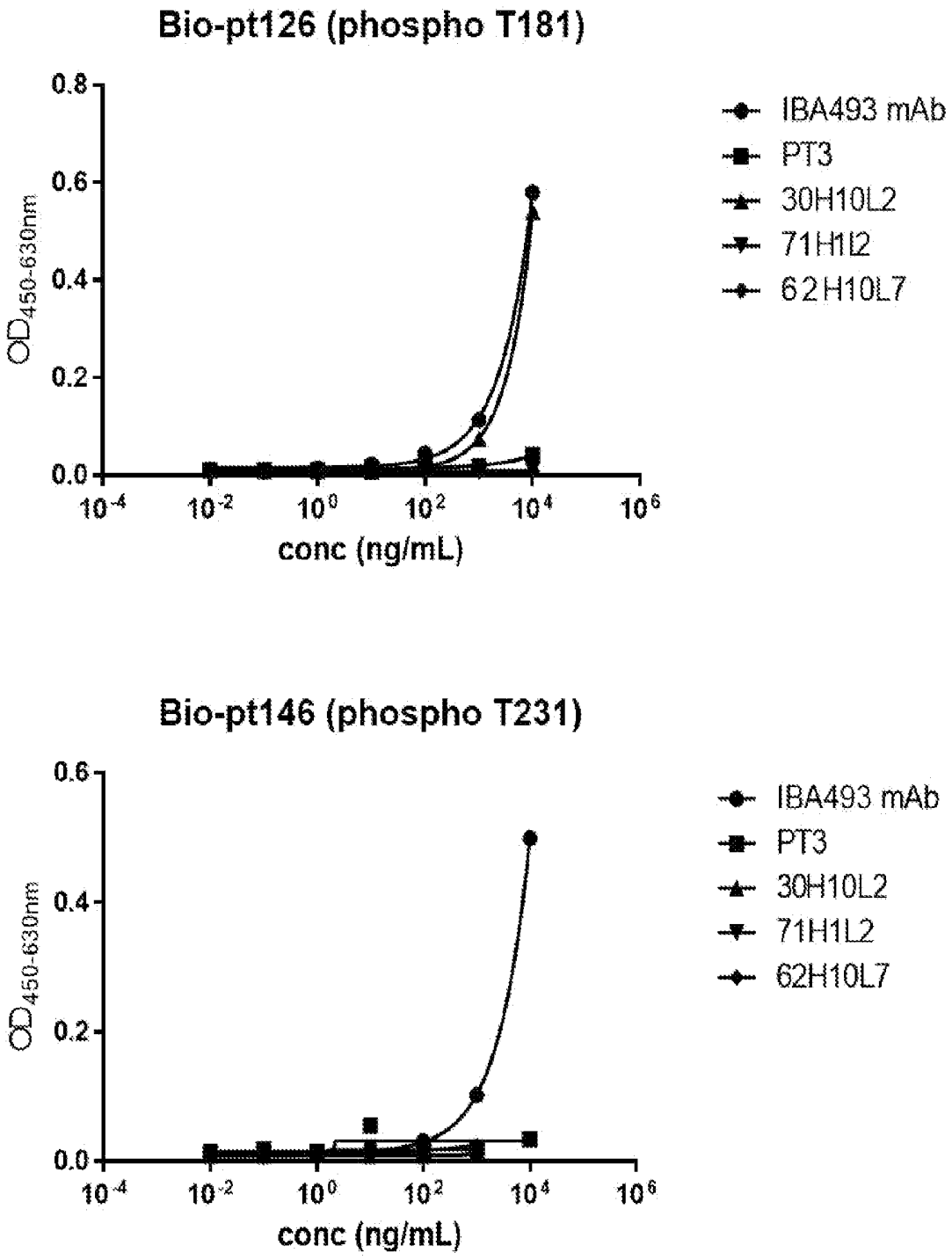
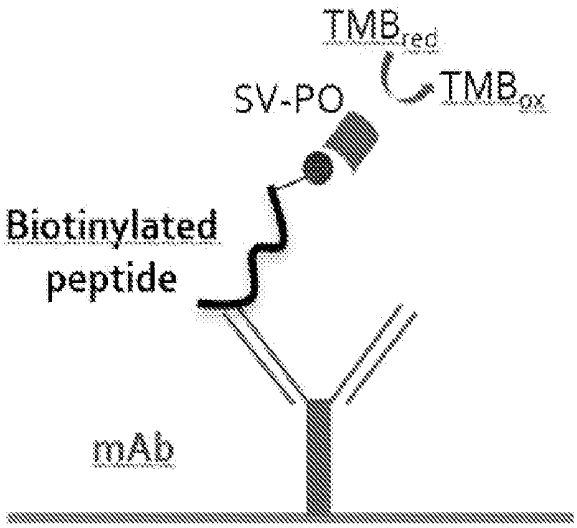

Bio-pt126 (phospho T181)
OD450-630nm
0.8
0.6
0.4
0.2
0.0
10-4
10-2
100
102
104
106
conc (ng/mL)
IBA493 mAb
PT3
30H10L2
71H1l2
62H10L7
Bio-pt146 (phospho T231)
OD450-630nm
0.6
0.4
0.2
0.0
10-4
10-2
100
102
104
106
conc (ng/mL)
IBA493 mAb
PT3
30H10L2
71H1L2
62H10L7

FIG. 27


TMB$_{red}$
TMB$_{ox}$
SV-PO
Biotinylated peptide
mAb

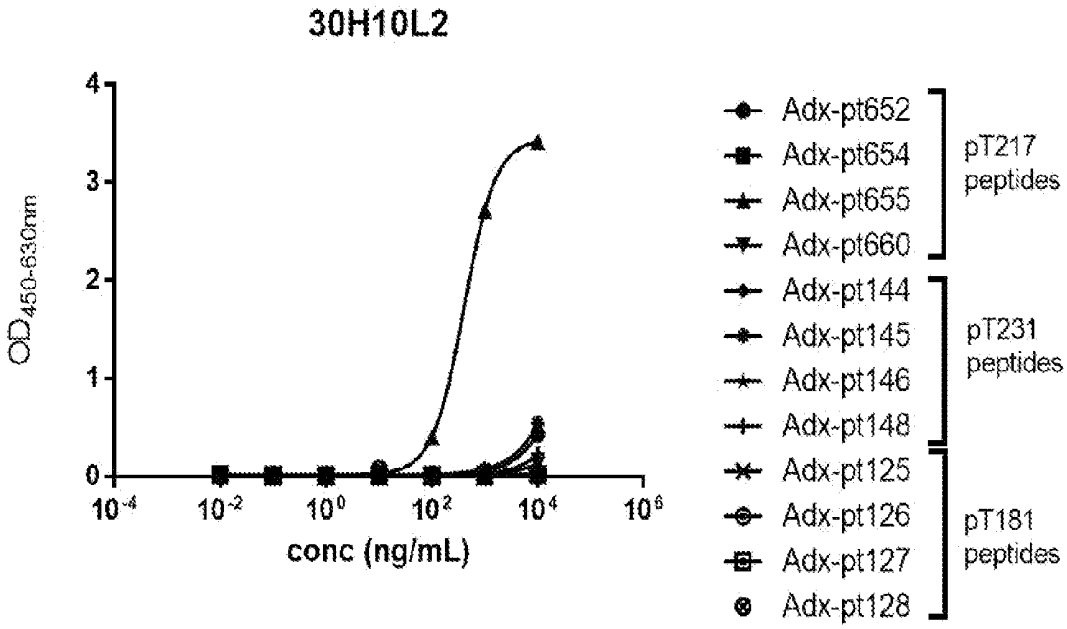
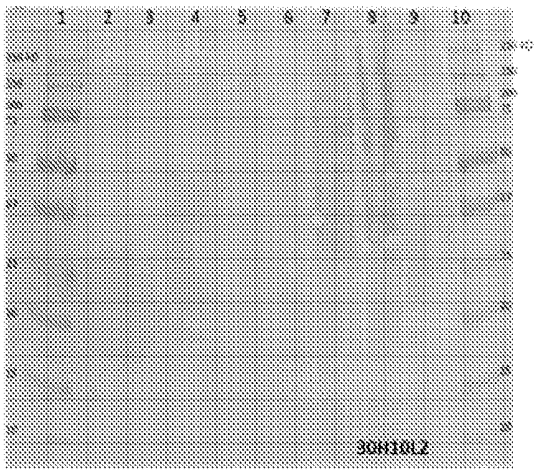
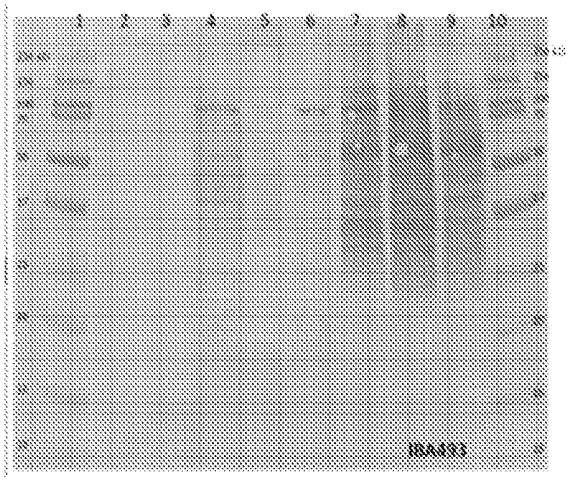
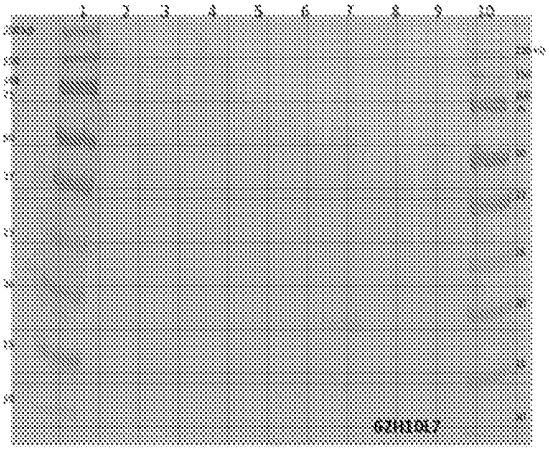

30H10L2
OD$_{450-630nm}$
conc (ng/mL)
Adx-pt652
Adx-pt654
Adx-pt655
Adx-pt660
pT217 peptides
Adx-pt144
Adx-pt145
Adx-pt146
Adx-pt148
pT231 peptides
Adx-pt125
Adx-pt126
Adx-pt127
Adx-pt128
pT181 peptides

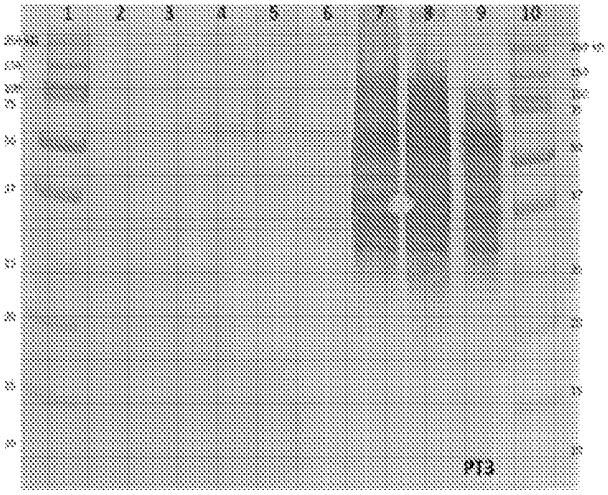
FIG. 28

Lane	Biomaterial	Loaded	Pathology	Lot#
1	PL			
2	Phosphatase treated pTau	0,05 µg		ADR21051
3	Tau441	0,05 µg		ADIP20939
4	BS18022	DF5	Control	ADIP19534
5	BS18024	DF5	Control	ADIP19534
6	BS18025	DF5	Control	ADIP19534
7	BS18027	DF5	AD	ADIP19534
8	BS18028	DF5	AD	ADIP19534
9	BS18029	DF5	AD	ADIP19534
10	PL			









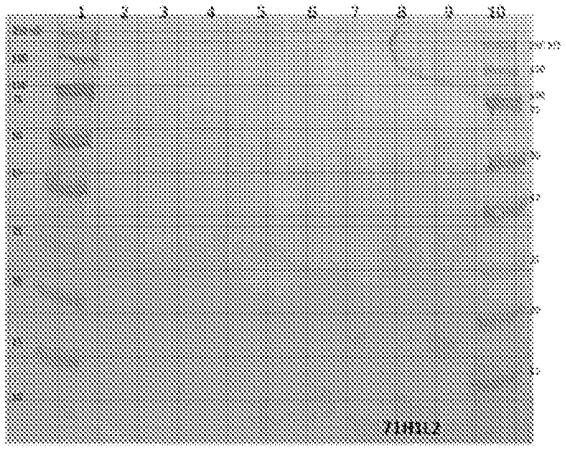


FIG. 29

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
16 March 2023 (16.03.2023)

WIPO | PCT

(10) International Publication Number
WO 2023/039107 A3

(51) International Patent Classification:
G01N 33/53 (2006.01) *C07K 16/30* (2006.01)

(21) International Application Number:
PCT/US2022/042963

(22) International Filing Date:
08 September 2022 (08.09.2022)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/242,437 09 September 2021 (09.09.2021) US

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(72) Inventors: **JEROMIN, Andreas**; c/o ALZPath, Inc., 7323 Black Swan Place, Carlsbad, California 92011 (US). **VENKAT, Krish**; c/o ALZPath, Inc., 7323 Black Swan Place, Carlsbad, California 92011 (US).

(74) Agent: **MAY, Scott R.**; Wilson Sonsini Goodrich & Rosati, 650 Page Mill Road, Palo alto, California 94304 (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, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, 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, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

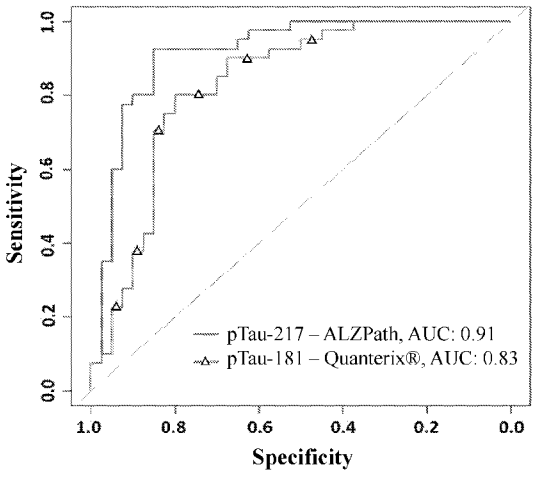
(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, 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).

Published:
— *with international search report (Art. 21(3))*

(88) Date of publication of the international search report:
05 October 2023 (05.10.2023)

(54) Title: PHOSPHO-TAU ANTIBODIES AND METHODS OF USE

(57) Abstract: Provided herein are compositions and methods relating to improved assays for establishing Alzheimer's disease. Further provided herein are compositions and methods comprising improved antibodies for assays including immunoassays.



	AD-dementia Median (IQR)	Controls Median	Differentiation Fold change	Differentiation AUC (95% CI)	AD-dementia vs Controls Cut off	% Sensitivity	% Specificity
pTau-217 ALZPath	5.2 (3.5 - 6.8)	1.3 (0.8 – 1.9)	4.2	0.91 (0.85 – 0.98)	2.26	92.5	85
pTau-181 Quanterix®	2.5 (2.2 - 3.3)	1.4 (1.1 – 1.9)	1.8	0.83 (0.74 – 0.920	2.14	80	80

FIG. 24

WO 2023/039107 A3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2022/042963

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - INV. - G01N 33/68; C07K 16/18; C12N 15/13 (2023.01)
ADD.

CPC - INV. - G01N 33/6896; C07K 16/18 (2023.05)

ADD. - C12N 15/11; G01N 2333/435 (2023.05)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic database consulted during the international search (name of database and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2017/0082641 A1 (BRISTOL-MYERS SQUIBB COMPANY) 23 March 2017 (23.03.2017) entire document	1-18, 44, 45, 51-53
A	US 2018/0321261 A1 (CELLCAP TECHNOLOGIES LTD) 08 November 2018 (08.11.2018) entire document	1-18, 44, 45, 51-53
A	US 2019/0169275 A1 (IPIERIAN INC.) 06 June 2019 (06.06.2019) entire document	1-18, 44, 45, 51-53
A	US 2018/0238910 A1 (KOREA INSTITUTE OF SCIENCE AND TECHNOLOGY) 23 August 2018 (23.08.2018) entire document	1-18, 44, 45, 51-53
A	US 2019/0033328 A1 (KYOTO PREFECTURAL PUBLIC UNIVERSITY CORPORATION et al.) 31 January 2019 (31.01.2019) entire document	1-18, 44, 45, 51-53

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
09 May 2023	JUN 14 2023
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Taina Matos Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2022/042963

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 19-43, 54-65
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See extra sheet(s).

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-18, 44, 45, 51-53

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Continued from Box No. III Observations where unity of invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I+: claims 1-18 and 44-53 are drawn to anti-phosphorylated tau antibodies and methods comprising the same.

The first invention of Group I+ is restricted to an antibody comprising a heavy chain variable domain (VH), and a light chain variable domain (VL), where the VH is selected to be SEQ ID NO: 30, which further comprises HCDR1 (SEQ ID NO: 1), HCDR2 (SEQ ID NO: 6), HCDR3 (SEQ ID NO: 10), and where the VL is selected to be SEQ ID NO: 35, which further comprises LCDR1 (SEQ ID NO: 14), LCDR2 (SEQ ID NO: 20), LCDR3 (SEQ ID NO: 24), and methods comprising the same. It is believed that claims 1-18, 44, 45, and 51-53 read on this first named invention and thus these claims will be searched without fee to the extent that they read on SEQ ID NOs: 30 and 35.

Applicant is invited to elect additional antibody VH, VL, and their respective, corresponding, SEQ ID NOs to be searched in a specific combination by paying additional fee for each set of election. An exemplary election would be an antibody comprising a heavy chain variable domain (VH), and a light chain variable domain (VL), where the VH is selected to be SEQ ID NO: 31, which further comprises HCDR1 (SEQ ID NO: 2), HCDR2 (SEQ ID NO: 7), HCDR3 (SEQ ID NO: 11), and where the VL is selected to be SEQ ID NO: 36, which further comprises LCDR1 (SEQ ID NO: 15), LCDR2 (SEQ ID NO: 21), LCDR3 (SEQ ID NO: 25), and methods comprising the same. Additional VH, VL, and their respective, corresponding, SEQ ID NOs will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The Groups I+ formulas do not share a significant structural element responsible for detecting phosphorylated tau, requiring the selection of alternative antibody VH and VL where "a method for detecting phosphorylated tau in a sample from an individual comprising: performing an immunoassay on the sample using an antibody or antibody fragment comprising a variable domain, heavy chain region (VH) and a variable domain, light chain region (VL), wherein the VH comprises an amino acid sequence at least about 90% identical to a sequence as set forth in any one of SEQ ID NOs: 30-34, and wherein the VL comprises an amino acid sequence at least about 90% identical to a sequence as set forth in any one of SEQ ID NOs: 35-40."

Additionally, even if Groups I+ were considered to share the technical features of a method for detecting phosphorylated tau in a sample from an individual comprising: performing an immunoassay on the sample using an antibody or antibody fragment comprising a variable domain, heavy chain region (VH) and a variable domain, light chain region (VL), an anti-tau antibody comprising i) a heavy chain comprising variable heavy chain (VH) domain and ii) a light chain comprising a variable light chain (VL) domain, and HCDR1, 2, and 3, and LCDR1, 2, and 3. However, these shared technical features do not represent a contribution over the prior art.

Specifically, US 2019/0169275 to Ipierian Inc. discloses a method for detecting phosphorylated tau in a sample from an individual (an in vitro method of detecting a Tau polypeptide in a biological sample obtained from an individual, Para. [0021]; [r]eduction in phosphorylated Tau can be determined using any known method, e.g., an immunological method using an anti-phospho-Tau antibody, Para. [0132]) comprising: performing an immunoassay on the sample using an antibody or antibody fragment reduction in phosphorylated Tau can be determined using any known method, e.g., an immunological method using an anti-phospho-Tau antibody, Para. [0132]) comprising a variable domain, heavy chain region (VH) and a variable domain, light chain region (VL) (an antibody that comprises: a) a light chain region comprising: i) a VL CDR1 comprising an amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 7; (ii) a VL CDR2 comprising an amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 8; and (iii) a VL CDR3 ...and b) a heavy chain region comprising: (i) a VH CDR1 comprising an amino acid sequence of SEQ ID NO: 4 or SEQ ID NO: 10; (ii) a VH CDR2 comprising an amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 11; and (iii) a VH CDR3, Para. [0008]), an anti-tau antibody comprising i) a heavy chain comprising variable heavy chain (VH) domain and ii) a light chain comprising a variable light chain (VL) domain, and HCDR1, 2, and 3, and LCDR1, 2, and 3 (an antibody that comprises: a) a light chain region comprising: i) a VL CDR1 comprising an amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 7; (ii) a VL CDR2 comprising an amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 8; and (iii) a VL CDR3 ... and b) a heavy chain region comprising: (i) a VH CDR1 comprising an amino acid sequence of SEQ ID NO: 4 or SEQ ID NO: 10; (ii) a VH CDR2 comprising an amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 11; and (iii) a VH CDR3, Para. [0008]).

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical features.

(19)国家知识产权局

(12)发明专利申请

(10)申请公布号 CN 118235043 A
(43)申请公布日 2024.06.21

(21)申请号 202280074834.X
(22)申请日 2022.09.08
(30)优先权数据
63/242,437 2021.09.09 US
(85)PCT国际申请进入国家阶段日
2024.05.09
(86)PCT国际申请的申请数据
PCT/US2022/042963 2022.09.08
(87)PCT国际申请的公布数据
WO2023/039107 EN 2023.03.16
(71)申请人 阿尔茨帕斯公司
地址 美国加利福尼亚州
(72)发明人 安德烈亚斯·赫罗明
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(74)专利代理机构 北京安信方达知识产权代理有限公司 11262
专利代理师 徐爱文 武晶晶
(51)Int.Cl.
G01N 33/53(2006.01)
C07K 16/30(2006.01)

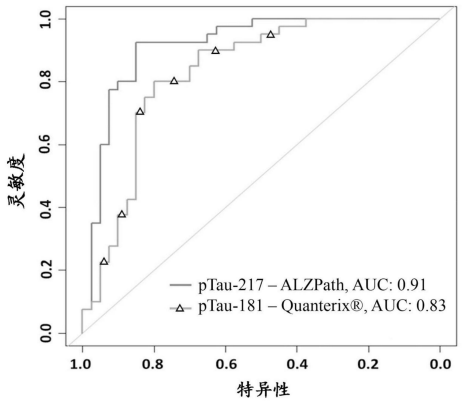
权利要求书4页 说明书81页 附图48页

(54)发明名称

磷酸化-TAU抗体和使用方法

(57)摘要

本文提供了与用于确定阿尔茨海默病的改善的测定相关的组合物和方法。本文还提供了包含用于包括免疫测定在内的测定的改善的抗体的组合物和方法。



	AD-痴呆	对照		微分	AD-痴呆相对于对照		
	中值(IQR)	中值	倍数变化	AUC (95% CI)	截止值	灵敏度%	特异性%
pTau-217 ALZPath	5.2 (3.5 - 6.8)	1.3 (0.8 – 1.9)	4.2	0.91 (0.85 – 0.98)	2.26	92.5	85
pTau-181 Quanterix®	2.5 (2.2 - 3.3)	1.4 (1.1 – 1.9)	1.8	0.83 (0.74 – 0.920	2.14	80	80

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1.一种用于检测来自个体的样品中的磷酸化tau的方法,所述方法包括:使用包含重链区可变结构域(VH)和轻链区可变结构域(VL)的抗体或抗体片段对所述样品进行免疫测定,其中所述VH包含与如SEQ ID NO:30-34中任一个中所示的序列具有至少约90%同一性的氨基酸序列,并且其中所述VL包含与如SEQ ID NO:35-40中任一个中所示的序列具有至少约90%同一性的氨基酸序列。

2.如权利要求1所述的方法,其中所述磷酸化tau选自pTau-181、pTau-212、pTau-217、pTau-231、pTau-214和pTau-220。

3.如权利要求1-2中任一项所述的方法,其中所述磷酸化tau是pTau-217。

4.如权利要求1-2中任一项所述的方法,其中所述磷酸化tau是pTau-231。

5.如权利要求2所述的方法,其中所述方法检测pTau-217和pTau-231。

6.如权利要求2所述的方法,其中所述方法检测pTau-212和pTau-217。

7.如权利要求2所述的方法,其中所述方法检测pTau-212和pTau-231。

8.如权利要求2所述的方法,其中所述方法检测pTau-181和pTau-217。

9.如权利要求2所述的方法,其中所述方法检测pTau-181和pTau-231。

10.如权利要求2所述的方法,其中所述方法检测pTau-181、pTau-217和pTau-231。

11.如权利要求2所述的方法,其中所述方法检测pTau-212、pTau-217和pTau-231。

12.如权利要求5所述的方法,其中所述方法检测选自血浆样品和血清样品的样品中的pTau-217和pTau-231。

13.如权利要求6所述的方法,其中所述方法检测选自血浆样品和血清样品的样品中的pTau-212和pTau-217。

14.如权利要求7所述的方法,其中所述方法检测选自血浆样品和血清样品的样品中的pTau-212和pTau-231。

15.如权利要求8所述的方法,其中所述方法检测选自血浆样品和血清样品的样品中的pTau-181和pTau-217。

16.如权利要求9所述的方法,其中所述方法检测选自血浆样品和血清样品的样品中的pTau-181和pTau-231。

17.如权利要求10所述的方法,其中所述方法检测选自血浆样品和血清样品的样品中的pTau-181、pTau-217和pTau-231。

18.如权利要求11所述的方法,其中所述方法检测选自血浆样品和血清样品的样品中的pTau-212、pTau-217和pTau-231。

19.如权利要求1-18中任一项所述的方法,其中所述VH包含根据SEQ ID NO:30-34中任一个的氨基酸序列。

20.如权利要求1-19中任一项所述的方法,其中所述VL包含根据SEQ ID NO:35-40中任一个的氨基酸序列。

21.如权利要求1-20中任一项所述的方法,其中所述VH包含根据SEQ ID NO:30-34中任一个的氨基酸序列,并且其中所述VL包含根据SEQ ID NO:35-40中任一个的氨基酸序列。

22.如权利要求1-21中任一项所述的方法,其中所述VH包含与SEQ ID NO:30具有至少约90%同一性的氨基酸序列,并且其中所述VL包含与SEQ ID NO:35具有至少约90%同一性的氨基酸序列。

23.如权利要求1-21中任一项所述的方法,其中所述VH包含与SEQ ID NO:31具有至少约90%同一性的氨基酸序列,并且其中所述VL包含与SEQ ID NO:36具有至少约90%同一性的氨基酸序列。

24.如权利要求1-21中任一项所述的方法,其中所述VH包含与SEQ ID NO:31具有至少约90%同一性的氨基酸序列,并且其中所述VL包含与SEQ ID NO:37具有至少约90%同一性的氨基酸序列。

25.如权利要求1-21中任一项所述的方法,其中所述VH包含与SEQ ID NO:32具有至少约90%同一性的氨基酸序列,并且其中所述VL包含与SEQ ID NO:38具有至少约90%同一性的氨基酸序列。

26.如权利要求1-21中任一项所述的方法,其中所述VH包含与SEQ ID NO:33具有至少约90%同一性的氨基酸序列,并且其中所述VL包含与SEQ ID NO:39具有至少约90%同一性的氨基酸序列。

27.如权利要求1-21中任一项所述的方法,其中所述VH包含与SEQ ID NO:34具有至少约90%同一性的氨基酸序列,并且其中所述VL包含与SEQ ID NO:40具有至少约90%同一性的氨基酸序列。

28.如权利要求1-27中任一项所述的方法,其中所述抗体或抗体片段包含与SEQ ID NO:41-51中任一个具有至少约90%同一性的氨基酸序列。

29.如权利要求1-28中任一项所述的方法,其还包括对所述样品进行测定,以确定选自以下的生物标志物的水平:Aβ42、Aβ40、Aβ38、BACE1、hFABP、TREM2、YKL-40、IP-10、神经颗粒蛋白、SNAP-25、突触结合蛋白、α-突触核蛋白、TDP-43、铁蛋白、VILIP-1、NfL、GFAP及其组合。

30.如权利要求1-29中任一项所述的方法,其中所述样品选自血液样品、血浆样品、血清样品和脑脊液(CSF)样品。

31.如权利要求1-30中任一项所述的方法,其还包括基于磷酸化tau的检测确定所述个体的阿尔茨海默病。

32.如权利要求1-31中任一项所述的方法,其还包括基于磷酸化tau的检测确定所述个体发展阿尔茨海默病的预后。

33.如权利要求32所述的方法,其还确定所述个体的年龄、基因型或生物标志物表达。

34.如权利要求33所述的方法,其中所述生物标志物选自Aβ42、Aβ40、Aβ38、BACE1、hFABP、TREM2、YKL-40、IP-10、神经颗粒蛋白、SNAP-25、突触结合蛋白、α-突触核蛋白、TDP-43、铁蛋白、VILIP-1、NfL、GFAP及其组合。

35.如权利要求1-34中任一项所述的方法,其中所述方法对于检测磷酸化tau具有至少约80%的特异性。

36.如权利要求1-34中任一项所述的方法,其中所述方法对于检测磷酸化tau具有至少约85%的特异性。

37.如权利要求1-34中任一项所述的方法,其中所述方法对于检测磷酸化tau具有至少约90%的特异性。

38.如权利要求1-37中任一项所述的方法,其中所述方法对于检测磷酸化tau具有至少约80%的灵敏度。

39.如权利要求1-37中任一项所述的方法,其中所述方法对于检测磷酸化tau具有至少约85%的灵敏度。

40.如权利要求1-37中任一项所述的方法,其中所述方法对于检测磷酸化tau具有至少约90%的灵敏度。

41.如权利要求1-40中任一项所述的方法,其中所述方法能够以至少约1.0皮克/毫升(pg/mL)的检测限值检测所述样品中的磷酸化tau。

42.如权利要求1-40中任一项所述的方法,其中所述方法能够以至少约1.5皮克/毫升(pg/mL)的检测限值检测所述样品中的磷酸化tau。

43.如权利要求1-40中任一项所述的方法,其中所述方法能够以至少约5皮克/毫升(pg/mL)的检测限值检测所述样品中的磷酸化tau。

44.一种抗tau抗体,其包含i)包含可变重链(VH)结构域的重链和ii)包含可变轻链(VL)结构域的轻链,其中所述VH结构域包含具有选自SEQ ID NO:1-5的序列的HCDR1序列、具有选自SEQ ID NO:6-9的序列的HCDR2序列、和具有选自SEQ ID NO:10-13的序列的HCDR3序列,并且VL结构域包含具有选自SEQ ID NO:14-19的序列的LCDR1序列、具有选自SEQ ID NO:20-23的序列的LCDR2序列、和具有选自SEQ ID NO:24-29的序列的LCDR3序列。

45.如权利要求44所述的抗tau抗体,其中所述HCDR1序列包含SEQ ID NO:1,HCDR2序列包含SEQ ID NO:6,HCDR3序列包含SEQ ID NO:10,LCDR1序列包含SEQ ID NO:14,LCDR2序列包含SEQ ID NO:20,并且LCDR3序列包含SEQ ID NO:24。

46.如权利要求44所述的抗tau抗体,其中所述HCDR1序列包含SEQ ID NO:2,HCDR2序列包含SEQ ID NO:7,HCDR3序列包含SEQ ID NO:11,LCDR1序列包含SEQ ID NO:15,LCDR2序列包含SEQ ID NO:21,并且LCDR3序列包含SEQ ID NO:25。

47.如权利要求44所述的抗tau抗体,其中所述HCDR1序列包含SEQ ID NO:2,HCDR2序列包含SEQ ID NO:7,HCDR3序列包含SEQ ID NO:11,LCDR1序列包含SEQ ID NO:16,LCDR2序列包含SEQ ID NO:22,并且LCDR3序列包含SEQ ID NO:26。

48.如权利要求44所述的抗tau抗体,其中所述HCDR1序列包含SEQ ID NO:3,HCDR2序列包含SEQ ID NO:8,HCDR3序列包含SEQ ID NO:10,LCDR1序列包含SEQ ID NO:17,LCDR2序列包含SEQ ID NO:20,并且LCDR3序列包含SEQ ID NO:27。

49.如权利要求44所述的抗tau抗体,其中所述HCDR1序列包含SEQ ID NO:4,HCDR2序列包含SEQ ID NO:7,HCDR3序列包含SEQ ID NO:12,LCDR1序列包含SEQ ID NO:18,LCDR2序列包含SEQ ID NO:23,并且LCDR3序列包含SEQ ID NO:28。

50.如权利要求44所述的抗tau抗体,其中所述HCDR1序列包含SEQ ID NO:5,HCDR2序列包含SEQ ID NO:9,HCDR3序列包含SEQ ID NO:13,LCDR1序列包含SEQ ID NO:19,LCDR2序列包含SEQ ID NO:21,并且LCDR3序列包含SEQ ID NO:29。

51.如权利要求44所述的抗tau抗体,其中所述VH结构域与选自SEQ ID NO:30-34的序列具有至少80%、至少85%、至少90%、至少95%序列同一性。

52.如权利要求44所述的抗tau抗体,其中所述VL结构域与选自SEQ ID NO:35-40的序列具有至少80%、至少85%、至少90%、至少95%序列同一性。

53.如权利要求44-52中任一项所述的抗tau抗体,其中所述抗tau抗体是嵌合抗体或其抗原结合片段。

54.如权利要求44-53中任一项所述的抗tau抗体,其中所述抗tau抗体包括IgG-scFv、纳米抗体、BiTE、双链抗体、DART、TandAb、scDiabody、scDiabody-CH3、三体、微型抗体、微抗体、TriBi微抗体、scFv-CH3 KIH、Fab-scFv-Fc KIH、Fab-scFv、scFv-CH-CL-scFv、Fab'、F(ab')2、F(ab')3、F(ab')2-scFv2、scFv、scFv-KIH、Fab-scFv-Fc、四价HCAb、scDiabody-Fc、双链抗体-Fc、串联scFv-Fc或内抗体。

55.如权利要求44-54中任一项所述的抗tau抗体,其中所述抗tau抗体是IgG1抗体。

56.如权利要求44-55中任一项所述的抗tau抗体,其中所述抗tau抗体是IgG2抗体。

57.如权利要求44-56中任一项所述的抗tau抗体,其中所述抗tau抗体是IgG4抗体。

58.如权利要求44-57中任一项所述的抗tau抗体,其中所述轻链是κ链。

59.如权利要求44-58中任一项所述的抗tau抗体,其中所述抗tau抗体对于人tau具有约100pM至约3nM的结合亲和力。

60.如权利要求44-59中任一项所述的抗tau抗体,其中所述抗tau抗体包含由核酸编码的VH结构域,所述核酸与选自SEQ ID NO:52-56的序列具有至少80%、至少85%、至少90%、至少95%序列同一性。

61.如权利要求44-60中任一项所述的抗tau抗体,其中所述抗tau抗体包含由核酸编码的VL结构域,所述核酸与选自SEQ ID NO:57-62的序列具有至少80%、至少85%、至少90%、至少95%序列同一性。

62.如权利要求44-61中任一项所述的抗tau抗体,其中所述抗tau抗体包含由与选自SEQ ID NO:52-56的序列具有至少80%、至少85%、至少90%、至少95%序列同一性的核酸编码的VH结构域和由与选自SEQ ID NO:57-62的序列具有至少80%、至少85%、至少90%、至少95%序列同一性的核酸编码的VL结构域。

63.如权利要求44-62中任一项所述的抗tau抗体,其中所述抗tau抗体包含由核酸编码的VH结构域,所述核酸包含与SEQ ID NO:52-56相同的序列。

64.如权利要求44-63中任一项所述的抗tau抗体,其中所述抗tau抗体包含由核酸编码的VL结构域,所述核酸包含与SEQ ID NO:57-62相同的序列。

65.如权利要求44-64中任一项所述的抗tau抗体,其中所述抗tau抗体包含由包含与SEQ ID NO:52-56相同的序列的核酸编码的VH结构域和由包含与SEQ ID NO:57-62相同的序列的核酸编码的VL结构域。

磷酸化-TAU抗体和使用方法

[0001] 交叉引用

[0002] 本申请要求2021年9月9日提交的美国临时专利申请号63/242,437的权益,所述专利申请通过引用整体并入。

背景技术

[0003] 阿尔茨海默病(AD)和其他tau蛋白病的生物标志物发现和筛查技术是一个正在进行开发的领域,其中这些工具可以应用于筛查群体,以确定哪些非痴呆个体患AD痴呆的风险最大并评估患者的疾病进展。已经通过各种手段来检测反映AD病理学的蛋白质,包括淀粉样蛋白β42($A\beta_{42}$)、神经丝轻链和各种tau亚型。tau的异常或过度磷酸化与病理正常的tau分子转化为指示各种tau蛋白病病理的配对螺旋丝(PHF)tau和神经原纤维缠结(NFT)相关联。

发明内容

[0004] Tau是一种重要的微管相关蛋白,在CNS神经元中大量表达,并且在正常细胞生理中发挥关键作用。还发现Tau在阿尔茨海默病和其他tau蛋白病中失调。tau蛋白的六种亚型通过选择性剪接由TAU基因生成。所述亚型彼此不同之处在于两个N末端插入物和一个被称为R2的重复序列的存在或不存在。tau的所有六种蛋白质亚型在正常和健康的细胞条件下都是高度可溶的,并且通常通过磷酸化和去磷酸化来调节。Tau已被证明与微管相互作用并促进微管组装。在神经元中,tau促进轴突微管的形成并使其稳定。Tau在驱动神经突生长方面具有额外的作用。tau与微管的相互作用受损可能是tau蛋白病的病理学、发展和进展的重要组成部分。tau的过度磷酸化是AD和其他tau蛋白病的标志性特征,并且过度磷酸化的程度通常与疾病进展相关。tau蛋白的过度磷酸化可能导致tau的配对螺旋丝和直丝的不溶性缠结的自组装。这些不溶性缠结聚集体,被称为神经原纤维缠结(NFT),包含过度磷酸化的tau,并且被认为是tau蛋白病的病理学标志物。

[0005] 分别从脑脊液(CSF)和/或血液中检测到的磷酸化tau(pTau)、总tau和$A\beta_{42}$是阿尔茨海默病和若干其他相关tau蛋白病的个体生物标志物。与年龄和性别匹配的对照相比,在后来被确认为患有AD的个体中,CSF pTau在前驱期和痴呆期均增加。在患有AD的个体中,CSF pTau水平与认知障碍的程度表现出强烈的相关性。实际上,CSF pTau水平可以以一定的精确度用作生物标志物来预测从认知未受损到轻度认知障碍(MCI)再到AD痴呆的进展。就作为预测AD进展的甚至相对早期阶段的生物标志物的效用而言,已经显示CSF pTau在来自临床前AD个体的样品中显著增加。pTau磷酸化程度的变化已经在AD的临床前散发病例和常染色体显性遗传AD的早期阶段中均得到证明。当在同一个个体内进行测定时,pTau、总tau和$A\beta_{42}$的血液水平通常低于CSF水平,并且如果这些生物标志物的血液水平可以以足够的特异性和精确性进行测定,则可以用作AD和其他相关tau蛋白病的信息性生物标志物。

[0006] 已经鉴定了导致聚集成NFT的过度磷酸化tau的若干磷酸化位点。在最长的tau亚型中,存在79个潜在的丝氨酸或苏氨酸磷酸化位点,并且这些位点中的至少30个已经被鉴

定为在NFT聚集体中磷酸化。用于测定tau分子的磷酸化状态的常见位点是苏氨酸-181。CSF液体含有一系列不同丰度的tau片段。来自tau多肽的N末端区域和中间区域的tau片段在CSF样品中比C末端tau片段丰富得多。来自个体的血浆样品也含有tau多肽和tau多肽片段，但它们往往以比在匹配的CSF样品中更低的浓度存在。能够检测与疾病病理学和进展相关的特定氨基酸残基处的tau磷酸化是诊断、疾病分期的关键组成部分，并且是测量AD和其他tau蛋白病的治疗功效的指标。检测和测量来自血浆样品的特定疾病相关残基处的pTau水平将大大有助于开发针对可能具有发展AD或其他tau蛋白病的风险或处于其早期阶段的个体的更敏感和精细的诊断、预后和疾病分析。在苏氨酸217处的tau磷酸化(pTau 217)是在一种在开发新的生物标志物和诊断测定中特别感兴趣的这样的残基。CSF和血浆中pTau生物标志物浓度的变化被认为先于AD和其他tau蛋白病中的可测量行为或认知变化。开发能够产生某些残基的tau磷酸化的特定点和程度的连续体的新测定无疑将有助于临床相关的医学诊断和治疗决策。将新测定的结果与现有测定的结果进行比较也可以产生进一步的医学信息性确定。基于血浆的tau生物标志物测定的结果可以与匹配的CSF样品(检测CSF pTau或CSF可溶性Aβ)进行比较，并且也可以与正电子发射断层扫描(PET)扫描进行比较，所述PET扫描检测Aβ聚集体的程度和位置，作为其实用性的指标，尤其是用于临床前或早期疾病阶段的分析。

[0007] 本文提供了用于检测来自个体的样品中的磷酸化tau的方法，所述方法包括：使用包含重链区可变结构域(VH)和轻链区可变结构域(VL)的抗体或抗体片段对所述样品进行免疫测定，其中所述VH包含与如SEQ ID NO:30-34中任一个中所示的序列具有至少约90％同一性的氨基酸序列，并且其中所述VL包含与如SEQ ID NO:35-40中任一个中所示的序列具有至少约90％同一性的氨基酸序列。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法，其中所述磷酸化tau选自pTau-181、pTau-212、pTau-217、pTau-231、pTau-214和pTau-220。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法，其中所述磷酸化tau是pTau-217。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法，其中所述磷酸化tau是pTau-231。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法，其中所述方法检测pTau-217和pTau-231。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法，其中所述方法检测pTau-212和pTau-217。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法，其中所述方法检测pTau-212和pTau-231。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法，其中所述方法检测pTau-181和pTau-217。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法，其中所述方法检测pTau-181和pTau-231。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法，其中所述方法检测pTau-181、pTau-217和pTau-231。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法，其中所述方法检测pTau-212、pTau-217和pTau-231。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法，其中所述方法检测选自血浆样品和血清样品的样品中的pTau-217和pTau-231。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法，其中所述方法检测选自血浆样品和血清样品的样品中的pTau-212和pTau-217。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法，其中所述方法检测选自血浆样品和血清样品的样品中的pTau-212和pTau-231。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法，其中所述方法检测选自血浆样品和血清样品的样品中的pTau-181和pTau-217。

本文还提供了用于检测来自个体的样品中的磷酸化tau的方法,其中所述方法检测选自血浆样品和血清样品的样品中的pTau-181和pTau-231。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法,其中所述方法检测选自血浆样品和血清样品的样品中的pTau-181、pTau-217和pTau-231。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法,其中所述方法检测选自血浆样品和血清样品的样品中的pTau-212、pTau-217和pTau-231。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法,其中所述VH包含根据SEQ ID NO:30-34中任一个的氨基酸序列。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法,其中所述VL包含根据SEQ ID NO:35-40中任一个的氨基酸序列。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法,其中所述VH包含根据SEQ ID NO:30-34中任一个的氨基酸序列,并且其中所述VL包含根据SEQ ID NO:35-40中任一个的氨基酸序列。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法,其中所述VH包含与SEQ ID NO:30具有至少约90%同一性的氨基酸序列,并且其中所述VL包含与SEQ ID NO:35具有至少约90%同一性的氨基酸序列。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法,其中所述VH包含与SEQ ID NO:31具有至少约90%同一性的氨基酸序列,并且其中所述VL包含与SEQ ID NO:36具有至少约90%同一性的氨基酸序列。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法,其中所述VH包含与SEQ ID NO:31具有至少约90%同一性的氨基酸序列,并且其中所述VL包含与SEQ ID NO:37具有至少约90%同一性的氨基酸序列。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法,其中所述VH包含与SEQ ID NO:32具有至少约90%同一性的氨基酸序列,并且其中所述VL包含与SEQ ID NO:38具有至少约90%同一性的氨基酸序列。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法,其中所述VH包含与SEQ ID NO:33具有至少约90%同一性的氨基酸序列,并且其中所述VL包含与SEQ ID NO:39具有至少约90%同一性的氨基酸序列。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法,其中所述VH包含与SEQ ID NO:34具有至少约90%同一性的氨基酸序列,并且其中所述VL包含与SEQ ID NO:40具有至少约90%同一性的氨基酸序列。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法,其中所述抗体或抗体片段包含与SEQ ID NO:41-51中任一个具有至少约90%同一性的氨基酸序列。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法,其还包括对所述样品进行测定,以确定选自以下的生物标志物的水平:Aβ42、Aβ40、Aβ38、BACE1、hFABP、TREM2、YKL-40、IP-10、神经颗粒蛋白、SNAP-25、突触结合蛋白、α-突触核蛋白、TDP-43、铁蛋白、VILIP-1、NfL、GFAP及其组合。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法,其中所述样品选自血液样品、血浆样品、血清样品和脑脊液(CSF)样品。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法,其还包括基于磷酸化tau的检测确定所述个体的阿尔茨海默病。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法,其还包括基于磷酸化tau的检测确定所述个体发展阿尔茨海默病的预后。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法,其还确定所述个体的年龄、基因型或生物标志物表达。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法,其中所述生物标志物选自Aβ42、Aβ40、Aβ38、BACE1、hFABP、TREM2、YKL-40、IP-10、神经颗粒蛋白、SNAP-25、突触结合蛋白、α-突触核蛋白、TDP-43、铁蛋白、VILIP-1、NfL、GFAP及其组合。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法,其中所述方法对于检测磷酸化tau具有至少约80%的特异

性。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法,其中所述方法对于检测磷酸化tau具有至少约85%的特异性。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法,其中所述方法对于检测磷酸化tau具有至少约90%的特异性。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法,其中所述方法对于检测磷酸化tau具有至少约80%的灵敏度。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法,其中所述方法对于检测磷酸化tau具有至少约85%的灵敏度。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法,其中所述方法对于检测磷酸化tau具有至少约90%的灵敏度。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法,其中所述方法能够以至少约1.0皮克/毫升(pg/mL)的检测限值检测所述样品中的磷酸化tau。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法,其中所述方法能够以至少约1.5皮克/毫升(pg/mL)的检测限值检测所述样品中的磷酸化tau。本文还提供了用于检测来自个体的样品中的磷酸化tau的方法,其中所述方法能够以至少约5皮克/毫升(pg/mL)的检测限值检测所述样品中的磷酸化tau。

[0008] 在某些实施方案中,本文还提供了抗tau抗体,其包含i)包含可变重链(VH)结构域的重链和ii)包含可变轻链(VL)结构域的轻链,其中所述VH结构域包含具有选自SEQ ID NO:1-5的序列的HCDR1序列、具有选自SEQ ID NO:6-9的序列的HCDR2序列、和具有选自SEQ ID NO:10-13的序列的HCDR3序列,并且VL结构域包含具有选自SEQ ID NO:14-19的序列的LCDR1序列、具有选自SEQ ID NO:20-23的序列的LCDR2序列、和具有选自SEQ ID NO:24-29的序列的LCDR3序列。在一些实施方案中,所述HCDR1序列包含SEQ ID NO:1,所述HCDR2序列包含SEQ ID NO:6,所述HCDR3序列包含SEQ ID NO:10,所述LCDR1序列包含SEQ ID NO:14,所述LCDR2序列包含SEQ ID NO:20,并且所述LCDR3序列包含SEQ ID NO:24。在一些实施方案中,所述HCDR1序列包含SEQ ID NO:2,所述HCDR2序列包含SEQ ID NO:7,所述HCDR3序列包含SEQ ID NO:11,所述LCDR1序列包含SEQ ID NO:15,所述LCDR2序列包含SEQ ID NO:21,并且所述LCDR3序列包含SEQ ID NO:25。在一些实施方案中,所述HCDR1序列包含SEQ ID NO:2,所述HCDR2序列包含SEQ ID NO:7,所述HCDR3序列包含SEQ ID NO:11,所述LCDR1序列包含SEQ ID NO:16,所述LCDR2序列包含SEQ ID NO:22,并且所述LCDR3序列包含SEQ ID NO:26。在一些实施方案中,所述HCDR1序列包含SEQ ID NO:3,所述HCDR2序列包含SEQ ID NO:8,所述HCDR3序列包含SEQ ID NO:10,所述LCDR1序列包含SEQ ID NO:17,所述LCDR2序列包含SEQ ID NO:20,并且所述LCDR3序列包含SEQ ID NO:27。在一些实施方案中,所述HCDR1序列包含SEQ ID NO:4,所述HCDR2序列包含SEQ ID NO:7,所述HCDR3序列包含SEQ ID NO:12,所述LCDR1序列包含SEQ ID NO:18,所述LCDR2序列包含SEQ ID NO:23,并且所述LCDR3序列包含SEQ ID NO:28。在一些实施方案中,所述HCDR1序列包含SEQ ID NO:5,所述HCDR2序列包含SEQ ID NO:9,所述HCDR3序列包含SEQ ID NO:13,所述LCDR1序列包含SEQ ID NO:19,所述LCDR2序列包含SEQ ID NO:21,并且所述LCDR3序列包含SEQ ID NO:29。在一些实施方案中,本文还提供了抗tau抗体,其包含i)包含可变重链(VH)结构域的重链和ii)包含可变轻链(VL)结构域的轻链,其中所述VH结构域与选自SEQ ID NO:30-34的序列具有至少80%、至少85%、至少90%、至少95%序列同一性。在一些实施方案中,本文还提供了抗tau抗体,其包含i)包含可变重链(VH)结构域的重链和ii)包含可变轻链(VL)结构域的轻链,其中所述VL结构域与选自SEQ ID NO:35-40的序列具有至少80%、至少85%、至少90%、

至少95％序列同一性。在一些实施方案中,本文所述的抗tau抗体是嵌合抗体或其抗原结合片段。在一些实施方案中,本文所述的抗tau抗体包括IgG-scFv、纳米抗体、BiTE、双链抗体、DART、TandAb、scDiabody、scDiabody-CH3、三体(triple body)、微型抗体(mini-antibody)、微抗体(minibody)、TriBi微抗体、scFv-CH3 KIH、Fab-scFv-Fc KIH、Fab-scFv、scFv-CH-CL-scFv、Fab'、F(ab')2、F(ab')3、F(ab')2-scFv2、scFv、scFv-KIH、Fab-scFv-Fc、四价HCAb、scDiabody-Fc、双链抗体-Fc、串联scFv-Fc或内抗体。在一些实施方案中,本文所述的抗tau抗体是IgG1抗体。在一些实施方案中,本文所述的抗tau抗体是IgG2抗体。在一些实施方案中,本文所述的抗tau抗体是IgG4抗体。在一些实施方案中,本文还提供了抗tau抗体,其包含i)包含可变重链(VH)结构域的重链和ii)包含可变轻链(VL)结构域的轻链,其中所述轻链是κ链。在一些实施方案中,本文还提供了抗tau抗体,其包含i)包含可变重链(VH)结构域的重链和ii)包含可变轻链(VL)结构域的轻链,其中所述抗tau抗体对于人tau具有约100pM至约3nM的结合亲和力。在一些实施方案中,本文提供了抗tau抗体,其包含由核酸编码的VH结构域,所述核酸与选自SEQ ID NO:52-56的序列具有至少80％、至少85％、至少90％、至少95％序列同一性。在一些实施方案中,本文提供了抗tau抗体,其包含由核酸编码的VL结构域,所述核酸与选自SEQ ID NO:57-62的序列具有至少80％、至少85％、至少90％、至少95％序列同一性。在一些实施方案中,本文提供了抗tau抗体,其包含由与选自SEQ ID NO:52-56的序列具有至少80％、至少85％、至少90％、至少95％序列同一性的核酸编码的VH结构域和由与选自SEQ ID NO:57-62的序列具有至少80％、至少85％、至少90％、至少95％序列同一性的核酸编码的VL结构域。在一些实施方案中,本文提供了抗tau抗体,其包含由核酸编码的VH结构域,所述核酸包含与SEQ ID NO:52-56相同的序列。在一些实施方案中,本文提供了抗tau抗体,其包含由核酸编码的VL结构域,所述核酸包含与SEQ ID NO:57-62相同的序列。在一些实施方案中,本文提供了抗tau抗体,其包含由包含与SEQ ID NO:52-56相同的序列的核酸编码的VH结构域和由包含与SEQ ID NO:57-62相同的序列的核酸编码的VL结构域。

[0009] 援引并入

[0010] 本说明书中提到的所有出版物、专利和专利申请都通过引用并入本文,其并入程度如同每个单独的出版物、专利或专利申请被明确且单独地指示通过引用并入。

附图说明

[0011] 本专利或申请文件含有至少一幅以彩色绘制的附图。在提出请求并支付必要费用后,专利局将提供具有一幅或多幅彩色附图的本专利或专利申请公布的副本。

[0012] 图1描绘了本文所用的用于测定本文所述的tau抗体的单分子阵列(Simoa®)方法的纲要。在将底物添加到样品中(珠上的夹心ELISA,1.1)之后,将样品添加到Simoa®圆盘(1.2)中。给予珠时间,使其沉淀到圆盘上的微阵列孔中(每个孔一个珠)(1.3)。然后,使用密封油去除多余的珠,以便进行成像(1.4)。具有夹心复合物的珠(阳性珠)将通过底物发出荧光并在成像过程中显示;没有夹心复合物的珠(阴性)仍将在成像中显示,但不会发出荧光(1.5)。将阳性珠的百分比转化为AEB(平均酶/珠)值。

[0013] 图2A-2D描绘了Simoa®测定中抗体1、抗体2、抗体3、抗体4、抗体5和抗体6的数

据。

[0014] 图3描绘了ELISA数据。

[0015] 图4A-4G描绘了抗体6的免疫组织化学染色的数据。

[0016] 图5A-5G描绘了抗体5的免疫组织化学染色的数据。

[0017] 图6A-6G描绘了抗体2的免疫组织化学染色的数据。

[0018] 图7描绘了间接ELISA测定的图和测定与pTau-217肽结合的抗体的ELISA数据的图。

[0019] 图8描绘了120个来源于血浆的临床样品的与pTau-217肽结合的抗体2的ELISA测定的信号/噪声(S/N)分析的图和120个来源于血浆的临床样品的与pTau-217肽结合的抗体2的ELISA测定的变异系数(CV%)的图。

[0020] 图9描绘了与使用ADx p204抗体的测定相比,对指定QTx的来源于脑脊液(68个CSF样品)和血浆(120个血浆样品)的临床样品的组(板)使用抗体2进行基于Simoa®的pTau-217测定的校准曲线(Cal曲线)的图。

[0021] 图10描绘了在来自样品个体的来源于血浆(Y轴)或CSF(X轴)的匹配样品中使用抗体2的基于Simoa®的pTau测定-217结果的图,以及对非阿尔茨海默病临床诊断、不确定诊断或阿尔茨海默病临床诊断的个体的相关结果的统计分析。

[0022] 图11描绘了使用抗体2/样品的基于Simoa®的pTau测定-217结果相对于使用抗体2/样品的基于Simoa®的pTau测定-181结果的图,以及相关结果的统计分析。

[0023] 图12描绘了使用抗体2/样品的基于Simoa®的pTau测定-217结果相对于使用Innotest pTau 181抗体/样品的基于Simoa®的Tau测定结果的图,以及相关结果的统计分析。

[0024] 图13描绘了使用抗体2作为捕获抗体、抗体ADx p204作为检测抗体和肽作为校准器的基于Simoa®的pTau测定结果的图,以及相关结果的统计分析。

[0025] 图14描绘了使用抗体2的将来自临床诊断为阿尔茨海默病的个体的样品和来自对照个体的来源于CSF或血浆的样品分组在一起的基于Simoa®的pTau测定-217结果的图。

[0026] 图15描绘了使用抗体2针对不同浓度的EDTA血浆样品的基于Simoa®的pTau测定-217结果的图,以及列出每个样品浓度的变异系数以说明测定的精确性的图表。

[0027] 图16描绘了使用抗体2的基于Simoa®的pTau测定-217结果的图,作为变异系数(CV%)相对于测量浓度进行作图。

[0028] 图17描绘了使用抗体2的基于Simoa®的pTau测定-217结果的图,和对并行性的统计分析,以确定稀释之后含有高内源性分析物浓度的实际样品是否在标准曲线中提供类似程度的检测。

[0029] 图18描绘了使用抗体2的基于Simoa®的pTau测定-217结果的图,和对线性的统计分析,以确定在四个样品加缓冲液尖峰的标准曲线范围内稀释之后,用高于检测上限的检测分析物加标的样品基质是否仍然可以提供可靠的定量。

[0030] 图19描绘了使用抗体2的基于Simoa®的pTau测定-217结果的图,和对线性的统计分析,以确定在三个样品加校准样品的标准曲线范围内稀释之后,用高于检测上限的检

测分析物加标的样品基质是否仍然可以提供可靠的定量。

[0031] 图20描绘了在记忆临床队列的临床验证中使用抗体2的基于Simoa®的pTau测定-217结果的图,以及针对pTau-217测定灵敏度作图的受试者操作特征(ROC)分析的图。

[0032] 图21描绘了使用抗体2针对来自对照和AD痴呆个体的组的基于Simoa®的pTau测定-217结果的图。

[0033] 图22描绘了使用来自Quanterix®的抗体针对来自对照和AD痴呆个体的组的基于Simoa®的pTau测定-181结果的图和样品分层的图表。

[0034] 图23描绘了使用抗体2的基于Simoa®的pTau测定-217结果和使用来自Quanterix®的抗体的基于Simoa®的pTau测定-181结果的图,示出了具有计算变异系数的精确性图。

[0035] 图24描绘了比较灵敏度和特异性的各种基于Simoa®的pTau测定的临床性能的图,和具有结果的统计分析的图表。

[0036] 图25描绘了指示各种蛋白质结构域的相对位置和苏氨酸残基的位置的Tau的示意图,其可以使用本文公开的方法测定磷酸化状态。

[0037] 图26描绘了在各种抗体的间接ELISA中对具有非磷酸化T217(Bio-pt654)和全长Tau(Tau441)的Tau片段的反应性的图。

[0038] 图27描绘了在各种抗体的间接ELISA中对具有磷酸化T181(Bio-pt126)和磷酸化T231(Bio-pt146)的Tau片段的反应性的图。

[0039] 图28描绘了利用pTau217单克隆抗体作为各种合成肽的捕获工具的测定的图,和使用抗体2作为捕获工具的此测定的结果的图。

[0040] 图29描绘了使用各种Tau抗体针对来自AD患者或对照对象的脑裂解液样品的蛋白质印迹分析。

具体实施方式

[0041] 阿尔茨海默病(AD)是一种复杂的疾病,并且有效的治疗需要准确诊断。本文描述了用于检测AD的改善的组合物和方法,其包含用于在诊断和/或预后测定中使用的改善的抗体。

[0042] 某些术语

[0043] 在整个本公开中,各种实施方案以范围形式呈现。应理解,范围格式的描述仅为了方便和简洁,而不应被解释为对任何实施方案的范围的严格限制。因此,除非上下文另有明确指示,否则应认为对范围的描述已具体公开了所有可能的子范围以及在此范围内直至下限单位的十分之一的单个数值。例如,对范围诸如从1至6的描述应视为已具体公开了子范围,诸如从1至3、从1至4、从1至5、从2至4、从2至6、从3至6等,以及此范围内的单个值,例如,1.1、2、2.3、5和5.9。无论范围的宽度如何,这都适用。这些中间范围的上限和下限可以独立地包括在较小的范围内,并且也包括在本发明内,受规定范围内任何明确排除的限制。当所述范围包括一个或两个限值时,除非上下文另外明确指出,否则不包含所包括的那些限值中的一个或两个的范围也包括在本发明中。

[0044] 本文所用的术语仅出于描述特定实施方案的目的,并且不旨在限制任何实施方

案。如本文所用,单数形式“一种(a/an)”、“一个(a/an)”和“所述(the)”旨在包括复数形式,除非上下文另有明确指出。还将理解,当在本说明书中使用术语“包括(comprises)”和/或“包含(comprising)”时,其指定了所述特征、整体、步骤、操作、要素和/或组件的存在,但并不排除一个或多个其他特征、整体、步骤、操作、要素、组件和/或其群组的存在或添加。如本文所用,术语“和/或”包括一个或多个相关联的所列项目的任何和所有组合。

[0045] 除非特别说明或从上下文可以明显看出,否则如本文所用,术语“约”在提及数值或数值范围时应理解为是指所述数值及其+/-10%的数值,或对于一个范围中列出的值,则指从比列出的下限低10%到比列出的上限高10%。

[0046] 术语“个体”、“患者”或“对象”可互换使用。所述术语均不要求或不限于以卫生保健工作者(例如,医生、注册护士、执业护士、医师助理、护理员或临终关怀工作者)进行监督(例如,持续或间断)为特征的情况。此外,这些术语是指人或动物对象。

[0047] 本文中的术语“抗体”以最广泛的意义使用并且包括单克隆抗体,包括完整抗体及其功能性(抗原结合)抗体片段,包括以下片段:抗原结合(Fab)片段、F(ab')2片段、Fab'片段、Fv片段、重组IgG(rIgG)片段、单链抗体片段(包括单链可变片段(sFv或scFv))和单结构域抗体(例如,sdAb、sdFv、纳米抗体)片段。所述术语涵盖免疫球蛋白的基因工程和/或以其他方式修饰的形式,诸如胞内抗体(intrabodies)、肽抗体(peptibodies)、嵌合抗体和杂缀合物抗体(heteroconjugate antibodies)、串联双-scFv、串联三-scFv。除非另有说明,否则术语“抗体”应理解成涵盖其功能性抗体片段。所述术语还涵盖完整抗体或全长抗体,包括任何类别或亚类的抗体,包括IgG及其亚类、IgM、IgE、IgA和IgD。抗体可以包含兔IgG1恒定区。抗体可以包含兔IgG4恒定区。抗体包括但不限于全长抗体和天然抗体以及其保留其结合特异性的片段和部分,诸如其任何特异性结合部分,包括具有任何数目的免疫球蛋白类别和/或同型(例如,IgG1、IgG2、IgG3、IgG4、IgM、IgA、IgD、IgE和IgM)的那些;及其生物学相关(抗原结合)片段或特异性结合部分,包括但不限于Fab、F(ab')2、Fv和scFv(单链或相关实体)。单克隆抗体通常是基本上均质的抗体的组合物内的一种;因此,单克隆抗体组合物内所包含的任何单个抗体都是相同的,除了可能以微量存在的可能天然存在的突变。单克隆抗体可以包含兔IgG1恒定区或兔IgG4恒定区。

[0048] 术语“互补决定区”或“CDR”是抗体可变区的区段,其在结构上与抗体结合的表位互补,并且比可变区域的其余部分更具可变性。因此,CDR有时被称为高变区。可变区包含三个CDR。CDR肽可以通过构建编码感兴趣的抗体的CDR的基因来获得。例如,通过使用聚合酶链反应从产生抗体的细胞的RNA合成可变区域来制备此类基因。参见例如Larrick等人,Methods:A Companion to Methods in Enzymology2:106(1991);Courtenay-Luck,“Genetic Manipulation of Monoclonal Antibodies,”Monoclonal Antibodies:Production,Engineering and Clinical Application,Ritter等人(编),第166-179页(Cambridge University Press 1995);以及Ward等人,“Genetic Manipulation and Expression of Antibodies,”Monoclonal Antibodies:Principles and Applications,Birch等人(编),第137-185页(Wiley-Liss,Inc.1995)。

[0049] 术语“Fab”是指含有轻链的恒定结构域和重链的第一恒定结构域(CH1)的蛋白质。Fab片段与Fab′片段的不同之处在于在重链CH1结构域的羧基末端处添加了一些残基,包括来自抗体铰链区的一个或多个半胱氨酸。Fab'-SH是本文中Fab′的名称,其中恒定结构域的

一个或多个半胱氨酸残基带有游离巯基。Fab′片段通过还原F(ab')2片段的重链二硫键来产生。抗体片段的其他化学偶联也是已知的。

[0050] “单链可变片段(scFv)”是抗体重链(VH)和轻链(VL)可变区的融合蛋白,通过10至约25个氨基酸的短接头肽连接。接头通常富含甘氨酸以提高柔性,以及丝氨酸或苏氨酸以提高溶解性,并且可以将VH的N末端与VL的C末端连接,或者反之亦然。所述蛋白质保留了原始抗体的特异性,尽管去除了恒定区并引入了接头。scFv抗体例如在Houston,J.S.,Methods in Enzymol.203(1991)46-96)中进行描述。另外,抗体片段包含具有以下VH结构域的特征,即能够与VL结构域一起,或VL结构域的特征,即能够与VH结构域一起组装到功能性抗原结合位点,并且从而提供全长抗体的抗原结合特性的单链多肽。

[0051] 如本文所用,关于序列的术语“氨基酸序列同一性百分比(%)”被定义为:在比对序列并引入空位(如果需要以实现最大序列同一性百分比)之后,并且不将任何保守取代视为序列同一性的一部分,候选序列中与特定序列中的氨基酸残基相同的氨基酸残基的百分比。用于确定氨基酸序列同一性百分比的比对可以本领域技术范围内的各种方式来实现,例如,使用可公开获得的计算机软件,诸如EMBOSS MATCHER、EMBOSS WATER、EMBOSS STRETCHER、EMBOSS NEEDLE、EMBOSS LALIGN、BLAST、BLAST-2、ALIGN或Megalign(DNASTAR)软件。本领域技术人员可以确定用于测量比对的适当参数,包括为了在被比较的序列的全长上实现最大比对所需要的任何算法。

[0052] 在ALIGN-2用于氨基酸序列比较的情形下,给定氨基酸序列A对于、与或相对于给定氨基酸序列B的氨基酸序列同一性%(其可以可替代地措词为给定氨基酸序列A对于、与或相对于给定氨基酸序列B具有或包含某一氨基酸序列同一性%)如下计算:100×分数X/Y,其中X为通过序列比对程序ALIGN-2在所述程序的A和B的比对中评分为相同匹配的氨基酸残基的数目,并且其中Y为B中的氨基酸残基的总数。应理解,在氨基酸序列A的长度不等于氨基酸序列B的长度的情况下,A与B的氨基酸序列同一性%将不等于B与A的氨基酸序列同一性%。除非另外明确说明,否则本文所用的全部氨基酸序列同一性%值如紧接上一段中所述那样使用ALIGN-2计算机程序获得。

[0053] 术语“互补决定区”和“CDR”(其与“高变区”或“HVR”同义)在本领域中已知是指抗体可变区内的非连续氨基酸序列,其赋予抗原特异性和/或结合亲和力。通常,在每个重链可变区中有三个CDR(CDR-H1、CDR-H2、CDR-H3),并且在每个轻链可变区中有三个CDR(CDR-L1、CDR-L2、CDR-L3)。“框架区”和“FR”在本领域中是已知的,指代重链和轻链的可变区的非CDR部分。通常,在每个全长重链可变区中有四个FR(FR-H1、FR-H2、FR-H3和FR-H4),并且在每个全长轻链可变区中有四个FR(FR-L1、FR-L2、FR-L3和FR-L4)。给定CDR或FR的精确氨基酸序列边界可以使用许多熟知方案中的任一种容易地确定,所述方案包括以下文献中所述的那些:Kabat等人(1991),“Sequences of Proteins of Immunological Interest,”第5版Public Health Service,National Institutes of Health,Bethesda,MD(“Kabat”编号方案);Al-Lazikani等人,(1997)JMB 273,927-948(“Chothia”编号方案);MacCallum等人,J.Mol.Biol.262:732-745(1996),“Antibody-antigen interactions:Contact analysis and binding site topography,”J.Mol.Biol.262,732-745.”(“Contact”编号方案);Lefranc MP等人,“IMGT unique numbering for immunoglobulin and Tcell receptor variabledomains and Ig superfamily V-like domains,”Dev Comp Immunol,2003年1

月;27(1):55-77(“IMGT”编号方案);Honegger A和Plückthun A,“Yet another numbering scheme for immunoglobulin variabledomains:an automatic modeling and analysis tool,”J Mol Biol,2001年6月8日;309(3):657-70(“Aho”编号方案);以及Whitelegg NR和Rees AR,“WAM:an improved algorithm for modelling antibodies on the WEB,”Protein Eng.2000年12月;13(12):819-24(“AbM”编号方案)。在某些实施方案中,本文所述的抗体的CDR可以通过选自Kabat、Chothia、IMGT、Aho、AbM或其组合的方法定义。

[0054] 给定CDR或FR的边界可能根据用于鉴定的方案而有差别。例如,Kabat方案是基于结构比对,而Chothia方案是基于结构信息。Kabat和Chothia方案的编号均是基于最常见的抗体区域序列长度,在一些抗体中出现插入(其通过插入字母适应,例如“30a”)和缺失。两个方案将某些插入和缺失(“插入缺失”)放置在不同的位置,得到不同的编号。Contact方案是基于对复杂的晶体结构的分析并且在许多方面中与Chothia编号方案相似。

[0055] 除非另外定义,否则本文所用的所有技术和科学术语具有与本文所述的方法和组合物所属领域的普通技术人员通常理解的相同的含义。尽管与本文所述的那些类似或等同的任何方法和材料也可用于实践或测试本文所述的方法和组合物,但现在描述代表性的示例性方法和材料。

[0056] Tau抗体

[0057] 本文提供了与tau结合的抗体。在一些情况下,与tau结合的抗体是单克隆抗体。在某些方面,本文公开了一种抗tau抗体。在一些情况下,所述抗tau抗体与哺乳动物tau特异性结合。在一些情况下,所述抗tau抗体与人tau特异性结合。在一些情况下,所述抗tau抗体与tau的N末端部分特异性结合。在一些情况下,所述抗tau抗体与人tau的N末端部分特异性结合。在一些情况下,所述抗tau抗体与tau的包含蛋白质结构域P2的部分特异性结合。在一些情况下,所述抗tau抗体与人tau的包含蛋白质结构域P2的部分特异性结合。在一些情况下,所述抗tau抗体与tau的包含蛋白质结构域P1的部分特异性结合。在一些情况下,所述抗tau抗体与人tau的包含蛋白质结构域P1的部分特异性结合。在一些情况下,所述抗tau抗体与tau的包含蛋白质结构域P1和P2的部分特异性结合。在一些情况下,所述抗tau抗体与人tau的包含蛋白质结构域P1和P2的部分特异性结合。

[0058] 在一些实施方案中,所述抗tau抗体包含i)包含可变重链(VH)结构域的重链和ii)包含可变轻链(VL)结构域的轻链。在一些实施方案中,VH结构域包含具有选自SEQ ID NO:1-5的序列的重链CDR1(HCDR1)序列、具有选自SEQ ID NO:6-9的序列的重链CDR2(HCDR2)序列、和具有选自SEQ ID NO:10-13的序列的重链CDR3(HCDR3)序列。在一些实施方案中,VL结构域包含具有选自SEQ ID NO:14-19的序列的轻链CDR1(LCDR1)序列、具有选自SEQ ID NO:20-23的序列的轻链CDR2(LCDR2)序列、和具有选自SEQ ID NO:24-29的序列的轻链CDR3(LCDR3)序列。

[0059] 在一些实施方案中,抗tau抗体的VH区包含选自表1的HCDR1、HCDR2和HCDR3序列。

[0060] 表1.HCDR氨基酸序列

[0061]

SEQ ID NO:	HCDR1序列
1	SQKVG
2	SYAMI
3	NYKVG

4	NYAMS
5	THAMT
SEQ ID NO:	HCDR2序列
6	IINNYGSTYYASWAKG
7	FISRSGITYYASWAKG
8	IINYYSQTYYASWAKG
9	VINPSGSAYYATWVNG
SEQ ID NO:	HCDR3序列
10	DPDGSIVFDI
11	EFGAVGSDYYRDAFNL
12	EFGAVGSDYYRDALRL
13	DYITAGDYYMDAFDP

[0062] 在一些实施方案中,VH区包含具有SEQ ID NO:1的HCDR1序列;具有SEQ ID NO:6的HCDR2序列;和具有SEQ ID NO:10的HCDR3序列。在一些实施方案中,VH区包含具有SEQ ID NO:2的HCDR1序列;具有SEQ ID NO:7的HCDR2序列;和具有SEQ ID NO:11的HCDR3序列。在一些实施方案中,VH区包含具有SEQ ID NO:3的HCDR1序列;具有SEQ ID NO:8的HCDR2序列;和具有SEQ ID NO:10的HCDR3序列。在一些实施方案中,VH区包含具有SEQ ID NO:4的HCDR1序列;具有SEQ ID NO:7的HCDR2序列;和具有SEQ ID NO:12的HCDR3序列。在一些实施方案中,VH区包含具有SEQ ID NO:5的HCDR1序列;具有SEQ ID NO:9的HCDR2序列;和具有SEQ ID NO:13的HCDR3序列。

[0063] 在一些实施方案中,抗tau抗体的VL区包含选自表2的LCDR1、LCDR2和LCDR3序列。

[0064] 表2.LCDR氨基酸序列

[0065]

SEQ ID NO:	LCDR1序列
14	QSSQSVVYNNRLS
15	QASESINSWLS
16	QASQNIYSNLA
17	QSSQSVYSNKRLA
18	QASQSIGSNLA
19	QASQSISNQLS
SEQ ID NO:	LCDR2序列
20	GASTLAS
21	RASTLAS
22	GASNLAS
23	GASTLES
SEQ ID NO:	LCDR3序列
24	LGSYDCSSGDCHA
25	QSYYEEDGIGYA
26	QGYDYSTAGAYP
27	AGGYDCSTGDCWT

28	QSYYEGSDIGYA
29	QQGYNRDNVDNL

[0066] 在一些实施方案中,VL区包含具有SEQ ID NO:14的LCDR1序列;具有SEQ ID NO:20的LCDR2序列;和具有SEQ ID NO:24的LCDR3序列。在一些实施方案中,VL区包含具有SEQ ID NO:15的LCDR1序列;具有SEQ ID NO:21的LCDR2序列;和具有SEQ ID NO:25的LCDR3序列。在一些实施方案中,VL区包含具有SEQ ID NO:16的LCDR1序列;具有SEQ ID NO:22的LCDR2序列;和具有SEQ ID NO:26的LCDR3序列。在一些实施方案中,VL区包含具有SEQ ID NO:17的LCDR1序列;具有SEQ ID NO:20的LCDR2序列;和具有SEQ ID NO:27的LCDR3序列。在一些实施方案中,VL区包含具有SEQ ID NO:18的LCDR1序列;具有SEQ ID NO:23的LCDR2序列;和具有SEQ ID NO:28的LCDR3序列。在一些实施方案中,VL区包含具有SEQ ID NO:19的LCDR1序列;具有SEQ ID NO:21的LCDR2序列;和具有SEQ ID NO:29的LCDR3序列。

[0067] 在一些实施方案中,所述抗tau抗体是其抗原结合片段。在一些实施方案中,所述抗tau抗体是嵌合抗体或其抗原结合片段。在一些实施方案中,所述抗tau抗体包括IgG-scFv、纳米抗体、微型抗体、微抗体、scFv-CH3 KIH、Fab-scFv-Fc KIH、Fab-scFv、scFv-CH-CL-scFv、Fab'、F(ab')2、F(ab')3、F(ab')2-scFv2、scFv、scFv-KIH、Fab-scFv-Fc或内抗体。在一些实施方案中,所述抗tau抗体包括双特异性抗体。在一些实施方案中,所述抗tau抗体包括多特异性抗体。在一些实施方案中,所述抗tau抗体是IgG1抗体。在一些实施方案中,所述抗tau抗体是IgG2抗体。在一些实施方案中,所述抗tau抗体是IgG4抗体。在一些实施方案中,所述抗tau抗体包含轻链,其中所述轻链是κ链。

[0068] 在一些实施方案中,所述抗tau抗体对于人tau具有约100pM至约3nM的结合亲和力。在一些实施方案中,所述抗tau抗体对于人tau具有约100pM至300pM的结合亲和力。在一些实施方案中,所述抗tau抗体对于人tau具有约100pM至500pM的结合亲和力。在一些实施方案中,所述抗tau抗体对于人tau具有约100pM至800pM的结合亲和力。在一些实施方案中,所述抗tau抗体对于人tau具有约300pM至600pM的结合亲和力。在一些实施方案中,所述抗tau抗体对于人tau具有约300pM至900pM的结合亲和力。在一些实施方案中,所述抗tau抗体对于人tau具有约400pM至1nM的结合亲和力。在一些实施方案中,所述抗tau抗体对于人tau具有约500pM至1.5nM的结合亲和力。在一些实施方案中,所述抗tau抗体对于人tau具有约500pM至2nM的结合亲和力。在一些实施方案中,所述抗tau抗体对于人tau具有约600pM至3nM的结合亲和力。在一些实施方案中,所述抗tau抗体对于人tau具有约100pM至约3nM的结合亲和力。

[0069] 在一些实施方案中,所述抗tau抗体对于磷酸化人tau具有约100pM至300pM的结合亲和力。在一些实施方案中,所述抗tau抗体对于磷酸化人tau具有约100pM至500pM的结合亲和力。在一些实施方案中,所述抗tau抗体对于磷酸化人tau具有约100pM至800pM的结合亲和力。在一些实施方案中,所述抗tau抗体对于磷酸化人tau具有约300pM至600pM的结合亲和力。在一些实施方案中,所述抗tau抗体对于磷酸化人tau具有约300pM至900pM的结合亲和力。在一些实施方案中,所述抗tau抗体对于磷酸化人tau具有约400pM至1nM的结合亲和力。在一些实施方案中,所述抗tau抗体对于磷酸化人tau具有约500pM至1.5nM的结合亲和力。在一些实施方案中,所述抗tau抗体对于磷酸化人tau具有约500pM至2nM的结合亲和力。在一些实施方案中,所述抗tau抗体对于磷酸化人tau具有约600pM至3nM的结合亲和力。

[0070] 本文描述了包含表3或表4中所示的任何序列的序列的抗体。

[0071] 表3.重链可变结构域

[0072]

名称	SEQ ID NO:	氨基酸序列
抗体1重链可变结构域	30	METGLRWLLLVAVLKGVQCQSLEESGGRLVTPGTPLTLT CTVSGFSLSSQKVGWVRQAPGKGLEWIGIINNYGSTYYAS WAKGRFTISKTSTTVDLRITSLTAEDTATYFCARDPDGSIV FDIWGPGTLVTVSL

抗体2和抗体3重链可变结构域	31	METGLRWLLLVAVLKGVQCQSVEESGGRLVTP GTPLTLT CTVSGFSLSSYAMIWVRQAPGKGLEWIGFISRSGI TYYASW AKGRFTISKTSTTVDLKMTSLTTEDTATYFCARE FGAVGS DYYRDAFNLWGPGTLVTVSS
抗体4重链可变结构域	32	METGLRWLLLVAVLKGVQCQSLEESGGRLVTPG TPLTLT CTVSGFSLNNYKVGWVRQAPGKGLEWIGIINYY SQTYYA SWAKGRFTISKTSTTVDLKLTSPTTEDTATYFCA RDPDGS IVFDIWGPGTLVTVSL
抗体5重链可变结构域	33	METGLRWLLLVAVLKGVQCQSVEESGGGLVTP GGTLTLT CTVSGFSLSNYAMSWVRQAPGKGLEWIGFISRS GITYYAS WAKGRFTISKTSTTVDLKITSPTTEDTAAYFCAR EFGAVGS DYYRDALRLWGPGTLVTVSS
抗体6重链可变结构域	34	METGLRWLLLVAVLKGVQCQSLEESGGRLVTPG TPLTLT CTVSGIDLSTHAMTWVRQAPGKGLEWIGVINPS GSAYYA TWVNGRFTISKTSTTVDLKITSPTTGDTAKYFCA RDYITA GDYYMDAFDPWGPGTLVTVSS

[0073]

[0074] 表4.轻链可变结构域

[0075]

名称	SEQ ID NO:	氨基酸序列
抗体1轻链可变结构域	35	MDTRAPTQLLGLLLLWLPGATFAQVLTQTASPV SAAVG GTVTINCQSSQSVVYNNRLSWFQQKPGQPPKLLI YGAST LASGVPSRFKGSGSGTQFTLTISDVQCDDAATYY CLGSY DCSSGDCHAFGGGTEVVVK
抗体2轻链可变结构域	36	MDMRAPTQLLGLLLLWLPGARCADIVMTQTPAS VEAA VGGTVTINCQASESINSWLSWYQQKPGQPPNLLI YRAST LASGVPSRFSGGGSGTEYTLTISDLECADAVTYY CQSYY EEDGIGYAFGGGTEVVVE
抗体3轻链可变结构域	37	MDMRAPTQLLGLLLLWLPGARCADIVMTQTPSS VSAA VGGTVTINCQASQNIYSNLAWYQQKPGQRPRLLI YGAS NLASGVPSRFKGSRSGTEFTLTISDLECADAATY YCQGY DYSTAGAYPFGGGTAVVVK
抗体4轻链可变结构	38	MDTRAPTQLLGLLLLWLPGATFAQVLTQTASPV SAAV GSTVTINCQSSQSVYSNKRLAWFQLKPGQPPKLL

[0076]

域		IYGAS TLASGVPSRFKGSGSGTQFTLTISDVQCDDAATY YCAGG YDCSTGDCWTFGGGTEVVVT
抗体5轻链可变结构域	39	MDMRAPTQLLGLLLLWLPGARCADIVMTQTPSS VSAA VGGTVTIKCQASQSIGSNLAWYQQKPGQPPKLLI YGAS TLESGVPSRFKGSGSGTEYTLTISDLECADAATY YCQSY YEGSDIGYAFGGGTEVVVE
抗体6轻链可变结构域	40	MDTRAPTQLLGLLLLWLPGARCADIVMTQTPAS VSAA VGGTVTIKCQASQSISNQLSWYQQKSGQPPKLLI YRAS TLASGVPSRFKGSGSGTEFTLTISDLECADAATY YCQQ GYNRDNVDNLFGGGTEVVVK

[0077] 在一些实施方案中,所述重链区可变结构域(VH)包含与根据SEQ ID NO:30-34中任一个的氨基酸序列具有至少70%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:30-34中任一个的氨基酸序列具有至少80%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:30-34中任一个的氨基酸序列具有至少85%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:30-34中任一个的氨基酸序列具有至少90%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:30-34中任一个的氨基酸序列具有至少91%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:30-34中任一个的氨基酸序列具有至少92%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:30-34中任一个的氨基酸序列具有至少93%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:30-34中任一个的氨基酸序列具有至少94%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:30-34中任一个的氨基酸序列具有至少95%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:30-34中任一个的氨基酸序列具有至少96%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:30-34中任一个的氨基酸序列具有至少97%序列同一性的氨

基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:30-34中任一个的氨基酸序列具有至少98％序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:30-34中任一个的氨基酸序列具有至少99％序列同一性的氨基酸序列。在一些实施方案中,所述VH包含根据SEQ ID NO:30-34中任一个的氨基酸序列。

[0078] 在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少50个连续氨基酸残基的氨基酸序列。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少60个连续氨基酸残基的氨基酸序列。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少70个连续氨基酸残基的氨基酸序列。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少80个连续氨基酸残基的氨基酸序列。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少90个连续氨基酸残基的氨基酸序列。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少100个连续氨基酸残基的氨基酸序列。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少105个连续氨基酸残基的氨基酸序列。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少110个连续氨基酸残基的氨基酸序列。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少115个连续氨基酸残基的氨基酸序列。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少120个连续氨基酸残基的氨基酸序列。

[0079] 在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少50个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:30-34中任一个的至少50个连续氨基酸残基具有至少80％序列同一性。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少60个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:30-34中任一个的至少60个连续氨基酸残基具有至少80％序列同一性。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少70个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:30-34中任一个的至少70个连续氨基酸残基具有至少80％序列同一性。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少80个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:30-34中任一个的至少80个连续氨基酸残基具有至少80％序列同一性。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少90个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:30-34中任一个的至少90个连续氨基酸残基具有至少80％序列同一性。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少100个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:30-34中任一个的至少100个连续氨基酸残基具有至少80％序列同一性。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少105个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:30-34中任一个的至少105个连续氨基酸残基具有至少80％序列同一性。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少110个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:30-34中任一个的至少110个连续氨基酸残基具有至少80％序列同一性。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少115个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:30-34中任一个的至少115个连续氨基酸残基具有至少80％序列同一性。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少120个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:30-34中任一个的至少120个连续氨基酸残基具有至少80％序列同一性。

[0080] 在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少50个连续氨基

酸残基的氨基酸序列,并且与SEQ ID NO:30-34中任一个的至少50个连续氨基酸残基具有至少90%序列同一性。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少60个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:30-34中任一个的至少60个连续氨基酸残基具有至少90%序列同一性。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少70个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:30-34中任一个的至少70个连续氨基酸残基具有至少90%序列同一性。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少80个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:30-34中任一个的至少80个连续氨基酸残基具有至少90%序列同一性。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少90个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:30-34中任一个的至少90个连续氨基酸残基具有至少90%序列同一性。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少100个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:30-34中任一个的至少100个连续氨基酸残基具有至少90%序列同一性。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少105个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:30-34中任一个的至少105个连续氨基酸残基具有至少90%序列同一性。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少110个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:30-34中任一个的至少110个连续氨基酸残基具有至少90%序列同一性。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少115个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:30-34中任一个的至少115个连续氨基酸残基具有至少90%序列同一性。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少120个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:30-34中任一个的至少120个连续氨基酸残基具有至少90%序列同一性。

[0081] 在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少50个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:30-34中任一个的至少50个连续氨基酸残基具有至少95%序列同一性。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少60个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:30-34中任一个的至少60个连续氨基酸残基具有至少95%序列同一性。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少70个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:30-34中任一个的至少70个连续氨基酸残基具有至少95%序列同一性。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少80个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:30-34中任一个的至少80个连续氨基酸残基具有至少95%序列同一性。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少90个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:30-34中任一个的至少90个连续氨基酸残基具有至少95%序列同一性。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少100个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:30-34中任一个的至少100个连续氨基酸残基具有至少95%序列同一性。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少105个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:30-34中任一个的至少105个连续氨基酸残基具有至少95%序列同一性。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少110个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:30-34中任一个的至少110个连续氨基酸残基具有至少95%序列同一性。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少115

个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:30-34中任一个的至少115个连续氨基酸残基具有至少95%序列同一性。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少120个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:30-34中任一个的至少120个连续氨基酸残基具有至少95%序列同一性。

[0082] 在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少100个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:30-34中任一个的至少100个连续氨基酸残基具有至少99%序列同一性。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少105个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:30-34中任一个的至少105个连续氨基酸残基具有至少99%序列同一性。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少110个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:30-34中任一个的至少110个连续氨基酸残基具有至少99%序列同一性。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少115个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:30-34中任一个的至少115个连续氨基酸残基具有至少99%序列同一性。在一些实施方案中,所述VH包含SEQ ID NO:30-34中任一个的至少120个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:30-34中任一个的至少120个连续氨基酸残基具有至少99%序列同一性。

[0083] 在一些实施方案中,所述轻链区可变结构域(VL)包含与根据SEQ ID NO:35-40中任一个的氨基酸序列具有至少70%序列同一性的氨基酸序列。在一些实施方案中,所述VL包含与根据SEQ ID NO:35-40中任一个的氨基酸序列具有至少80%序列同一性的氨基酸序列。在一些实施方案中,所述VL包含与根据SEQ ID NO:35-40中任一个的氨基酸序列具有至少85%序列同一性的氨基酸序列。在一些实施方案中,所述VL包含与根据SEQ ID NO:35-40中任一个的氨基酸序列具有至少90%序列同一性的氨基酸序列。在一些实施方案中,所述VL包含与根据SEQ ID NO:35-40中任一个的氨基酸序列具有至少91%序列同一性的氨基酸序列。在一些实施方案中,所述VL包含与根据SEQ ID NO:35-40中任一个的氨基酸序列具有至少92%序列同一性的氨基酸序列。在一些实施方案中,所述VL包含与根据SEQ ID NO:35-40中任一个的氨基酸序列具有至少93%序列同一性的氨基酸序列。在一些实施方案中,所述VL包含与根据SEQ ID NO:35-40中任一个的氨基酸序列具有至少94%序列同一性的氨基酸序列。在一些实施方案中,所述VL包含与根据SEQ ID NO:35-40中任一个的氨基酸序列具有至少95%序列同一性的氨基酸序列。在一些实施方案中,所述VL包含与根据SEQ ID NO:35-40中任一个的氨基酸序列具有至少96%序列同一性的氨基酸序列。在一些实施方案中,所述VL包含与根据SEQ ID NO:35-40中任一个的氨基酸序列具有至少97%序列同一性的氨基酸序列。在一些实施方案中,所述VL包含与根据SEQ ID NO:35-40中任一个的氨基酸序列具有至少98%序列同一性的氨基酸序列。在一些实施方案中,所述VL包含与根据SEQ ID NO:35-40中任一个的氨基酸序列具有至少99%序列同一性的氨基酸序列。在一些实施方案中,所述VL包含根据SEQ ID NO:35-40中任一个的氨基酸序列。

[0084] 在一些实施方案中,所述VL包含SEQ ID NO:35-40中任一个的至少50个连续氨基酸残基的氨基酸序列。在一些实施方案中,所述VL包含SEQ ID NO:35-40中任一个的至少60个连续氨基酸残基的氨基酸序列。在一些实施方案中,所述VL包含SEQ ID NO:35-40中任一个的至少70个连续氨基酸残基的氨基酸序列。在一些实施方案中,所述VL包含SEQ ID NO:35-40中任一个的至少80个连续氨基酸残基的氨基酸序列。在一些实施方案中,所述VL包含

SEQ ID NO:35-40中任一个的至少90个连续氨基酸残基的氨基酸序列。在一些实施方案中,所述VL包含SEQ ID NO:35-40中任一个的至少100个连续氨基酸残基的氨基酸序列。在一些实施方案中,所述VL包含SEQ ID NO:35-40中任一个的至少105个连续氨基酸残基的氨基酸序列。

[0085] 在一些实施方案中,所述VL包含SEQ ID NO:35-40中任一个的至少50个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:35-40中任一个的至少50个连续氨基酸残基具有至少80%序列同一性。在一些实施方案中,所述VL包含SEQ ID NO:35-40中任一个的至少60个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:35-40中任一个的至少60个连续氨基酸残基具有至少80%序列同一性。在一些实施方案中,所述VL包含SEQ ID NO:35-40中任一个的至少70个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:35-40中任一个的至少70个连续氨基酸残基具有至少80%序列同一性。在一些实施方案中,所述VL包含SEQ ID NO:35-40中任一个的至少80个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:35-40中任一个的至少80个连续氨基酸残基具有至少80%序列同一性。在一些实施方案中,所述VL包含SEQ ID NO:35-40中任一个的至少90个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:35-40中任一个的至少90个连续氨基酸残基具有至少80%序列同一性。在一些实施方案中,所述VL包含SEQ ID NO:35-40中任一个的至少100个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:35-40中任一个的至少100个连续氨基酸残基具有至少80%序列同一性。在一些实施方案中,所述VL包含SEQ ID NO:35-40中任一个的至少105个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:35-40中任一个的至少105个连续氨基酸残基具有至少80%序列同一性。

[0086] 在一些实施方案中,所述VL包含SEQ ID NO:35-40中任一个的至少50个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:35-40中任一个的至少50个连续氨基酸残基具有至少90%序列同一性。在一些实施方案中,所述VL包含SEQ ID NO:35-40中任一个的至少60个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:35-40中任一个的至少60个连续氨基酸残基具有至少90%序列同一性。在一些实施方案中,所述VL包含SEQ ID NO:35-40中任一个的至少70个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:35-40中任一个的至少70个连续氨基酸残基具有至少90%序列同一性。在一些实施方案中,所述VL包含SEQ ID NO:35-40中任一个的至少80个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:35-40中任一个的至少80个连续氨基酸残基具有至少90%序列同一性。在一些实施方案中,所述VL包含SEQ ID NO:35-40中任一个的至少90个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:35-40中任一个的至少90个连续氨基酸残基具有至少90%序列同一性。在一些实施方案中,所述VL包含SEQ ID NO:35-40中任一个的至少100个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:35-40中任一个的至少100个连续氨基酸残基具有至少90%序列同一性。在一些实施方案中,所述VL包含SEQ ID NO:35-40中任一个的至少105个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:35-40中任一个的至少105个连续氨基酸残基具有至少90%序列同一性。

[0087] 在一些实施方案中,所述VL包含SEQ ID NO:35-40中任一个的至少50个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:35-40中任一个的至少50个连续氨基酸残基具有至少95%序列同一性。在一些实施方案中,所述VL包含SEQ ID NO:35-40中任一个的至少60

个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:35-40中任一个的至少60个连续氨基酸残基具有至少95%序列同一性。在一些实施方案中,所述VL包含SEQ ID NO:35-40中任一个的至少70个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:35-40中任一个的至少70个连续氨基酸残基具有至少95%序列同一性。在一些实施方案中,所述VL包含SEQ ID NO:35-40中任一个的至少80个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:35-40中任一个的至少80个连续氨基酸残基具有至少95%序列同一性。在一些实施方案中,所述VL包含SEQ ID NO:35-40中任一个的至少90个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:35-40中任一个的至少90个连续氨基酸残基具有至少95%序列同一性。在一些实施方案中,所述VL包含SEQ ID NO:35-40中任一个的至少100个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:35-40中任一个的至少100个连续氨基酸残基具有至少95%序列同一性。在一些实施方案中,所述VL包含SEQ ID NO:35-40中任一个的至少105个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:35-40中任一个的至少105个连续氨基酸残基具有至少95%序列同一性。在一些实施方案中,所述VL包含SEQ ID NO:35-40中任一个的至少100个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:35-40中任一个的至少100个连续氨基酸残基具有至少99%序列同一性。在一些实施方案中,所述VL包含SEQ ID NO:35-40中任一个的至少105个连续氨基酸残基的氨基酸序列,并且与SEQ ID NO:35-40中任一个的至少105个连续氨基酸残基具有至少99%序列同一性。

[0088] 在一些实施方案中,所述VH包含与根据SEQ ID NO:30-34中任一个的氨基酸序列具有至少70%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:35-40中任一个的氨基酸序列具有至少70%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:30-34中任一个的氨基酸序列具有至少80%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:35-40中任一个的氨基酸序列具有至少80%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:30-34中任一个的氨基酸序列具有至少85%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:35-40中任一个的氨基酸序列具有至少85%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:30-34中任一个的氨基酸序列具有至少90%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:35-40中任一个的氨基酸序列具有至少90%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:30-34中任一个的氨基酸序列具有至少91%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:35-40中任一个的氨基酸序列具有至少91%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:30-34中任一个的氨基酸序列具有至少92%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:35-40中任一个的氨基酸序列具有至少92%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:30-34中任一个的氨基酸序列具有至少93%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:35-40中任一个的氨基酸序列具有至少93%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:30-34中任一个的氨基酸序列具有至少94%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:35-40中任一个的氨基酸序列具有至少94%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:30-34中任一个的氨基酸序列具有至少95%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID

NO:35-40中任一个的氨基酸序列具有至少95％序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:30-34中任一个的氨基酸序列具有至少96％序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:35-40中任一个的氨基酸序列具有至少96％序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:30-34中任一个的氨基酸序列具有至少97％序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:35-40中任一个的氨基酸序列具有至少97％序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:30-34中任一个的氨基酸序列具有至少98％序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:35-40中任一个的氨基酸序列具有至少98％序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:30-34中任一个的氨基酸序列具有至少99％序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:35-40中任一个的氨基酸序列具有至少99％序列同一性的氨基酸序列。

[0089] 在一些实施方案中,所述VH包含与根据SEQ ID NO:30的氨基酸序列具有至少70％序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:35的氨基酸序列具有至少70％序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:30的氨基酸序列具有至少80％序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:35的氨基酸序列具有至少80％序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:30的氨基酸序列具有至少85％序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:35的氨基酸序列具有至少85％序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:30的氨基酸序列具有至少90％序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:35的氨基酸序列具有至少90％序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:30的氨基酸序列具有至少91％序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:35的氨基酸序列具有至少91％序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:30的氨基酸序列具有至少92％序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:35的氨基酸序列具有至少92％序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:30的氨基酸序列具有至少93％序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:35的氨基酸序列具有至少93％序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:30的氨基酸序列具有至少94％序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:35的氨基酸序列具有至少94％序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:30的氨基酸序列具有至少95％序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:35的氨基酸序列具有至少95％序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:30的氨基酸序列具有至少96％序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:35的氨基酸序列具有至少96％序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:30的氨基酸序列具有至少97％序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:35的氨基酸序列具有至少97％序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:30的氨基酸序列具有至少98％序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:35的氨基酸序列具有至少98％序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:30的氨基酸序列具有至少99％序列同一性的

氨基酸序列;并且所述VL包含与根据SEQ ID NO:35的氨基酸序列具有至少99%序列同一性的氨基酸序列。

[0090] 在一些实施方案中,所述VH包含与根据SEQ ID NO:31的氨基酸序列具有至少70%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:36的氨基酸序列具有至少70%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:31的氨基酸序列具有至少80%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:36的氨基酸序列具有至少80%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:31的氨基酸序列具有至少85%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:36的氨基酸序列具有至少85%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:31的氨基酸序列具有至少90%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:36的氨基酸序列具有至少90%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:31的氨基酸序列具有至少91%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:36的氨基酸序列具有至少91%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:31的氨基酸序列具有至少92%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:36的氨基酸序列具有至少92%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:31的氨基酸序列具有至少93%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:36的氨基酸序列具有至少93%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:31的氨基酸序列具有至少94%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:36的氨基酸序列具有至少94%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:31的氨基酸序列具有至少95%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:36的氨基酸序列具有至少95%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:31的氨基酸序列具有至少96%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:36的氨基酸序列具有至少96%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:31的氨基酸序列具有至少97%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:36的氨基酸序列具有至少97%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:31的氨基酸序列具有至少98%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:36的氨基酸序列具有至少98%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:31的氨基酸序列具有至少99%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:36的氨基酸序列具有至少99%序列同一性的氨基酸序列。

[0091] 在一些实施方案中,所述VH包含与根据SEQ ID NO:31的氨基酸序列具有至少70%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:37的氨基酸序列具有至少70%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:31的氨基酸序列具有至少80%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:37的氨基酸序列具有至少80%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:31的氨基酸序列具有至少85%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:37的氨基酸序列具有至少85%序列同一性的氨基酸序列。在一些实施方案

中,所述VH包含与根据SEQ ID NO:31的氨基酸序列具有至少90%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:37的氨基酸序列具有至少90%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:31的氨基酸序列具有至少91%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:37的氨基酸序列具有至少91%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:31的氨基酸序列具有至少92%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:37的氨基酸序列具有至少92%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:31的氨基酸序列具有至少93%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:37的氨基酸序列具有至少93%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:31的氨基酸序列具有至少94%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:37的氨基酸序列具有至少94%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:31的氨基酸序列具有至少95%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:37的氨基酸序列具有至少95%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:31的氨基酸序列具有至少96%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:37的氨基酸序列具有至少96%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:31的氨基酸序列具有至少97%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:37的氨基酸序列具有至少97%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:31的氨基酸序列具有至少98%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:37的氨基酸序列具有至少98%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:31的氨基酸序列具有至少99%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:37的氨基酸序列具有至少99%序列同一性的氨基酸序列。

[0092] 在一些实施方案中,所述VH包含与根据SEQ ID NO:32的氨基酸序列具有至少70%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:38的氨基酸序列具有至少70%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:32的氨基酸序列具有至少80%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:38的氨基酸序列具有至少80%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:32的氨基酸序列具有至少85%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:38的氨基酸序列具有至少85%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:32的氨基酸序列具有至少90%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:38的氨基酸序列具有至少90%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:32的氨基酸序列具有至少91%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:38的氨基酸序列具有至少91%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:32的氨基酸序列具有至少92%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:38的氨基酸序列具有至少92%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:32的氨基酸序列具有至少93%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:38的氨基酸序列具有至少93%序列同一性的氨基酸序列。在一些实施方案中,

所述VH包含与根据SEQ ID NO:32的氨基酸序列具有至少94％序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:38的氨基酸序列具有至少94％序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:32的氨基酸序列具有至少95％序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:38的氨基酸序列具有至少95％序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:32的氨基酸序列具有至少96％序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:38的氨基酸序列具有至少96％序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:32的氨基酸序列具有至少97％序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:38的氨基酸序列具有至少97％序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:32的氨基酸序列具有至少98％序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:38的氨基酸序列具有至少98％序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:32的氨基酸序列具有至少99％序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:38的氨基酸序列具有至少99％序列同一性的氨基酸序列。

[0093] 在一些实施方案中,所述VH包含与根据SEQ ID NO:33的氨基酸序列具有至少70％序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:39的氨基酸序列具有至少70％序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:33的氨基酸序列具有至少80％序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:39的氨基酸序列具有至少80％序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:33的氨基酸序列具有至少85％序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:39的氨基酸序列具有至少85％序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:33的氨基酸序列具有至少90％序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:39的氨基酸序列具有至少90％序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:33的氨基酸序列具有至少91％序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:39的氨基酸序列具有至少91％序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:33的氨基酸序列具有至少92％序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:39的氨基酸序列具有至少92％序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:33的氨基酸序列具有至少93％序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:39的氨基酸序列具有至少93％序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:33的氨基酸序列具有至少94％序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:39的氨基酸序列具有至少94％序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:33的氨基酸序列具有至少95％序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:39的氨基酸序列具有至少95％序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:33的氨基酸序列具有至少96％序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:39的氨基酸序列具有至少96％序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:33的氨基酸序列具有至少97％序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:39的氨基酸序列具有至少97％序列同一性的氨基酸序列。在一些实施方案中,所述

VH包含与根据SEQ ID NO:33的氨基酸序列具有至少98%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:39的氨基酸序列具有至少98%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:33的氨基酸序列具有至少99%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:39的氨基酸序列具有至少99%序列同一性的氨基酸序列。

[0094] 在一些实施方案中,所述VH包含与根据SEQ ID NO:34的氨基酸序列具有至少70%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:40的氨基酸序列具有至少70%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:34的氨基酸序列具有至少80%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:40的氨基酸序列具有至少80%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:34的氨基酸序列具有至少85%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:40的氨基酸序列具有至少85%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:34的氨基酸序列具有至少90%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:40的氨基酸序列具有至少90%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:34的氨基酸序列具有至少91%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:40的氨基酸序列具有至少91%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:34的氨基酸序列具有至少92%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:40的氨基酸序列具有至少92%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:34的氨基酸序列具有至少93%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:40的氨基酸序列具有至少93%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:34的氨基酸序列具有至少94%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:40的氨基酸序列具有至少94%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:34的氨基酸序列具有至少95%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:40的氨基酸序列具有至少95%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:34的氨基酸序列具有至少96%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:40的氨基酸序列具有至少96%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:34的氨基酸序列具有至少97%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:40的氨基酸序列具有至少97%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:34的氨基酸序列具有至少98%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:40的氨基酸序列具有至少98%序列同一性的氨基酸序列。在一些实施方案中,所述VH包含与根据SEQ ID NO:34的氨基酸序列具有至少99%序列同一性的氨基酸序列;并且所述VL包含与根据SEQ ID NO:40的氨基酸序列具有至少99%序列同一性的氨基酸序列。

[0095] 在一些实施方案中,本文描述了抗体或抗体片段,其包含与如SEQ ID NO:41、43、46、48和50中任一个中所示的序列具有至少约90%同一性的重链序列。在一些情况下,抗体或抗体片段包含与SEQ ID NO:41、43、46、48和50中任一个具有至少或约70%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%序列同一性的重链序列。

[0096] 在一些实施方案中,本文描述了抗体或抗体片段,其包含与如SEQ ID NO:42、44、45、47、49和51中任一个中所示的序列具有至少约90%同一性的轻链序列。在一些情况下,抗体或抗体片段包含与SEQ ID NO:42、44、45、47、49和51中任一个具有至少或约70%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%序列同一性的轻链序列。

[0097] 在一些实施方案中,本文描述了抗体或抗体片段,其包含与如SEQ ID NO:41、43、46、48和50中任一个中所示的序列具有至少约90%同一性的重链序列,和与如SEQ ID NO:42、44、45、47、49和51中任一个中所示的序列具有至少约90%同一性的轻链序列。在一些情况下,所述抗体或抗体片段包含与SEQ ID NO:41、43、46、48和50中任一个具有至少或约70%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%序列同一性的重链序列,和与SEQ ID NO:42、44、45、47、49和51中任一个具有至少或约70%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%序列同一性的轻链序列。

[0098] 表5.重链和轻链序列

[0099]

名称	SEQ ID NO:	氨基酸序列
抗体1重链	41	METGLRWLLLVAVLKGVQCQSLEESGGRLVTPGTPLTLTCTVSG FSLSSQKVGWVRQAPGKGLEWIGIINNYGSTYYASWAKGRFTIS KTSTTVDLRITSLTAEDTATYFCARDPDGSIVFDIWGPGTLVTVSL GQPKAPSVFPLAPCCGDTPSSTVTLGCLVKGYLPEPVTVTWNSG TLTNGVRTFPSVRQSSGLYSLSSVVSVTSSSQPVTCNVAHPATNT KVDKTVAPSTCSKPTCPPPELLGRSSVFIFPPKPKDTLMISRTPEV TCVVVDVSQDDPEVQFTWYINNEQVRTARPPLREQQFNSTIRVV STLPIAHQDWLRGKEFKCKVHNKALPAPIEKTISKARGQPLEPKV YTMGPPREELSSRSVSLTCMINGFYPSDISVEWEKNGKAEDNYK TTPAVLDSDGSYFLYSKLSVPTSEWQRGDVFTCSVMHEALHNH YTQKSISRSPGK
抗体1轻链	42	MDTRAPTQLLGLLLLWLPGATFAQVLTQTASPVSAAVGGTVTI NCQSSQSVVYNNRLSWFQQKPGQPPKLLIYGASTLASGVPSRF

[0100]

		KGSGSGTQFTLTISDVQCDDAATYYCLGSYDCSSGD CHAFGGG TEVVVKGDPVAPTVLIFPPAADQVATGTVTIVCVAN KYFPDVT VTWEVDGTTQTTGIENSKTPQNSADCTYNLSSTLTL TSTQYNSH KEYTCKVTQGTTSVVQSFNRGDC
抗体2和抗体3重链	43	METGLRWLLLVAVLKGVQCQSVEESGGRLVTPGTP LTLTCTVSG FSLSSYAMIWVRQAPGKGLEWIGFISRSGITYYASW AKGRFTISK TSTTVDLKMTSLTTEDTATYFCAREFGAVGSDYYR DAFNLWGP GTLVTVSSGQPKAPSVFPLAPCCGDTPSSTVTLGCL VKGYLPEPV TVTWNSGTLTNGVRTFPSVRQSSGLYSLSSVVSVTS SSQPVTCNV AHPATNTKVDKTVAPSTCSKPTCPPPELLGRSSVFIF PPKPKDTL MISRTPEVTCVVVDVSQDDPEVQFTWYINNEQVRT ARPPLREQQ FNSTIRVVSTLPIAHQDWLRGKEFKCKVHNKALPAP IEKTISKAR GQPLEPKVYTMGPPREELSSRSVSLTCMINGFYPSDI SVEWEKNG KAEDNYKTTPAVLDSDGSYFLYSKLSVPTSEWQRG DVFTCSVM HEALHNHYTQKSISRSPGK

[0101]

抗体2轻链	44	MDMRAPTQLLGLLLLWLPGARCADIVMTQTPASVE AAVGGTV TINCQASESINSWLSWYQQKPGQPPNLLIYRASTLAS GVPSRFSG GGSGTEYTLTISDLECADAVTYYCQSYYEEDGIGYA FGGGTEVV VEGDPVAPTVLIFPPAADQVATGTVTIVCVANKYFP DVTVTWE VDGTTQTTGIENSKTPQNSADCTYNLSSTLTLTSTQ YNSHKEYT CKVTQGTTSVVQSFNRGDC
抗体3轻链	45	MDMRAPTQLLGLLLLWLPGARCADIVMTQTPSSVS AAVGGTVT INCQASQNIYSNLAWYQQKPGQRPRLLIYGASNLAS GVPSRFKG SRSGTEFTLTISDLECADAATYYCQGYDYSTAGAYP FGGGTAVV VKGDPVAPTVLIFPPAADQVATGTVTIVCVANKYFP DVTVTWEV DGTTQTTGIENSKTPQNSADCTYNLSSTLTLTSTQY NSHKEYTCK VTQGTTSVVQSFNRGDC
抗体4重链	46	METGLRWLLLVAVLKGVQCQSLEESGGRLVTPGTP LTLTCTVS GFSLNNYKVGWVRQAPGKGLEWIGIINYYSQTYYA SWAKGRF TISKTSTTVDLKLTSPTTEDTATYFCARDPDGSIVFDI WGPGTLV TVSLGQPKAPSVFPLAPCCGDTPSSTVTLGCLVKGY

[0102]

		LPEPVTVT WNSGTLTNGVRTFPSVRQSSGLYSLSSVVSVTSSSQ PVTCNVAH PATNTKVDKTVVPSTCSKPTCPPPELLGRSSVFIFPP KPKDTLMI SRTPEVTCVVVDVSQDDPEVQFTWYINNEQVRTAR PPLREQQF NSTIRVVSTLPIAHQDWLRGKEFKCKVHNKALPAPI EKTISKAR GQPLEPKVYTMGPPREELSSRSVSLTCMINGFYPSDI SVEWEKN GKAEDNYKTTPAVLDSDGSYFLYSKLSVPTSEWQR GDVFTCSV MHEALHNHYTQKSISRSPGK
抗体4轻链	47	MDTRAPTQLLGLLLLWLPGATFAQVLTQTASPVSA AVGSTVTIN CQSSQSVYSNKRLAWFQLKPGQPPKLLIYGASTLAS GVPSRFKG SGSGTQFTLTISDVQCDDAATYYCAGGYDCSTGDC WTFGGGTE VVVTGDPVAPTVLIFPPAADQVATGTVTIVCVANK YFPDVTVT WEVDGTTQTTGIENSKTPQNSADCTYNLSSTLTLTS TQYNSHKE YTCKVTQGTTSVVQSFNRGDC

[0103]

抗体5重链	48	METGLRWLLLVAVLKGVQCQSVEESGGGLVTPGGTLTLTCTVS GFSLSNYAMSWVRQAPGKGLEWIGFISRSGITYYASWAKGRFT ISKTSTTVDLKITSPTTEDTAAYFCAREFGAVGSDYYRDALRLW GPGTLVTVSSGQPKAPSVFPLAPCCGDTPSSTVTLGCLVKGYLP EPVTVTWNSGTLTNGVRTFPSVRQSSGLYSLSSVVSVTSSSQPVT CNVAHPATNTKVDKTVAPSTCSKPTCPPPELLGRSSVFIFPPKPK DTLMISRTPEVTCVVVDVSQDDPEVQFTWYINNEQVRTARPPL REQQFNSTIRVVSTLPIAHQDWLRGKEFKCKVHNKALPAPIEKT ISKARGQPLEPKVYTMGPPREELSSRSVSLTCMINGFYPSDISVE WEKNGKAEDNYKTTPAVLDSDGSYFLYSKLSVPTSEWQRGDV FTCSVMHEALHNHYTQKSISRSPGK
抗体5轻链	49	MDMRAPTQLLGLLLLWLPGARCADIVMTQTPSSVSAAVGGTVT IKCQASQSIGSNLAWYQQKPGQPPKLLIYGASTLESGVPSRFKGS GSGTEYTLTISDLECADAATYYCQSYYEGSDIGYAFGGGTEVVV EGDPVAPTVLIFPPAADQVATGTVTIVCVANKYFPDVTVTWEVD

[0104]

		GTTQTTGIENSKTPQNSADCTYNLSSTLTLTSTQYNS HKEYTCKV TQGTTSVVQSFNRGDC
抗体6 重链	50	METGLRWLLLVAVLKGVQCQSLEESGGRLVTPGTP LTLTCTVS GIDLSTHAMTWVRQAPGKGLEWIGVINPSGSAYYA TWVNGRF TISKTSTTVDLKITSPTTGDTAKYFCARDYITAGDYY MDAFDPW GPGTLVTVSSGQPKAPSVFPLAPCCGDTPSSTVTLG CLVKGYLP EPVTVTWNSGTLTNGVRTFPSVRQSSGLYSLSSVVS VTSSSQPV TCNVAHPATNTKVDKTVAPSTCSKPTCPPPELLGRS SVFIFPPKP KDTLMISRTPEVTCVVVDVSQDDPEVQFTWYINNE QVRTARPP LREQQFNSTIRVVSTLPIAHQDWLRGKEFKCKVHNK ALPAPIEK TISKARGQPLEPKVYTMGPPREELSSRSVSLTCMING FYPSDISV EWEKNGKAEDNYKTTPAVLDSDGSYFLYSKLSVPT SEWQRGD VFTCSVMHEALHNHYTQKSISRSPGK
抗体6 轻链	51	MDTRAPTQLLGLLLLWLPGARCADIVMTQTPASVS AAVGGTV TIKCQASQSISNQLSWYQQKSGQPPKLLIYRASTLAS GVPSRFK GSGSGTEFTLTISDLECADAATYYCQQGYNRDNVD

[0105]

		NLFGGGT EVVVKGDPVAPTVLIFPPAADQVATGTVTIVCVAN KYFPDVTV TWEVDGTTQTTGIENSKTPQNSADCTYNLSSTLTLT STQYNSH KEYTCKVTQGTTSVVQSFNRGDC

[0106] 在一些实施方案中,所述抗tau抗体包含由核酸编码的VH结构域,所述核酸与选自SEQ ID NO:52-56的序列具有至少80%、至少85%、至少90%、至少95%序列同一性。在一些实施方案中,所述抗tau抗体包含由核酸编码的VL结构域,所述核酸与选自SEQ ID NO:57-62的序列具有至少80%、至少85%、至少90%、至少95%序列同一性。在此所述的抗tau-tau抗体的VH结构域的核酸序列在表6中列出,并且在此所述的抗tau-tau抗体的VL结构域的核酸序列在表7中列出。在一些实施方案中,所述抗tau-tau抗体包含由核酸编码的VH结构域,所述核酸与SEQ ID NO:52具有至少90%序列同一性。在一些实施方案中,所述抗tau-tau抗体包含由核酸编码的VH结构域,所述核酸与SEQ ID NO:53具有至少90%序列同一性。在一些实施方案中,所述抗tau-tau抗体包含由核酸编码的VH结构域,所述核酸与SEQ ID NO:54具有至少90%序列同一性。在一些实施方案中,所述抗tau-tau抗体包含由核酸编码的VH结构域,所述核酸与SEQ ID NO:55具有至少90%序列同一性。在一些实施方案中,所述抗tau-tau抗体包含由核酸编码的VH结构域,所述核酸与SEQ ID NO:56具有至少90%序列同一性。在一些实施方案中,所述抗tau-tau抗体包含由核酸编码的VL结构域,所述核酸与SEQ ID NO:57具有至少90%序列同一性。在一些实施方案中,所述抗tau-tau抗体包含由核酸编码的VL结构域,所述核酸与SEQ ID NO:58具有至少90%序列同一性。在一些实施方案中,所述抗tau抗体包含由核酸编码的VL结构域,所述核酸与SEQ ID NO:59具有至少90%序列同一性。在一些实施方案中,所述抗tau抗体包含由核酸编码的VL结构域,所述核酸与SEQ ID NO:60具有至少90%序列同一性。在一些实施方案中,所述抗tau抗体包含由核酸编码的VL结构域,所述核酸与SEQ ID NO:61具有至少90%序列同一性。在一些实施方案中,所述抗tau抗体包含由核酸编码的VL结构域,所述核酸与SEQ ID NO:62具有至少90%序列同一性。在一些实施方案中,所述抗tau抗体包含由核酸编码的VH结构域,所述核酸与SEQ ID NO:52具有至少90%序列同一性;和由核酸编码的VL结构域,所述核酸与SEQ ID NO:57具有至少90%序列同一性。在一些实施方案中,所述抗tau抗体包含由核酸编码的VH结构域,所述核酸与SEQ ID NO:53具有至少90%序列同一性;和由核酸编码的VL结构域,所述核酸与SEQ ID NO:58具有至少90%序列同一性。在一些实施方案中,所述抗tau抗体包含由核酸编码的VH结构域,所述核酸与SEQ ID NO:53具有至少90%序列同一性;和由核酸编码的VL结构域,所述核酸与SEQ ID NO:59具有至少90%序列同一性。在一些实施方案中,所述抗tau抗体包含由核酸编码的VH结构域,所述核酸与SEQ ID NO:54具有至少90%序列同一性;和由核酸编码的VL结构域,所述核酸与SEQ ID NO:60具有至少90%序列同一性。在一些实施方案中,所述抗tau抗体包含由核酸编码的VH结构域,所述核酸与SEQ ID NO:55具有至少90%序列同一性;和由核酸编码的VL结构域,所述核酸与SEQ ID NO:61具有至少90%序列同一性。在

一些实施方案中,所述抗tau抗体包含由核酸编码的VH结构域,所述核酸与SEQ ID NO:563具有至少90％序列同一性;和由核酸编码的VL结构域,所述核酸与SEQ ID NO:62具有至少90％序列同一性。在一些实施方案中,所述抗tau抗体包含由核酸编码的VH结构域,所述核酸包含与SEQ ID NO:52相同的序列。在一些实施方案中,所述抗tau抗体包含由核酸编码的VH结构域,所述核酸包含与SEQ ID NO:53相同的序列。在一些实施方案中,所述抗tau抗体包含由核酸编码的VH结构域,所述核酸包含与SEQ ID NO:54相同的序列。在一些实施方案中,所述抗tau抗体包含由核酸编码的VH结构域,所述核酸包含与SEQ ID NO:55相同的序列。在一些实施方案中,所述抗tau抗体包含由核酸编码的VH结构域,所述核酸包含与SEQ ID NO:56相同的序列。在一些实施方案中,所述抗tau抗体包含由核酸编码的VL结构域,所述核酸包含与SEQ ID NO:57相同的序列。在一些实施方案中,所述抗tau抗体包含由核酸编码的VL结构域,所述核酸包含与SEQ ID NO:58相同的序列。在一些实施方案中,所述抗tau抗体包含由核酸编码的VL结构域,所述核酸包含与SEQ ID NO:59相同的序列。在一些实施方案中,所述抗tau抗体包含由核酸编码的VL结构域,所述核酸包含与SEQ ID NO:60相同的序列。在一些实施方案中,所述抗tau抗体包含由核酸编码的VL结构域,所述核酸包含与SEQ ID NO:61相同的序列。在一些实施方案中,所述抗tau抗体包含由核酸编码的VL结构域,所述核酸包含与SEQ ID NO:62相同的序列。在一些实施方案中,所述抗tau抗体包含由核酸编码的VH结构域,所述核酸包含与SEQ ID NO:52相同的序列;和由核酸编码的VL结构域,所述核酸包含与SEQ ID NO:57相同的序列。在一些实施方案中,所述抗tau抗体包含由核酸编码的VH结构域,所述核酸包含与SEQ ID NO:53相同的序列;和由核酸编码的VL结构域,所述核酸包含与SEQ ID NO:58相同的序列。在一些实施方案中,所述抗tau抗体包含由核酸编码的VH结构域,所述核酸包含与SEQ ID NO:53相同的序列;和由核酸编码的VL结构域,所述核酸包含与SEQ ID NO:59相同的序列。在一些实施方案中,所述抗tau抗体包含由核酸编码的VH结构域,所述核酸包含与SEQ ID NO:54相同的序列;和由核酸编码的VL结构域,所述核酸包含与SEQ ID NO:60相同的序列。在一些实施方案中,所述抗tau抗体包含由核酸编码的VH结构域,所述核酸包含与SEQ ID NO:55相同的序列;和由核酸编码的VL结构域,所述核酸包含与SEQ ID NO:61相同的序列。在一些实施方案中,所述抗tau抗体包含由核酸编码的VH结构域,所述核酸包含与SEQ ID NO:56相同的序列;和由核酸编码的VL结构域,所述核酸包含与SEQ ID NO:62相同的序列。

[0107] 表6.编码VH结构域的核酸序列

[0108]

SEQ ID NO:	编码 VH 结构域的核酸序列
52	ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGG TGTCCAGTGTCAGTCGCTGGAGGAGTCCGGGGGTCGCCTGGTCACG CCTGGGACACCCCTGACACTCACCTGCACAGTCTCTGGATTTTCCCT CAGTAGCCAGAAAGTGGGCTGGGTCCGCCAGGCTCCAGGGAAGGGG CTGGAATGGATCGGAATCATTAATAATTATGGTAGCACATACTACGC GAGCTGGGCGAAAGGCCGATTCACCATCTCGAAAACCTCGACCACA GTGGATCTGAGAATCACCAGTCTGACGGCCGAGGACACGGCCACCT ATTTCTGTGCCCGTGATCCTGATGGTAGTATTGTCTTTGACATCTGGG GCCCAGGCACCCTTGTCACCGTCTCCTTG
53	ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGG TGTCCAGTGTCAGTCGGTGGAGGAGTCCGGGGGTCGCCTGGTCACGC CTGGGACACCCCTGACACTCACCTGCACCGTCTCTGGATTCTCCCTC

[0109]

	AGTAGCTATGCAATGATCTGGGTCCGCCAGGCTCCAGGGAAGGGGC TGGAATGGATCGGATTCATTAGTCGTAGTGGTATCACATACTACGCG AGCTGGGCAAAAGGCCGATTCACCATCTCCAAAACCTCGACCACGG TGGATCTGAAAATGACCAGTCTGACAACCGAGGACACGGCCACCTA TTTCTGTGCCAGAGAATTCGGTGCTGTTGGTAGTGATTATTATAGGG ACGCCTTTAACTTGTGGGGCCCAGGCACCCTGGTCACCGTCTCCTCA
54	ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGG TGTCCAGTGTCAGTCGCTGGAGGAGTCCGGGGGTCGCCTGGTCACG CCTGGGACACCCCTGACACTCACCTGCACAGTCTCTGGATTTTCCCT AAATAACTACAAAGTGGGCTGGGTCCGCCAGGCTCCAGGAAAGGG GCTGGAATGGATCGGAATCATTAACTATTATAGTCAGACATACTAC GCGAGCTGGGCCAAAGGCCGATTCACCATCTCGAAAACCTCGACC ACGGTG GATCTGAAGCTCACCAGTCCGACAACCGAAGACACGGCCACCTATTTCTGTGCCCGTGATCCTGATGGTAGTATTGTCTTTGACAT CTGGGGCCCAGGCACCCTTGTCACCGTCTCCTTG

[0110]

55	ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGG TGTCCAGTGTCAGTCGGTGGAGGAGTCCGGAGGAGGCCTGGTAACG CCTGGAGGAACCCTGACACTCACCTGCACCGTCTCTGGATTCTCCCT CAGTAACTATGCAATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGG CTGGAATGGATCGGATTCATTAGTCGTAGTGGTATTACATACTACGC GAGCTGGGCAAAAGGCCGATTCACCATCTCCAAAACCTCGACCACG GTGGATCTGAAAATCACCAGTCCGACGACCGAGGACACGGCCGCCT ATTTCTGTGCCAGAGAATTCGGTGCTGTTGGTAGTGATTATTATAGG GACGCCTTGAGGTTGTGGGGCCCAGGCACCCTGGTCACCGTCTCCT CA
56	ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAAGG TGTCCAGTGTCAGTCGCTGGAGGAGTCCGGGGGTCGCCTGGTAACG CCTGGGACACCCCTGACACTCACCTGCACAGTCTCTGGAATCGACCT CAGTACCCATGCAATGACCTGGGTCCGCCAGGCTCCAGGAAAGGGG CTGGAATGGATCGGAGTCATTAATCCTAGTGGTAGCGCATACTACG

[0111]

	CGACCTGGGTGAATGGCCGATTCACCATCTCCAAAACCTC GACCACG GTGGATCTGAAAATCACCAGTCCGACAACCGGGGACACGG CCAAGT ATTTCTGTGCCAGAGATTATATTACTGCGGGTGATTATTAT ATGGAT GCTTTTGATCCCTGGGGCCCAGGCACCCTGGTCACCGTCTC CTCA

[0112] 表7.编码VL结构域的核酸序列

SEQ ID NO:	**编码 VL 结构域的核酸序列**
57	ATGGACACGAGGGCCCCCACTCAGCTGCTGGGGCTCCTG CTGCTC TGGCTCCCAGGTGCCACATTTGCCCAAGTGCTGACCCAG ACTGCA TCCCCCGTGTCTGCGGCTGTTGGAGGCACAGTCACCATC AATTGC CAGTCCAGTCAGAGTGTTGTATATAACAACCGCTTATCCT GGTTT CAACAGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTAT GGTGCA TCCACTCTGGCATCTGGGGTCCCATCGCGGTTCAAAGGC AGTGGA TCTGGGACACAGTTCACTCTCACCATCAGCGACGTGCAG TGTGAC

[0114]

	GATGCTGCCACTTACTACTGTCTAGGCTCCTATGATTGTAGTAGT GGTGATTGCCATGCTTTCGGCGGAGGGACCGAGGTGGTGGTCAAA
58	ATGGACATGAGGGCCCCCACTCAGCTGCTGGGGCTCCTACTGCTC TGGCTCCCAGGTGCCAGATGTGCTGACATTGTGATGACCCAGACT CCAGCCTCCGTGGAGGCAGCTGTGGGAGGCACAGTCACCATCAA TTGCCAAGCCAGTGAGAGCATTAATAGTTGGTTGTCCTGGTATCA GCAGAAACCAGGGCAGCCTCCCAACCTCCTGATCTACAGGGCATC CACTCTGGCATCTGGGGTCCCATCGCGGTTCAGTGGCGGTGGATC TGGGACAGAGTACACTCTCACCATCAGCGACCTGGAGTGTGCCGA TGCTGTCACTTATTACTGTCAAAGCTATTATGAGGAGGATGGTAT TGGTTATGCTTTCGGCGGAGGGACCGAGGTGGTGGTCGAA
59	ATGGACATGAGGGCCCCCACTCAGCTGCTGGGGCTCCTGCTGCTCTG GCTCCCAGGTGCCAGATGTGCTGACATTGTGATGACCCAGACTCCAT CCTCCGTGTCTGCAGCTGTGGGAGGCACAGTCACCATCAATTGCCAG GCCAGTCAGAACATTTACAGCAATTTAGCCTGGTATCAG

[0115]

	CAGAAAC CAGGGCAGCGTCCCAGGCTCCTGATCTATGGCGCATCCA ATCTGGCA TCTGGGGTCCCATCGCGGTTCAAAGGCAGTAGATCTGGG ACAGAGTT CACTCTCACCATCAGCGACCTGGAGTGTGCCGATGCTGC CACTTACT ACTGTCAAGGCTATGATTATAGTACTGCTGGTGCCTATCC TTTCGGC GGAGGGACCGCGGTGGTGGTCAAA
60	ATGGACACGAGGGCCCCCACTCAGCTGCTGGGGCTCCTG CTGCTCTG GCTCCCAGGTGCCACATTTGCCCAAGTGCTGACCCAGAC TGCATCGC CCGTGTCTGCGGCTGTGGGAAGCACAGTCACCATCAATT GCCAGTCC AGTCAGAGCGTTTATAGTAACAAGCGCTTAGCCTGGTTT CAGCTGAA ACCAGGGCAGCCTCCCAAGCTCCTGATCTATGGTGCATC CACACTGG CATCTGGGGTCCCATCGCGATTCAAGGGCAGTGGATCTG GGACACAG TTCACTCTCACCATCAGCGACGTGCAGTGTGACGATGCT GCCACTTA CTACTGTGCAGGCGGTTATGATTGTAGTACTGGTGATTGT TGGACTTT CGGCGGAGGGACCGAGGTGGTGGTCACA
61	ATGGACATGAGGGCCCCCACTCAGCTGCTGGGGCTCCTG CTGCTC

[0116]

	TGGCTCCCAGGTGCCAGATGTGCTGACATCGTGATGACCCAGACT CCATCCTCCGTGTCTGCAGCTGTGGGAGGCACAGTCACCATCAAG TGCCAGGCCAGTCAGAGCATTGGTAGTAATTTAGCCTGGTATCAG CAGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTATGGTGCATCC ACTCTGGAATCTGGGGTCCCATCGCGGTTTAAAGGCAGTGGATCT GGGACAGAGTACACTCTCACCATCAGCGACCTGGAGTGTGCCGAT GCTGCCACTTACTACTGTCAAAGCTATTATGAGGGTAGTGATATT GGTTATGCTTTCGGCGGAGGGACCGAGGTGGTGGTCGAA
62	ATGGACACGAGGGCCCCCACTCAGCTGCTGGGGCTCCTGCTGCTC TGGCTCCCAGGTGCCAGATGTGCTGACATCGTGATGACCCAGACT CCAGCCTCTGTGTCTGCAGCTGTGGGAGGCACAGTCACCATCAAG TGCCAGGCCAGTCAGAGCATTAGCAACCAACTATCCTGGTATCAG CAGAAATCAGGGCAGCCTCCCAAGCTCCTGATCTACAGGGCATCT ACTCTGGCATCTGGGGTCCCATCGCGGTTCAAAGGCAGTGGATCT GGGACAGAGTTCACTCTCACCATCAGCGACCTGGAGTGTGCCGAT

[0117]

	GCTGCCACTTACTACTGTCAACAGGGTTATAATAGAGATAATGTT GATAATCTTTTCGGCGGAGGGACCGAGGTGGTGGTCAAA

[0118] 在一些实施方案中,所述抗tau抗体包含由核酸编码的重链,所述核酸包含与选自SEQ ID NO:63-67的序列相同的序列。在一些实施方案中,所述抗tau抗体包含由核酸编码的轻链,所述核酸包含与选自SEQ ID NO:68-73的序列相同的序列。在此所述的抗tau抗体的重链的核酸序列在表8中列出,并且在此所述的抗tau抗体的轻链的核酸序列在表9中列出。在表8和表9中列出的核酸序列可以在本文所述的抗体的体外生产过程中使用。在一些实施方案中,所述抗tau抗体包含由核酸编码的重链,所述核酸包含与SEQ ID NO:63相同的序列。在一些实施方案中,所述抗tau抗体包含由核酸编码的重链,所述核酸包含与SEQ ID NO:64相同的序列。在一些实施方案中,所述抗tau抗体包含由核酸编码的重链,所述核酸包含与SEQ ID NO:65相同的序列。在一些实施方案中,所述抗tau抗体包含由核酸编码的重链,所述核酸包含与SEQ ID NO:66相同的序列。在一些实施方案中,所述抗tau抗体包含由核酸编码的重链,所述核酸包含与SEQ ID NO:67相同的序列。在一些实施方案中,所述抗tau抗体包含由核酸编码的轻链,所述核酸包含与SEQ ID NO:68相同的序列。在一些实施方案中,所述抗tau抗体包含由核酸编码的轻链,所述核酸包含与SEQ ID NO:69相同的序列。在一些实施方案中,所述抗tau抗体包含由核酸编码的轻链,所述核酸包含与SEQ ID NO:70相同的序列。在一些实施方案中,所述抗tau抗体包含由核酸编码的轻链,所述核酸包含与SEQ ID NO:71相同的序列。在一些实施方案中,所述抗tau抗体包含由核酸编码的轻链,所述核酸包含与SEQ ID NO:72相同的序列。在一些实施方案中,所述抗tau抗体包含由核酸编码的轻链,所述核酸包含与SEQ ID NO:73相同的序列。在一些实施方案中,所述抗tau抗体包含由核酸编码的重链,所述核酸包含与SEQ ID NO:63相同的序列;和由核酸编码的轻链,所述核酸包含与SEQ ID NO:68相同的序列。在一些实施方案中,所述抗tau抗体包含由核酸编码的重链,所述核酸包含与SEQ ID NO:64相同的序列;和由核酸编码的轻链,所述核酸包含与SEQ ID NO:69相同的序列。在一些实施方案中,所述抗tau抗体包含由核酸编码的重链,所述核酸包含与SEQ ID NO:64相同的序列;和由核酸编码的轻链,所述核酸包含与SEQ ID NO:70相同的序列。在一些实施方案中,所述抗tau抗体包含由核酸编码的重链,所述核酸包含与SEQ ID NO:65相同的序列;和由核酸编码的轻链,所述核酸包含与SEQ ID NO:71相同的序列。在一些实施方案中,所述抗tau抗体包含由核酸编码的重链,所述核酸包含与SEQ ID NO:66相同的序列;和由核酸编码的轻链,所述核酸包含与SEQ ID NO:72相同的序列。在一些实施方案中,所述抗tau抗体包含由核酸编码的重链,所述核酸包含与SEQ ID NO:67相同的序列;和由核酸编码的轻链,所述核酸包含与SEQ ID NO:73相同的序列。

[0119] 表8.编码重链的核酸序列

[0120]

SEQ ID NO:	编码重链的核酸序列
63	ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGC TCAAAGGTG TCCAGTGTCAGTCGCTGGAGGAGTCCGGGGGTCGCCTGG TCACGCCTGG GACACCCCTGACACTCACCTGCACAGTCTCTGGATTTTCC CTCAGTAGC CAGAAAGTGGGCTGGGTCCGCCAGGCTCCAGGGAAGGG GCTGGAATGG ATCGGAATCATTAATAATTATGGTAGCACATACTACGCG AGCTGGGCG

[0121]

AAAGGCCGATTCACCATCTCGAAAACCTCGACCACAGTG
GATCTGAGA
ATCACCAGTCTGACGGCCGAGGACACGGCCACCTATTTC
TGTGCCCGTG
ATCCTGATGGTAGTATTGTCTTTGACATCTGGGGCCCAGG
CACCCTTGTC
ACCGTCTCCTTGGGGCAACCTAAGGCTCCATCAGTCTTCC
CACTGGCCC
CCTGCTGCGGGGACACACCCAGCTCCACGGTGACCCTGG
GCTGCCTGGT
CAAAGGCTACCTCCCGGAGCCAGTGACCGTGACCTGGAA
CTCGGGCAC
CCTCACCAATGGGGTACGCACCTTCCCGTCCGTCCGGCA
GTCCTCAGGC
CTCTACTCGCTGAGCAGCGTGGTGAGCGTGACCTCAAGC
AGCCAGCCC
GTCACCTGCAACGTGGCCCACCCAGCCACCAACACCAAA
GTGGACAAG
ACCGTTGCGCCCTCGACATGCAGCAAGCCCACGTGCCCA
CCCCCTGAA
CTCCTGGGGCGATCCTCTGTCTTCATCTTCCCCCCAAAAC
CCAAGGACA
CCCTCATGATCTCACGCACCCCCGAGGTCACATGCGTGG
TGGTGGACG
TGAGCCAGGATGACCCCGAGGTGCAGTTCACATGGTACA
TAAACAAC
GAGCAGGTGCGCACCGCCCGGCCGCCGCTACGGGAGCA
GCAGTTCAAC
AGCACGATCCGCGTGGTCAGCACCCTCCCCATCGCGCAC

[0122]

	CAGGACTGG CTGAGGGGCAAGGAGTTCAAGTGCAAAGTCCACAACAA GGCACTCCC GGCCCCCATCGAGAAAACCATCTCCAAAGCCAGAGGGCA GCCCCTGG AGCCGAAGGTCTACACCATGGGCCCTCCCCGGGAGGAGC TGAGCAGC AGGTCGGTCAGCCTGACCTGCATGATCAACGGCTTCTAC CCTTCCGAC ATCTCGGTGGAGTGGGAGAAGAACGGGAAGGCAGAGGA CAACTACAA GACCACGCCGGCCGTGCTGGACAGCGACGGCTCCTACTT CCTCTACAG CAAGCTCTCAGTGCCCACGAGTGAGTGGCAGCGGGGCGA CGTCTTCAC CTGCTCCGTGATGCACGAGGCCTTGCACAACCACTACAC GCAGAAGTC CATCTCCCGCTCTCCGGGTAAATGA

[0123]

64	ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGC TCAAAGG TGTCCAGTGTCAGTCGGTGGAGGAGTCCGGGGGTCGCCT GGTCACGC CTGGGACACCCCTGACACTCACCTGCACCGTCTCTGGATT CTCCCTC AGTAGCTATGCAATGATCTGGGTCCGCCAGGCTCCAGGG AAGGGGC TGGAATGGATCGGATTCATTAGTCGTAGTGGTATCACAT ACTACGCG AGCTGGGCAAAAGGCCGATTCACCATCTCCAAAACCTCG ACCACGG TGGATCTGAAAATGACCAGTCTGACAACCGAGGACACGG CCACCTA TTTCTGTGCCAGAGAATTCGGTGCTGTTGGTAGTGATTAT TATAGGGA CGCCTTTAACTTGTGGGGCCCAGGCACCCTGGTCACCGTC TCCTCAGG GCAACCTAAGGCTCCATCAGTCTTCCCACTGGCCCCCTGC TGCGGGGA CACACCCAGCTCCACGGTGACCCTGGGCTGCCTGGTCAA AGGCTACCT CCCGGAGCCAGTGACCGTGACCTGGAACTCGGGCACCCT CACCAATGG GGTACGCACCTTCCCGTCCGTCCGGCAGTCCTCAGGCCTC TACTCGCTG AGCAGCGTGGTGAGCGTGACCTCAAGCAGCCAGCCCGTC ACCTGCAAC GTGGCCCACCCAGCCACCAACACCAAAGTGGACAAGACC

[0124]

	GTTGCGCCC TCGACATGCAGCAAGCCCACGTGCCCACCCCCTGAACTC CTGGGGCGA TCCTCTGTCTTCATCTTCCCCCCAAAACCCAAGGACACCC TCATGATCT CACGCACCCCCGAGGTCACATGCGTGGTGGTGGACGTGA GCCAGGAT GACCCCGAGGTGCAGTTCACATGGTACATAAACAACGAG CAGGTGCG CACCGCCCGGCCGCCGCTACGGGAGCAGCAGTTCAACAG CACGATCC GCGTGGTCAGCACCCTCCCCATCGCGCACCAGGACTGGC TGAGGGGC AAGGAGTTCAAGTGCAAAGTCCACAACAAGGCACTCCCG GCCCCCAT CGAGAAAACCATCTCCAAAGCCAGAGGGCAGCCCCTGG AGCCGAAG GTCTACACCATGGGCCCTCCCCGGGAGGAGCTGAGCAGC AGGTCGGT CAGCCTGACCTGCATGATCAACGGCTTCTACCCTTCCGAC ATCTCGGT GGAGTGGGAGAAGAACGGGAAGGCAGAGGACAACTACA AGACCACG CCGGCCGTGCTGGACAGCGACGGCTCCTACTTCCTCTAC AGCAAGCTC TCAGTGCCCACGAGTGAGTGGCAGCGGGGCGACGTCTTC ACCTGCTCC GTGATGCACGAGGCCTTGCACAACCACTACACGCAGAAG TCCATCTC

[0125]

	CCGCTCTCCGGGTAAATGA

[0126]

65	ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGC TCAAAGG TGTCCAGTGTCAGTCGCTGGAGGAGTCCGGGGGTCGCCT GGTCACG CCTGGGACACCCCTGACACTCACCTGCACAGTCTCTGGA TTTTCCCT AAATAACTACAAAGTGGGCTGGGTCCGCCAGGCTCCAGG AAAGGG GCTGGAATGGATCGGAATCATTAACTATTATAGTCAGAC ATACTAC GCGAGCTGGGCCAAAGGCCGATTCACCATCTCGAAAACC TCGACC ACGGTG GATCTGAAGCTCACCAGTCCGACAACCGAAGACACGGCC ACCTATTTCTGTGCCCGTGATCCTGATGGTAGTATTGTCT TTGACAT CTGGGGCCCAGGCACCCTTGTCACCGTCTCCTTGGGGCA ACCTAAGG CTCCATCAGTCTTCCCACTGGCCCCCTGCTGCGGGGACAC ACCCAGC TCCACGGTGACCCTGGGCTGCCTGGTCAAAGGCTACCTC CCGGAGCC AGTGACCGTGACCTGGAACTCGGGCACCCTCACCAATGG GGTACGCA CCTTCCCGTCCGTCCGGCAGTCCTCAGGCCTCTACTCGCT GAGCAGCG TGGTGAGCGTGACCTCAAGCAGCCAGCCCGTCACCTGCA ACGTGGCC CACCCAGCCACCAACACCAAAGTGGACAAGACCGTTGTG

[0127]

	CCCTCGAC ATGCAGCAAGCCCACGTGCCCACCCCCTGAACTCCTGGG GCGATCCT CTGTCTTCATCTTCCCCCCAAAACCCAAGGACACCCTCAT GATCTCAC GCACCCCCGAGGTCACATGCGTGGTGGTGGACGTGAGCC AGGATGAC CCCGAGGTGCAGTTCACATGGTACATAAACAACGAGCAG GTGCGCAC CGCCCGGCCGCCGCTACGGGAGCAGCAGTTCAACAGCAC GATCCGCG TGGTCAGCACCCTCCCCATCGCGCACCAGGACTGGCTGA GGGGCAAG GAGTTCAAGTGCAAAGTCCACAACAAGGCACTCCCGGCC CCCATCGA GAAAACCATCTCCAAAGCCAGAGGGCAGCCCCTGGAGCC GAAGGTCT ACACCATGGGCCCTCCCCGGGAGGAGCTGAGCAGCAGGT CGGTCAGC CTGACCTGCATGATCAACGGCTTCTACCCTTCCGACATCT CGGTGGAG TGGGAGAAGAACGGGAAGGCAGAGGACAACTACAAGAC CACGCCGG CCGTGCTGGACAGCGACGGCTCCTACTTCCTCTACAGCA AGCTCTCAG TGCCCACGAGTGAGTGGCAGCGGGGCGACGTCTTCACCT GCTCCGTGA TGCACGAGGCCTTGCACAACCACTACACGCAGAAGTCCA TCTCCCGCT

[0128]

	CTCCGGGTAAATGA

[0129]

66	ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGC TCAAAGG TGTCCAGTGTCAGTCGGTGGAGGAGTCCGGAGGAGGCCT GGTAACG CCTGGAGGAACCCTGACACTCACCTGCACCGTCTCTGGA TTCTCCCT CAGTAACTATGCAATGAGCTGGGTCCGCCAGGCTCCAGG GAAGGGG CTGGAATGGATCGGATTCATTAGTCGTAGTGGTATTACAT ACTACGC GAGCTGGGCAAAAGGCCGATTCACCATCTCCAAAACCTC GACCACG GTGGATCTGAAAATCACCAGTCCGACGACCGAGGACACG GCCGCCT ATTTCTGTGCCAGAGAATTCGGTGCTGTTGGTAGTGATTA TTATAGG GACGCCTTGAGGTTGTGGGGCCCAGGCACCCTGGTCACC GTCTCCTC AGGGCAACCTAAGGCTCCATCAGTCTTCCCACTGGCCCC CTGCTGCG GGGACACACCCAGCTCCACGGTGACCCTGGGCTGCCTGG TCAAAGG CTACCTCCCGGAGCCAGTGACCGTGACCTGGAACTCGGG CACCCTCA CCAATGGGGTACGCACCTTCCCGTCCGTCCGGCAGTCCTC AGGCCTC TACTCGCTGAGCAGCGTGGTGAGCGTGACCTCAAGCAGC CAGCCCGT CACCTGCAACGTGGCCCACCCAGCCACCAACACCAAAGT

[0130]

	GGACAAGA CCGTTGCGCCCTCGACATGCAGCAAGCCCACGTGCCCAC CCCCTGAAC TCCTGGGGCGATCCTCTGTCTTCATCTTCCCCCCAAAACC CAAGGACA CCCTCATGATCTCACGCACCCCCGAGGTCACATGCGTGG TGGTGGACG TGAGCCAGGATGACCCCGAGGTGCAGTTCACATGGTACA TAAACAAC GAGCAGGTGCGCACCGCCCGGCCGCCGCTACGGGAGCA GCAGTTCAA CAGCACGATCCGCGTGGTCAGCACCCTCCCCATCGCGCA CCAGGACTG GCTGAGGGGCAAGGAGTTCAAGTGCAAAGTCCACAACA AGGCACTCC CGGCCCCCATCGAGAAAACCATCTCCAAAGCCAGAGGGC AGCCCCTG GAGCCGAAGGTCTACACCATGGGCCCTCCCCGGGAGGAG CTGAGCAG CAGGTCGGTCAGCCTGACCTGCATGATCAACGGCTTCTA CCCTTCCGA CATCTCGGTGGAGTGGGAGAAGAACGGGAAGGCAGAGG ACAACTAC AAGACCACGCCGGCCGTGCTGGACAGCGACGGCTCCTAC TTCCTCTA CAGCAAGCTCTCAGTGCCCACGAGTGAGTGGCAGCGGGG CGACGTC TTCACCTGCTCCGTGATGCACGAGGCCTTGCACAACCACT ACACGCA

[0131]

	GAAGTCCATCTCCCGCTCTCCGGGTAAATGA

[0132]

67	ATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGC TCAAAGG TGTCCAGTGTCAGTCGCTGGAGGAGTCCGGGGGTCGCCT GGTAACG CCTGGGACACCCCTGACACTCACCTGCACAGTCTCTGGA ATCGACCT CAGTACCCATGCAATGACCTGGGTCCGCCAGGCTCCAGG AAAGGGG CTGGAATGGATCGGAGTCATTAATCCTAGTGGTAGCGCA TACTACG CGACCTGGGTGAATGGCCGATTCACCATCTCCAAAACCT CGACCACG GTGGATCTGAAAATCACCAGTCCGACAACCGGGGACACG GCCAAGT ATTTCTGTGCCAGAGATTATATTACTGCGGGTGATTATTA TATGGAT GCTTTTGATCCCTGGGGCCCAGGCACCCTGGTCACCGTCT CCTCAGG GCAACCTAAGGCTCCATCAGTCTTCCCACTGGCCCCCTGC TGCGGGG ACACACCCAGCTCCACGGTGACCCTGGGCTGCCTGGTCA AAGGCTAC CTCCCGGAGCCAGTGACCGTGACCTGGAACTCGGGCACC CTCACCAA TGGGGTACGCACCTTCCCGTCCGTCCGGCAGTCCTCAGG CCTCTACTC GCTGAGCAGCGTGGTGAGCGTGACCTCAAGCAGCCAGCC CGTCACCT GCAACGTGGCCCACCCAGCCACCAACACCAAAGTGGACA

[0133]

	AGACCGTT GCGCCCTCGACATGCAGCAAGCCCACGTGCCCACCCCCT GAACTCCT GGGGCGATCCTCTGTCTTCATCTTCCCCCCAAAACCCAAG GACACCC TCATGATCTCACGCACCCCCGAGGTCACATGCGTGGTGG TGGACGTG AGCCAGGATGACCCCGAGGTGCAGTTCACATGGTACATA AACAACG AGCAGGTGCGCACCGCCCGGCCGCCGCTACGGGAGCAGC AGTTCAA CAGCACGATCCGCGTGGTCAGCACCCTCCCCATCGCGCA CCAGGACT GGCTGAGGGGCAAGGAGTTCAAGTGCAAAGTCCACAAC AAGGCACT CCCGGCCCCCATCGAGAAAACCATCTCCAAAGCCAGAGG GCAGCCC CTGGAGCCGAAGGTCTACACCATGGGCCCTCCCCGGGAG GAGCTGA GCAGCAGGTCGGTCAGCCTGACCTGCATGATCAACGGCT TCTACCC TTCCGACATCTCGGTGGAGTGGGAGAAGAACGGGAAGG CAGAGGA CAACTACAAGACCACGCCGGCCGTGCTGGACAGCGACGG CTCCTA CTTCCTCTACAGCAAGCTCTCAGTGCCCACGAGTGAGTG GCAGCGG GGCGACGTCTTCACCTGCTCCGTGATGCACGAGGCCTTG CACAACC

[0134]

	ACTACACGCAGAAGTCCATCTCCCGCTCTCCGGGTAAATGA

[0135] 表9.编码轻链的核酸序列

[0136]

SEQ ID NO:	编码轻链的核酸序列
68	ATGGACACGAGGGCCCCCACTCAGCTGCTGGGGCTCCTGCTGCTCTG GCTCCCAGGTGCCACATTTGCCCAAGTGCTGACCCAGACTGCATCCC CCGTGTCTGCGGCTGTTGGAGGCACAGTCACCATCAATTGCCAGTCC AGTCAGAGTGTTGTATATAACAACCGCTTATCCTGGTTTC

[0137]

	AACAGAA ACCAGGGCAGCCTCCCAAGCTCCTGATCTATGGTGCATC CACTCTGG CATCTGGGGTCCCATCGCGGTTCAAAGGCAGTGGATCTG GGACACA GTTCACTCTCACCATCAGCGACGTGCAGTGTGACGATGC TGCCACTT ACTACTGTCTAGGCTCCTATGATTGTAGTAGTGGTGATTG CCATGCT TTCGGCGGAGGGACCGAGGTGGTGGTCAAAGGTGATCCA GTTGCAC CTACTGTCCTCATCTTCCCACCAGCTGCTGATCAGGTGGC AACTGGA ACAGTCACCATCGTGTGTGTGGCGAATAAATACTTTCCC GATGTCAC CGTCACCTGGGAGGTGGATGGCACCACCCAAACAACTGG CATCGAG AACAGTAAAACACCGCAGAATTCTGCAGATTGTACCTAC AACCTCA GCAGCACTCTGACACTGACCAGCACACAGTACAACAGCC ACAAAG AGTACACCTGCAAGGTGACCCAGGGCACGACCTCAGTCG TCCAGAG CTTCAATAGGGGTGACTGTTAG
69	ATGGACATGAGGGCCCCCACTCAGCTGCTGGGGCTCCTA CTGCTCTG GCTCCCAGGTGCCAGATGTGCTGACATTGTGATGACCCA GACTCCAG CCTCCGTGGAGGCAGCTGTGGGAGGCACAGTCACCATCA

[0138]

	ATTGCCAA GCCAGTGAGAGCATTAATAGTTGGTTGTCCTGGTATCAG CAGAAACC AGGGCAGCCTCCCAACCTCCTGATCTACAGGGCATCCAC TCTGGCAT CTGGGGTCCCATCGCGGTTCAGTGGCGGTGGATCTGGGA CAGAGTAC ACTCTCACCATCAGCGACCTGGAGTGTGCCGATGCTGTC ACTTATTA CTGTCAAAGCTATTATGAGGAGGATGGTATTGGTTATGC TTTCGGCG GAGGGACCGAGGTGGTGGTCGAAGGTGATCCAGTTGCAC CTACTGT CCTCATCTTCCCACCAGCTGCTGATCAGGTGGCAACTGG AACAGTCA CCATCGTGTGTGTGGCGAATAAATACTTTCCCGATGTCAC CGTCACC TGGGAGGTGGATGGCACCACCCAAACAACTGGCATCGA GAACAGTA AAACACCGCAGAATTCTGCAGATTGTACCTACAACCTCA GCAGCACT CTGACACTGACCAGCACACAGTACAACAGCCACAAAGA GTACACCT GCAAGGTGACCCAGGGCACGACCTCAGTCGTCCAGAGCT TCAATAG GGGTGACTGTTAG
70	ATGGACATGAGGGCCCCCACTCAGCTGCTGGGGCTCCTG CTGCTCTG GCTCCCAGGTGCCAGATGTGCTGACATTGTGATGACCCA

[0139]

	GACTCCAT CCTCCGTGTCTGCAGCTGTGGGAGGCACAGTCACCATCA ATTGCCAG GCCAGTCAGAACATTTACAGCAATTTAGCCTGGTATCAG CAGAAACC AGGGCAGCGTCCCAGGCTCCTGATCTATGGCGCATCCAA TCTGGCAT CTGGGGTCCCATCGCGGTTCAAAGGCAGTAGATCTGGGA CAGAGTT CACTCTCACCATCAGCGACCTGGAGTGTGCCGATGCTGC CACTTACT ACTGTCAAGGCTATGATTATAGTACTGCTGGTGCCTATCC TTTCGGC GGAGGGACCGCGGTGGTGGTCAAAGGTGATCCAGTTGCA CCTACTG TCCTCATCTTCCCACCAGCTGCTGATCAGGTGGCAACTGG AACAGTC ACCATCGTGTGTGTGGCGAATAAATACTTTCCCGATGTC ACCGTCAC CTGGGAGGTGGATGGCACCACCCAAACAACTGGCATCGA GAACAG TAAAACACCGCAGAATTCTGCAGATTGTACCTACAACCT CAGCAGC ACTCTGACACTGACCAGCACACAGTACAACAGCCACAAA GAGTAC ACCTGCAAGGTGACCCAGGGCACGACCTCAGTCGTCCAG AGCTTCA ATAGGGGTGACTGTTAG
71	ATGGACACGAGGGCCCCCACTCAGCTGCTGGGGCTCCTG

[0140]

	CTGCTCTG GCTCCCAGGTGCCACATTTGCCCAAGTGCTGACCCAGAC TGCATCGC CCGTGTCTGCGGCTGTGGGAAGCACAGTCACCATCAATT GCCAGTCC AGTCAGAGCGTTTATAGTAACAAGCGCTTAGCCTGGTTT CAGCTGAA ACCAGGGCAGCCTCCCAAGCTCCTGATCTATGGTGCATC CACACTGG CATCTGGGGTCCCATCGCGATTCAAGGGCAGTGGATCTG GGACACAG TTCACTCTCACCATCAGCGACGTGCAGTGTGACGATGCT GCCACTTA CTACTGTGCAGGCGGTTATGATTGTAGTACTGGTGATTGT TGGACTTT CGGCGGAGGGACCGAGGTGGTGGTCACAGGTGATCCAG TTGCACCT ACTGTCCTCATCTTCCCACCAGCTGCTGATCAGGTGGCAA CTGGAAC AGTCACCATCGTGTGTGTGGCGAATAAATACTTTCCCGA TGTCACCG TCACCTGGGAGGTGGATGGCACCACCCAAACAACTGGCA TCGAGAA CAGTAAAACACCGCAGAATTCTGCAGATTGTACCTACAA CCTCAGCA GCACTCTGACACTGACCAGCACACAGTACAACAGCCACA AAGAGTA CACCTGCAAGGTGACCCAGGGCACGACCTCAGTCGTCCA GAGCTTC

[0141]

	AATAGGGGTGACTGTTAG
72	ATGGACATGAGGGCCCCCACTCAGCTGCTGGGGCTCCTG CTGCTCTG GCTCCCAGGTGCCAGATGTGCTGACATCGTGATGACCCA GACTCCAT CCTCCGTGTCTGCAGCTGTGGGAGGCACAGTCACCATCA AGTGCCAG GCCAGTCAGAGCATTGGTAGTAATTTAGCCTGGTATCAG CAGAAACC AGGGCAGCCTCCCAAGCTCCTGATCTATGGTGCATCCAC TCTGGAAT CTGGGGTCCCATCGCGGTTTAAAGGCAGTGGATCTGGGA CAGAGTA CACTCTCACCATCAGCGACCTGGAGTGTGCCGATGCTGC CACTTACT ACTGTCAAAGCTATTATGAGGGTAGTGATATTGGTTATG CTTTCGGC GGAGGGACCGAGGTGGTGGTCGAAGGTGATCCAGTTGC ACCTACTG TCCTCATCTTCCCACCAGCTGCTGATCAGGTGGCAACTGG AACAGTC ACCATCGTGTGTGTGGCGAATAAATACTTTCCCGATGTC ACCGTCAC CTGGGAGGTGGATGGCACCACCCAAACAACTGGCATCGA GAACAGT AAAACACCGCAGAATTCTGCAGATTGTACCTACAACCTC AGCAGCA CTCTGACACTGACCAGCACACAGTACAACAGCCACAAAG AGTACAC

[0142]

	CTGCAAGGTGACCCAGGGCACGACCTCAGTCGTCCAGAG CTTCAAT AGGGGTGACTGTTAG
73	ATGGACACGAGGGCCCCCACTCAGCTGCTGGGGCTCCTG CTGCTCTG GCTCCCAGGTGCCAGATGTGCTGACATCGTGATGACCCA GACTCCAG CCTCTGTGTCTGCAGCTGTGGGAGGCACAGTCACCATCA AGTGCCAG GCCAGTCAGAGCATTAGCAACCAACTATCCTGGTATCAG CAGAAAT CAGGGCAGCCTCCCAAGCTCCTGATCTACAGGGCATCTA CTCTGGCA TCTGGGGTCCCATCGCGGTTCAAAGGCAGTGGATCTGGG ACAGAGTT CACTCTCACCATCAGCGACCTGGAGTGTGCCGATGCTGC CACTTACT ACTGTCAACAGGGTTATAATAGAGATAATGTTGATAATC TTTTCGGC GGAGGGACCGAGGTGGTGGTCAAAGGTGATCCAGTTGC ACCTACTG TCCTCATCTTCCCACCAGCTGCTGATCAGGTGGCAACTGG AACAGTC ACCATCGTGTGTGTGGCGAATAAATACTTTCCCGATGTC ACCGTCAC CTGGGAGGTGGATGGCACCACCCAAACAACTGGCATCGA GAACAGT AAAACACCGCAGAATTCTGCAGATTGTACCTACAACCTC AGCAGCA

[0143]

	CTCTGACACTGACCAGCACACAGTACAACAGCCACAAAG AGTACAC
	CTGCAAGGTGACCCAGGGCACGACCTCAGTCGTCCAGAG CTTCAAT
	AGGGGTGACTGTTAG

[0144] 本公开的方法

[0145] 本文公开了用于使用本文所述的抗体检测来自个体的样品中的磷酸化tau的方法。在一些实施方案中,所述磷酸化tau选自pTau-212、pTau-217、pTau-231、pTau-214和pTau-220。在一些实施方案中,用于使用本文所述的抗体检测来自个体的样品中的磷酸化tau的方法包括改善的特异性和灵敏度。

[0146] 本文描述了用于检测来自个体的样品中的磷酸化tau的方法,所述方法包括:使用与磷酸化tau结合的抗体或抗体片段对所述样品进行测定。本文描述了用于检测来自个体的样品中的磷酸化tau的方法,所述方法包括:使用与磷酸化tau结合的抗体或抗体片段对所述样品进行免疫测定。在一些实施方案中,所述磷酸化tau选自pTau-212、pTau-217、pTau-231、pTau-214、pTau-220和pTau-181。在一些实施方案中,所述磷酸化tau选自pTau-212、pTau-217、pTau-231、pTau-214和pTau-220。在一些实施方案中,所述磷酸化tau是pTau-217。在一些实施方案中,所述磷酸化tau是pTau-231。在一些实施方案中,所述磷酸化tau是pTau-181。在一些实施方案中,所述磷酸化tau是pTau-212。在一些实施方案中,所述磷酸化tau是pTau-217。在一些实施方案中,所述磷酸化tau是pTau-214。在一些实施方案中,所述磷酸化tau是pTau-220。在一些实施方案中,所述磷酸化tau是pTau-181和pTau-217。在一些实施方案中,所述磷酸化tau是pTau-181和pTau-231。在一些实施方案中,所述磷酸化tau是pTau-217和pTau-231。在一些实施方案中,所述磷酸化tau是pTau-181、pTau-217和pTau-231。

[0147] 本文还描述了用于检测来自个体的样品中的磷酸化tau的方法,所述方法包括:使用与多种磷酸化tau蛋白结合的抗体或抗体片段对所述样品进行测定。在一些实施方案中,所述方法检测pTau-217和pTau-231。在一些实施方案中,所述方法检测pTau-212和pTau-217。在一些实施方案中,所述方法检测pTau-212和pTau-231。在一些实施方案中,所述方法检测pTau-212、pTau-217和pTau-231。

[0148] 本文描述了用于检测来自个体的样品中的磷酸化tau的方法,其中所述方法检测选自血浆样品和血清样品的样品中的pTau-217和pTau-231。在一些实施方案中,所述方法检测选自血浆样品和血清样品的样品中的pTau-212和pTau-217。在一些实施方案中,所述方法检测选自血浆样品和血清样品的样品中的pTau-212和pTau-231。在一些实施方案中,所述方法检测选自血浆样品和血清样品的样品中的pTau-212、pTau-217和pTau-231。

[0149] 如本文所述的方法可以包括对样品进行测定,其中所述样品选自血浆样品和血清样品。在一些情况下,所述样品是血液样品。在一些情况下,所述样品是脑脊液样品。样品可以是通过静脉抽血获得的血液样品。样品可以是从手指穿刺抽血获得的血液样品。样品可以通过卫生保健提供者或对象获得。所述方法可以包括从对象获得样品。在一些情况下,样

品是在就诊诊所或医院期间从对象获得的。

[0150] 在一些实施方案中,本文还描述了确定生物标志物的水平的方法,所述生物标志物选自Aβ42、Aβ40、Aβ38、BACE1、hFABP、TREM2、YKL-40、IP-10、神经颗粒蛋白、SNAP-25、突触结合蛋白、α-突触核蛋白、TDP-43、铁蛋白、VILIP-1、NfL、GFAP及其组合。在一些情况下,所述生物标志物是Aβ42。在一些情况下,所述生物标志物是Aβ40。在一些情况下,所述生物标志物是Aβ42和Aβ40。在一些情况下,所述生物标志物是APOE。在一些情况下,所述生物标志物选自APOE2、APOE3和APOE4。在一些情况下,所述生物标志物是APOE4。

[0151] 在一些实施方案中,用于检测样品中的磷酸化tau的方法包括使用本文所述的抗体或抗体片段进行的免疫测定或配体测定。在一些情况下,所述测定选自酶联免疫吸附测定(ELISA)、比色免疫测定、均相免疫测定、非光学免疫测定、荧光免疫测定、化学发光免疫测定、电化学发光免疫测定、荧光共振能量转移(FRET)免疫测定、时间分辨荧光免疫测试、侧流免疫测定、微点免疫测定(microspot immunoassay)、表面等离子体共振测定、配体测定、凝集测定、色谱法测定和免疫捕获结合质谱法。在一些情况下,所述测定包括免疫测定。在一些情况下,所述测定选自蛋白质印迹、酶联免疫吸附测定(ELISA)和色谱法。在一些情况下,所述免疫测定是单重的。在一些情况下,所述免疫测定是多重的。

[0152] 如本文所述的方法可以包括使用本文所述的抗体或抗体片段进行的多个免疫测定。在一些情况下,所述多个免疫测定是相同的免疫测定(例如,四个或更多个ELISA测定)。当多个免疫测定是相同的免疫测定时,多个免疫测定中的每一个可以检测不同的磷酸化tau。当多个免疫测定是相同的免疫测定时,多个免疫测定中的每一个可以在相同的反应室或不同的反应室中进行。反应室可以是用于进行免疫测定的任何合适的空间。反应室的实例包括但不限于微孔板中的孔、Eppendorf管或液滴。

[0153] 在一些情况下,所述多个免疫测定是不同的免疫测定。当多个免疫测定是不同的免疫测定时,多个免疫测定中的每一个可以检测不同的磷酸化tau。当多个免疫测定是不同的免疫测定时,多个免疫测定中的每一个可以在相同的反应室或不同的反应室中进行。

[0154] 在一些情况下,所述测定包括非免疫测定。在一些情况下,所述测定选自高效液相色谱法(HPLC)、高效液相质谱法(HPLC-MS)、气相色谱-质谱法(GC-MS)、液相色谱-质谱法(LC-MS)、液相色谱-串联质谱法(LC-MS/MS)、免疫组织化学(IHC)、聚合酶链式反应(PCR)、定量PCR(qPCR)及其组合。

[0155] 使用本文所述的抗体进行的如本文所述的方法可以用于基于磷酸化tau的检测确定个体的阿尔茨海默病。在一些实施方案中,如果在来自个体的样品中检测到pTau-212、pTau-217、pTau-231、pTau-214、pTau-220或其组合,则确定个体的阿尔茨海默病。

[0156] 使用本文所述的抗体进行的如本文所述的方法可以用于基于磷酸化tau的检测对个体发展阿尔茨海默病进行预后。在一些实施方案中,如果在来自个体的样品中检测到pTau-212、pTau-217、pTau-231、pTau-214、pTau-220或其组合,则确定个体发展阿尔茨海默病的预后。

[0157] 与选自以下的疾病或病症或神经和认知未受损的病状相比,使用本文所述的抗体进行的如本文所述的方法可以用于准确地且特异性地确定个体的阿尔茨海默病(AD):非阿尔茨海默病(AD)神经退行性疾病、Aβ阴性非AD神经退行性疾病、Aβ阳性非AD神经退行性疾病、行为变异型额颞叶痴呆(BvFTD)、原发性进行性失语(PPA)、血管性痴呆(VaD)、帕金森病

(PD)、PD伴痴呆(PDD)、多系统萎缩(MSA)、进行性核上性麻痹(PSP)、皮质基底综合征(CBS)、Aβ阴性认知受损或未受损对照及其组合。在一些实施方案中,与疾病或病症或神经和认知未受损的病状相比,使用本文所述的抗体进行的如本文所述的方法包括确定AD的至少或约70%、80%、90%、95%、99%或更大的改善准确性或特异性。

[0158] 与神经病理学检查或临床诊断相比,使用本文所述的抗体进行的如本文所述的方法可以用于准确地且特异性地确定个体的阿尔茨海默病(AD)。在一些实施方案中,与神经病理学检查或临床诊断相比,使用本文所述的抗体进行的如本文所述的方法包括确定AD的至少或约70%、80%、90%、95%、99%或更大的改善准确性或特异性。在一些实施方案中,神经病理学检查或临床诊断包括神经测试、心理检查或脑成像(例如,MRI、CT或PET扫描)。

[0159] 使用本文所述的抗体进行的如本文所述的方法可以能够以检测下限检测样品中的磷酸化tau。在一些实施方案中,使用本文所述的抗体进行的如本文所述的方法能够以至少约1.5皮克/毫升(pg/mL)的检测限值检测所述样品中的磷酸化tau。在一些实施方案中,使用本文所述的抗体进行的如本文所述的方法能够以至少约5皮克/毫升(pg/mL)的检测限值检测所述样品中的磷酸化tau。在一些实施方案中,使用本文所述的抗体进行的如本文所述的方法能够以以下范围内的检测限值检测所述样品中的磷酸化tau:约0.5pg/mL至约10pg/mL、约1pg/mL至约8pg/mL、约1.5pg/mL至约7pg/mL、约2pg/mL至约6pg/mL、或约3pg/mL至约5pg/mL。

[0160] Tau抗体的产生

[0161] 在一些实施方案中,本文所述的抗体或抗体片段使用本领域已知的可用于合成抗体或抗体片段的任何方法,具体地通过化学合成或通过重组表达来产生,并且优选地通过重组表达技术来产生。

[0162] 在一些情况下,抗体或其结合片段是重组表达的,并且编码所述抗体或其结合片段的核酸由化学合成的寡核苷酸组装而成(例如,如Kutmeier等人,1994,BioTechniques 17:242中所述),这涉及合成含有编码抗体的序列的一部分的重叠寡核苷酸,对这些寡核苷酸进行退火和连接,然后通过PCR扩增连接的寡核苷酸。

[0163] 可替代地,编码抗体的核酸分子任选地通过使用与序列的3′和5′端可杂交的合成引物进行PCR扩增,或通过使用对特定基因序列具有特异性的寡核苷酸探针进行克隆从合适的来源(例如,抗体cDNA文库,或从表达免疫球蛋白的任何组织或细胞生成的cDNA文库)生成。

[0164] 在一些情况下,抗体或其结合任选地通过对动物(诸如小鼠)进行免疫以生成多克隆抗体或更优选地通过生成单克隆抗体来生成,例如,如由Kohler和Milstein(1975,Nature 256:495-497)或如由Kozbor等人(1983,Immunology Today 4:72)或Cole等人(1985Monoclonal Antibodies and Cancer Therapy,Alan R.Liss,Inc.,第77-96页)所述。可替代地,编码所述抗体的至少Fab部分的克隆任选地通过筛选Fab表达文库(例如,如在Huse等人,1989,Science 246:1275-1281中所述)以获得结合特定抗原的Fab片段的克隆或通过筛选抗体文库(参见例如Clackson等人,1991,Nature 352:624;Hane等人,1997Proc.Natl.Acad.Sci.USA 94:4937)来获得。

[0165] 在一些实施方案中,使用通过剪接来自具有适当抗原特异性的小鼠抗体分子的基因以及来自具有适当生物活性的人抗体分子的基因来产生“嵌合抗体”而开发的技术

(Morrison等人,1984,Proc.Natl.Acad.Sci.81:851-855;Neuberger等人,1984,Nature 312:604-608;Takeda等人,1985,Nature 314:452-454)。嵌合抗体是一种分子,其中不同部分衍生自不同的动物物种,诸如具有衍生自鼠单克隆抗体的可变区和人免疫球蛋白恒定区的那些分子。

[0166] 在一些实施方案中,针对单链抗体的产生描述的技术(美国专利号4,694,778;Bird,1988,Science 242:423-42;Huston等人,1988,Proc.Natl.Acad.Sci.USA 85:5879-5883;以及Ward等人,1989,Nature334:544-54)适于产生单链抗体。单链抗体是通过经由氨基酸桥连接Fv区的重链和轻链片段、从而产生单链多肽而形成的。还任选地使用用于在大肠杆菌中组装功能性Fv片段的技术(Skerra等人,1988,Science 242:1038-1041)。

[0167] 在一些实施方案中,通过常规技术(例如,电穿孔、脂质体转染和磷酸钙沉淀)将包含抗体的核苷酸序列的表达载体或抗体的核苷酸序列转移到宿主细胞中,并且然后通过常规技术培养转染的细胞以产生抗体。在具体实施方案中,抗体的表达由组成型、诱导型或组织特异性启动子调节。

[0168] 在一些实施方案中,利用多种宿主表达载体系统来表达本文所述的抗体或其结合片段。此类宿主表达系统代表借以产生并随后纯化抗体的编码序列的媒介物,而且还代表当用适当的核苷酸编码序列转化或转染时原位表达抗体或其结合片段的细胞。这些包括但不限于用含有抗体或其结合片段编码序列的重组噬菌体DNA、质粒DNA或黏粒DNA表达载体转化的微生物,诸如细菌(例如,大肠杆菌和枯草芽孢杆菌);用含有抗体或其结合片段编码序列的重组酵母表达载体转化的酵母(例如,毕赤酵母);用含有抗体或其结合片段编码序列的重组病毒表达载体(例如,杆状病毒)感染的昆虫细胞系统;用重组病毒表达载体(例如,花椰菜花叶病毒(CaMV)和烟草花叶病毒(TMV))感染或用含有抗体或其结合片段编码序列的重组质粒表达载体(例如,Ti质粒)转化的植物细胞系统;或携带含有衍生自哺乳动物细胞基因组(例如,金属硫蛋白启动子)或哺乳动物病毒(例如,腺病毒晚期启动子;牛痘病毒7.5K启动子)的启动子的重组表达构建体的哺乳动物细胞系统(例如,COS、CHO、BH、293、293T、3T3细胞)。

[0169] 对于重组蛋白的长期高产率产生,稳定表达是优选的。在一些情况下,任选地对稳定表达抗体的细胞系进行工程化。用受适当的表达控制元件(例如,启动子、增强子、序列、转录终止子、聚腺苷酸化位点等)控制的DNA和可选择标志物转化宿主细胞,而不是使用含有病毒复制起点的表达载体。在引入外来DNA后,然后使工程化细胞在富集培养基中生长1-2天,然后转换到选择性培养基。重组质粒中的可选择标志物赋予选择抗性并允许细胞将质粒稳定整合到其染色体中,并且生长以形成基因座,细胞转而进行克隆并扩增成细胞系。此方法可以有利地用于对表达抗体或其结合片段的细胞系进行工程化。

[0170] 在一些情况下,使用多种选择系统,包括但不限于分别在tk-、hgprt-或aprt-细胞中采用单纯疱疹病毒胸苷激酶(Wigler等人,1977,Cell 11:223)、次黄嘌呤-鸟嘌呤磷酸核糖基转移酶(Szybalska和Szybalski,192,Proc.Natl.Acad.Sci.USA 48:202)以及腺嘌呤磷酸核糖基转移酶(Lowy等人,1980,Cell 22:817)基因。另外,使用抗代谢物抗性作为以下基因的选择基础:dhfr,其赋予对于甲氨蝶呤的抗性(Wigler等人,1980,Proc.Natl.Acad.Sci.USA 77:357;O'Hare等人,1981,Proc.Natl.Acad.Sci.USA 78:1527);gpt,其赋予对于霉酚酸的抗性(Mulligan和Berg,1981,Proc.Natl.Acad.Sci.USA

78:2072);neo,其赋予对于氨基糖苷G-418的抗性(Clinical Pharmacy 12:488-505;Wu和Wu,1991,Biotherapy 3:87-95;Tolstoshev,1993,Ann.Rev.Pharmacol.Toxicol.32:573-596;Mulligan,1993,Science 260:926-932;以及Morgan和Anderson,1993,Ann.Rev.Biochem.62:191-217;1993年5月,TIB TECH 11(5):155-215)以及hygro,其赋予对于潮霉素的抗性(Santerre等人,1984,Gene 30:147)。可以使用的重组DNA技术领域中通常已知的方法在以下中描述:Ausubel等人(编,1993,Current Protocols in Molecular Biology,John Wiley&Sons,NY;Kriegler,1990,Gene Transfer and Expression,A Laboratory Manual,Stockton Press,NY;以及第12和13章,Dracopoli等人(编),1994,Current Protocols in Human Genetics,John Wiley&Sons,NY.;Colberre-Garapin等人,1981,J.Mol.Biol.150:1)。

[0171] 在一些情况下,抗体的表达水平通过载体扩增而增加(关于综述,参见Bebbington和Hentschel,the use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning,第3卷.(Academic Press,New York,1987))。当表达抗体的载体系统中的标志物是可扩增的时,宿主细胞的培养物中存在的抑制剂的水平增加将使标志物基因的拷贝数增加。因为扩增区与抗体的核苷酸序列相关联,所以所述抗体的产生也将增加(Crouse等人,1983,Mol.Cell Biol.3:257)。

[0172] 在一些情况下,使用本领域中已知的用于抗体纯化的任何方法,例如,通过色谱法(例如,离子交换色谱法、亲和色谱法(特别是通过对于蛋白A之后特定抗原的亲和力)和定径柱色谱法)、离心、差别溶解度或者通过用于纯化蛋白质的任何其他标准技术。

[0173] 表达载体

[0174] 在一些实施方案中,载体包括衍生自真核或原核来源的任何合适的载体。在一些情况下,载体是从细菌(例如,大肠杆菌)、昆虫、酵母(例如,毕赤酵母)、藻类或哺乳动物来源获得的。示例性细菌载体包括pACYC177、pASK75、pBAD载体系列、pBADM载体系列、pET载体系列、pETM载体系列、pGEX载体系列、pHAT、pHAT2、pMal-c2、pMal-p2、pQE载体系列、pRSET A、pRSET B、pRSET C、pTrcHis2系列、pZA31-Luc、pZE21-MCS-1、pFLAG ATS、pFLAG CTS、pFLAG MAC、pFLAG Shift-12c、pTAC-MAT-1、pFLAG CTC或pTAC-MAT-2。

[0175] 示例性昆虫载体包括pFastBac1、pFastBac DUAL、pFastBac ET、pFastBac HTa、pFastBac HTb、pFastBac HTc、pFastBac M30a、pFastBact M30b、pFastBac、M30c、pVL1392、pVL1393、pVL1393 M10、pVL1393M11、pVL1393 M12、FLAG载体诸如pPolh-FLAG1或pPolh-MAT2或者MAT载体诸如pPolh-MAT1或pPolh-MAT2。

[0176] 在一些情况下,酵母载体包括Gateway®pDEST™14载体、Gateway®pDEST™15载体、Gateway®pDEST™17载体、Gateway®pDEST™24载体、Gateway®pYES-DEST52载体、pBAD-DEST49Gateway®目的载体、pAO815毕赤酵母属载体、pFLD1巴斯德毕赤酵母载体、pGAPZ A、B和C巴斯德毕赤酵母载体、pPIC3.5K毕赤酵母属载体、pPIC6 A、B和C毕赤酵母属载体、pPIC9K毕赤酵母属载体、pTEF1/Zeo、pYES2酵母载体、pYES2/CT酵母载体、pYES2/NTA、B和C酵母载体或pYES3/CT酵母载体。

[0177] 示例性藻类载体包括pChlamy-4载体或MCS载体。

[0178] 哺乳动物载体的实例包括瞬时表达载体或稳定表达载体。哺乳动物瞬时表达载体可以包括pRK5、p3xFLAG-CMV 8、pFLAG-Myc-CMV 19、pFLAG-Myc-CMV 23、pFLAG-CMV 2、

pFLAG-CMV 6a,b,c、pFLAG-CMV 5.1、pFLAG-CMV 5a,b,c、p3xFLAG-CMV 7.1、pFLAG-CMV 20、p3xFLAG-Myc-CMV 24、pCMV-FLAG-MAT1、pCMV-FLAG-MAT2、pBICEP-CMV 3或pBICEP-CMV 4。哺乳动物稳定表达载体可以包括pFLAG-CMV 3、p3xFLAG-CMV 9、p3xFLAG-CMV 13、pFLAG-Myc-CMV 21、p3xFLAG-Myc-CMV 25、pFLAG-CMV 4、p3xFLAG-CMV 10、p3xFLAG-CMV 14、pFLAG-Myc-CMV 22、p3xFLAG-Myc-CMV 26、pBICEP-CMV 1或pBICEP-CMV 2。

[0179] 在一些情况下,无细胞系统是来自细胞的细胞质和/或细胞核组分的混合物,并且用于体外核酸合成。在一些情况下,无细胞系统利用原核细胞组分或真核细胞组分。有时,核酸合成是在基于例如果蝇细胞、非洲爪蟾卵或HeLa细胞的无细胞系统中获得的。示例性无细胞系统包括但不限于大肠杆菌S30提取系统、大肠杆菌T7 S30系统或PURExpress®。

[0180] 宿主细胞

[0181] 在一些实施方案中,宿主细胞包括任何合适的细胞,诸如天然来源的细胞或遗传修饰的细胞。在一些情况下,宿主细胞是生产宿主细胞。在一些情况下,宿主细胞是真核细胞。在其他情况下,宿主细胞是原核细胞。在一些情况下,真核细胞包括真菌(例如,酵母细胞)、动物细胞或植物细胞。在一些情况下,原核细胞是细菌细胞。细菌细胞的实例包括革兰氏阳性细菌或革兰氏阴性细菌。有时,革兰氏阴性细菌是厌氧的、杆状的或两者兼有。

[0182] 在一些情况下,革兰氏阳性细菌包括放线菌门、厚壁菌门或软壁菌门。在一些情况下,革兰氏阳性细菌包括产水菌门(Aquificae)、异常球菌-栖热菌门(Deinococcus-Thermus)、纤维杆菌门-绿菌门/拟杆菌门(Fibrobacteres-Chlorobi/Bacteroidetes)(FCB group)、梭杆菌门(Fusobacteria)、芽单胞菌门(Gemmatimonadetes)、硝化螺旋菌门(Nitrospirae)、浮霉菌门-疣微菌门/衣原体门(Planctomycetes-Verrucomicrobia/Chlamydiae)(PVC group)、变形菌门(Proteobacteria)、螺旋体门(Spirochaetes)或互养菌门(Synergistetes)。其他细菌可以是酸杆菌门(Acidobacteria)、绿弯菌门(Chloroflexi)、产金菌门(Chrysiogenetes)、蓝细菌(Cyanobacteria)、脱铁杆菌门(Deferribacteres)、网团菌门(Dictyoglomi)、热脱硫杆菌门(Thermodesulfobacteria)或热袍菌门(Thermotogae)。细菌细胞可以是大肠杆菌(大肠埃希氏菌)、肉毒杆菌(Clostridium botulinum)或大肠杆菌(Coli bacilli)。

[0183] 示例性原核宿主细胞包括但不限于BL21、Mach1™、DH10B™、TOP10、DH5α、DH10Bac™、OmniMax™、MegaX™、DH12S™、INV110、TOP10F'、INVαF、TOP10/P3、ccdB Survival、PIR1、PIR2、Stbl2™、Stbl3™或Stbl4™。

[0184] 在一些情况下,动物细胞包括来自脊椎动物或无脊椎动物的细胞。在一些情况下,动物细胞包括来自海洋无脊椎动物、鱼类、昆虫、两栖动物、爬行动物或哺乳动物的细胞。在一些情况下,真菌细胞包括酵母细胞,诸如啤酒酵母、面包酵母或葡萄酒酵母。

[0185] 真菌包括子囊菌诸如酵母、霉菌、丝状真菌、担子菌或接合菌。在一些情况下,酵母包括子囊菌门或担子菌门。在一些情况下,子囊菌门包括酵母亚门(Saccharomycotina)(真酵母,例如酿酒酵母(面包酵母))或外囊菌亚门(Taphrinomycotina)(例如,裂殖酵母纲(裂殖酵母))。在一些情况下,担子菌门包括伞菌亚门(Agaricomycotina)(例如,银耳纲(Tremellomycetes))或柄锈菌亚门(Pucciniomycotina)(例如,微球黑粉菌纲(Microbotryomycetes))。

[0186] 示例性酵母或丝状真菌包括例如以下属:酵母菌属(Saccharomyces)、裂殖酵母属(Schizosaccharomyces)、念珠菌属(Candida)、毕赤酵母属(Pichia)、汉逊酵母属(Hansenula)、克鲁维酵母属(Kluyveromyces)、接合酵母(Zygosaccharomyces)、耶氏酵母属(Yarrowia)、丝孢酵母属(Trichosporon)、红冬孢酵母属(Rhodosporidi)、曲霉属(Aspergillus)、镰孢霉属(Fusarium)或木霉属(Trichoderma)。示例性酵母或丝状真菌包括例如以下物种:酿酒酵母(Saccharomyces cerevisiae)、粟酒裂殖酵母(Schizosaccharomyces pombe)、产朊假丝酵母(Candida utilis)、博伊丁假丝酵母(Candida boidini)、白假丝酵母(Candida albicans)、热带假丝酵母(Candida tropicalis)、星形念珠菌(Candida stellatoidea)、光滑念珠菌(Candida glabrata)、克柔念珠菌(Candida krusei)、近平滑念珠菌(Candida parapsilosis)、季蒙假丝酵母(Candida guilliermondii)、维斯假丝酵母(Candida viswanathii)、葡萄牙假丝酵母(Candida lusitaniae)、胶红酵母(Rhodotorula mucilaginosa)、甲醇毕赤酵母(Pichia metanolica)、安格斯毕赤酵母(Pichia angusta)、巴斯德毕赤酵母(Pichia pastoris)、异常毕赤酵母(Pichia anomala)、多形汉逊酵母(Hansenula polymorpha)、乳酸克鲁维酵母(Kluyveromyces lactis)、鲁氏接合酵母(Zygosaccharomyces rouxii)、解脂耶氏酵母(Yarrowia lipolytica)、茁芽丝孢酵母(Trichosporon pullulans)、圆红冬孢酵母-黑曲霉(Rhodosporidium toru-Aspergillus niger)、构巢曲霉(Aspergillus nidulans)、泡盛曲霉(Aspergillus awamori)、米曲霉(Aspergillus oryzae)、里氏木霉(Trichoderma reesei)、解脂耶氏酵母(Yarrowia lipolytica)、布鲁塞尔酒香酵母(Brettanomyces bruxellensis)、星形假丝酵母(Candida stellata)、粟酒裂殖酵母(Schizosaccharomyces pombe)、孢圆酵母(Torulaspora delbrueckii)、拜耳接合酵母(Zygosaccharomyces bailii)、新型隐球菌(Cryptococcus neoformans)、加特隐球酵母(Cryptococcus gattii)或布拉氏酵母(Saccharomyces boulardii)。

[0187] 示例性酵母宿主细胞包括但不限于巴斯德毕赤酵母菌株,诸如GS115、KM71H、SMD1168、SMD1168H和X-33;以及酿酒酵母菌株,诸如INVSc1。

[0188] 在一些情况下,额外的动物细胞包括从软体动物、节肢动物、蜥蜴或海绵动物中获得的细胞。在一些情况下,额外的动物细胞是哺乳动物细胞,例如,来自灵长类动物、猿类、马、牛、猪、犬科动物、猫科动物或啮齿动物。在一些情况下,啮齿动物包括小鼠、大鼠、仓鼠、沙鼠、仓鼠、栗鼠、花枝鼠或豚鼠。

[0189] 示例性哺乳动物宿主细胞包括但不限于293A细胞系、293FT细胞系、293F细胞、293H细胞、CHO DG44细胞、CHO-S细胞、CHO-K1细胞、FUT8 KO CHOK1、Expi293F™细胞、Flp-In™T-REx™293细胞系、Flp-In™-293细胞系、Flp-In™-3T3细胞系、Flp-In™-BHK细胞系、Flp-In™-CHO细胞系、Flp-In™-CV-1细胞系、Flp-In™-Jurkat细胞系、FreeStyle™293-F细胞、FreeStyle™CHO-S细胞、GripTite™293MSR细胞系、GS-CHO细胞系、HepaRG™细胞、T-REx™Jurkat细胞系、Per.C6细胞、T-REx™-293细胞系、T-REx™-CHO细胞系和T-REx™-HeLa细胞系。

[0190] 在一些情况下,哺乳动物宿主细胞是稳定细胞系,或者是将感兴趣的遗传物质整合到其自身基因组中并且能够在多代细胞分裂之后表达遗传物质的产物的细胞系。在一些情况下,哺乳动物宿主细胞是瞬时细胞系,或者是没有将感兴趣的遗传物质整合到其自身

基因组中并且不能在多代细胞分裂之后表达遗传物质的产物的细胞系。

[0191] 示例性昆虫宿主细胞包括但不限于果蝇S2细胞、Sf9细胞、Sf21细胞、High Five™细胞和expresSF+®细胞。

[0192] 在一些情况下,植物细胞包括来自藻类的细胞。示例性昆虫细胞系包括但不限于来自莱茵衣藻137c(Chlamydomonas reinhardtii 137c)或细长聚球藻PPC 7942(Synechococcus elongatus PPC 7942)的菌株。

[0193] 编号实施方案

[0194] 编号实施方案1包括一种用于检测来自个体的样品中的磷酸化tau的方法,所述方法包括:使用包含重链区可变结构域(VH)和轻链区可变结构域(VL)的抗体或抗体片段对所述样品进行免疫测定,其中所述VH包含与如SEQ ID NO:30-34中任一个中所示的序列具有至少约90%同一性的氨基酸序列,并且其中所述VL包含与如SEQ ID NO:35-40中任一个中所示的序列具有至少约90%同一性的氨基酸序列。编号实施方案2包括如编号实施方案1所述的方法,其中所述磷酸化tau选自pTau-181、pTau-212、pTau-217、pTau-231、pTau-214和pTau-220。编号实施方案3包括如编号实施方案1-2所述的方法,其中所述磷酸化tau是pTau-217。编号实施方案4包括如编号实施方案1-2所述的方法,其中所述磷酸化tau是pTau-231。编号实施方案5包括如编号实施方案2所述的方法,其中所述方法检测pTau-217和pTau-231。编号实施方案6包括如编号实施方案2所述的方法,其中所述方法检测pTau-212和pTau-217。编号实施方案7包括如编号实施方案2所述的方法,其中所述方法检测pTau-212和pTau-231。编号实施方案8包括如编号实施方案2所述的方法,其中所述方法检测pTau-181和pTau-217。编号实施方案9包括如编号实施方案2所述的方法,其中所述方法检测pTau-181和pTau-231。编号实施方案10包括如编号实施方案2所述的方法,其中所述方法检测pTau-181、pTau-217和pTau-231。编号实施方案11包括如编号实施方案2所述的方法,其中所述方法检测pTau-212、pTau-217和pTau-231。编号实施方案12包括如编号实施方案5所述的方法,其中所述方法检测选自血浆样品和血清样品的样品中的pTau-217和pTau-231。编号实施方案13包括如编号实施方案6所述的方法,其中所述方法检测选自血浆样品和血清样品的样品中的pTau-212和pTau-217。编号实施方案14包括如编号实施方案7所述的方法,其中所述方法检测选自血浆样品和血清样品的样品中的pTau-212和pTau-231。编号实施方案15包括如编号实施方案11所述的方法,其中所述方法检测选自血浆样品和血清样品的样品中的pTau-181和pTau-217。编号实施方案16包括如编号实施方案11所述的方法,其中所述方法检测选自血浆样品和血清样品的样品中的pTau-181和pTau-231。编号实施方案17包括如编号实施方案11所述的方法,其中所述方法检测选自血浆样品和血清样品的样品中的pTau-181、pTau-217和pTau-231。编号实施方案18包括如编号实施方案11所述的方法,其中所述方法检测选自血浆样品和血清样品的样品中的pTau-212、pTau-217和pTau-231。编号实施方案19包括如编号实施方案1-18所述的方法,其中所述VH包含根据SEQ ID NO:30-34中任一个的氨基酸序列。编号实施方案20包括如编号实施方案1-19所述的方法,其中所述VL包含根据SEQ ID NO:35-40中任一个的氨基酸序列。编号实施方案21包括如编号实施方案1-20所述的方法,其中所述VH包含根据SEQ ID NO:30-34中任一个的氨基酸序列,并且其中所述VL包含根据SEQ ID NO:35-40中任一个的氨基酸序列。编号实施方案22包括如编号实施方案1-21所述的方法,其中所述VH包含与SEQ ID NO:30具有至少约90%同

一性的氨基酸序列,并且其中所述VL包含与SEQ ID NO:35具有至少约90%同一性的氨基酸序列。编号实施方案23包括如编号实施方案1-21所述的方法,其中所述VH包含与SEQ ID NO:31具有至少约90%同一性的氨基酸序列,并且其中所述VL包含与SEQ ID NO:36具有至少约90%同一性的氨基酸序列。编号实施方案24包括如编号实施方案1-21所述的方法,其中所述VH包含与SEQ ID NO:31具有至少约90%同一性的氨基酸序列,并且其中所述VL包含与SEQ ID NO:37具有至少约90%同一性的氨基酸序列。编号实施方案25包括如编号实施方案1-21所述的方法,其中所述VH包含与SEQ ID NO:32具有至少约90%同一性的氨基酸序列,并且其中所述VL包含与SEQ ID NO:38具有至少约90%同一性的氨基酸序列。编号实施方案26包括如编号实施方案1-21所述的方法,其中所述VH包含与SEQ ID NO:33具有至少约90%同一性的氨基酸序列,并且其中所述VL包含与SEQ ID NO:39具有至少约90%同一性的氨基酸序列。编号实施方案27包括如编号实施方案1-21所述的方法,其中所述VH包含与SEQ ID NO:34具有至少约90%同一性的氨基酸序列,并且其中所述VL包含与SEQ ID NO:40具有至少约90%同一性的氨基酸序列。编号实施方案28包括如编号实施方案1-27所述的方法,其中所述抗体或抗体片段包含与SEQ ID NO:41-51中任一个具有至少约90%同一性的氨基酸序列。编号实施方案29包括如编号实施方案1-28所述的方法,其还包括对所述样品进行测定,以确定选自以下的生物标志物的水平:Aβ42、Aβ40、Aβ38、BACE1、hFABP、TREM2、YKL-40、IP-10、神经颗粒蛋白、SNAP-25、突触结合蛋白、α-突触核蛋白、TDP-43、铁蛋白、VILIP-1、NfL、GFAP及其组合。编号实施方案30包括如编号实施方案1-29所述的方法,其中所述样品选自血液样品、血浆样品、血清样品和脑脊液(CSF)样品。编号实施方案31包括如编号实施方案1-30所述的方法,其还包括基于磷酸化tau的检测确定所述个体的阿尔茨海默病。编号实施方案32包括如编号实施方案1-31所述的方法,其还包括基于磷酸化tau的检测确定所述个体发展阿尔茨海默病的预后。编号实施方案33包括如编号实施方案32所述的方法,其还确定所述个体的年龄、基因型或生物标志物表达。编号实施方案34包括如编号实施方案33所述的方法,其中所述生物标志物选自Aβ42、Aβ40、Aβ38、BACE1、hFABP、TREM2、YKL-40、IP-10、神经颗粒蛋白、SNAP-25、突触结合蛋白、α-突触核蛋白、TDP-43、铁蛋白、VILIP-1、NfL、GFAP及其组合。编号实施方案35包括如编号实施方案1-34所述的方法,其中所述方法对于检测磷酸化tau具有至少约80%的特异性。编号实施方案36包括如编号实施方案1-34所述的方法,其中所述方法对于检测磷酸化tau具有至少约85%的特异性。编号实施方案37包括如编号实施方案1-34所述的方法,其中所述方法对于检测磷酸化tau具有至少约90%的特异性。编号实施方案38包括如编号实施方案1-37所述的方法,其中所述方法对于检测磷酸化tau具有至少约80%的灵敏度。编号实施方案39包括如编号实施方案1-37所述的方法,其中所述方法对于检测磷酸化tau具有至少约85%的灵敏度。编号实施方案40包括如编号实施方案1-37所述的方法,其中所述方法对于检测磷酸化tau具有至少约90%的灵敏度。编号实施方案41包括如编号实施方案1-40所述的方法,其中所述方法能够以至少约1.0皮克/毫升(pg/mL)的检测限值检测所述样品中的磷酸化tau。编号实施方案42包括如编号实施方案1-40所述的方法,其中所述方法能够以至少约1.5皮克/毫升(pg/mL)的检测限值检测所述样品中的磷酸化tau。编号实施方案43包括如编号实施方案1-40所述的方法,其中所述方法能够以至少约5皮克/毫升(pg/mL)的检测限值检测所述样品中的磷酸化tau。

[0195] 编号实施方案44包括一种抗tau抗体,其包含i)包含可变重链(VH)结构域的重链

和ii)包含可变轻链(VL)结构域的轻链,其中所述VH结构域包含具有选自SEQ ID NO:1-5的序列的HCDR1序列、具有选自SEQ ID NO:6-9的序列的HCDR2序列、和具有选自SEQ ID NO:10-13的序列的HCDR3序列,并且VL结构域包含具有选自SEQ ID NO:14-19的序列的LCDR1序列、具有选自SEQ ID NO:20-23的序列的LCDR2序列、和具有选自SEQ ID NO:24-29的序列的LCDR3序列。编号实施方案45包括如编号实施方案44所述的抗tau抗体,其中所述HCDR1序列包含SEQ ID NO:1,HCDR2序列包含SEQ ID NO:6,HCDR3序列包含SEQ ID NO:10,LCDR1序列包含SEQ ID NO:14,LCDR2序列包含SEQ ID NO:20,并且LCDR3序列包含SEQ ID NO:24。编号实施方案46包括如编号实施方案44所述的抗tau抗体,其中所述HCDR1序列包含SEQ ID NO:2,HCDR2序列包含SEQ ID NO:7,HCDR3序列包含SEQ ID NO:11,LCDR1序列包含SEQ ID NO:15,LCDR2序列包含SEQ ID NO:21,并且LCDR3序列包含SEQ ID NO:25。编号实施方案47包括如编号实施方案44所述的抗tau抗体,其中所述HCDR1序列包含SEQ ID NO:2,HCDR2序列包含SEQ ID NO:7,HCDR3序列包含SEQ ID NO:11,LCDR1序列包含SEQ ID NO:16,LCDR2序列包含SEQ ID NO:22,并且LCDR3序列包含SEQ ID NO:26。编号实施方案48包括如编号实施方案44所述的抗tau抗体,其中所述HCDR1序列包含SEQ ID NO:3,HCDR2序列包含SEQ ID NO:8,HCDR3序列包含SEQ ID NO:10,LCDR1序列包含SEQ ID NO:17,LCDR2序列包含SEQ ID NO:20,并且LCDR3序列包含SEQ ID NO:27。编号实施方案49包括如编号实施方案44所述的抗tau抗体,其中所述HCDR1序列包含SEQ ID NO:4,HCDR2序列包含SEQ ID NO:7,HCDR3序列包含SEQ ID NO:12,LCDR1序列包含SEQ ID NO:18,LCDR2序列包含SEQ ID NO:23,并且LCDR3序列包含SEQ ID NO:28。编号实施方案50包括如编号实施方案44所述的抗tau抗体,其中所述HCDR1序列包含SEQ ID NO:5,HCDR2序列包含SEQ ID NO:9,HCDR3序列包含SEQ ID NO:13,LCDR1序列包含SEQ ID NO:19,LCDR2序列包含SEQ ID NO:21,并且LCDR3序列包含SEQ ID NO:29。编号实施方案51包括如编号实施方案44所述的抗tau抗体,其中所述VH结构域与选自SEQ ID NO:30-34的序列具有至少80%、至少85%、至少90%、至少95%序列同一性。编号实施方案52包括如编号实施方案44所述的抗tau抗体,其中所述VL结构域与选自SEQ ID NO:35-40的序列具有至少80%、至少85%、至少90%、至少95%序列同一性。

[0196] 编号实施方案53包括如编号实施方案44-52所述的抗tau抗体,其中所述抗tau抗体是嵌合抗体或其抗原结合片段。编号实施方案54包括如编号实施方案44-53所述的抗tau抗体,其中所述抗tau抗体包括IgG-scFv、纳米抗体、BiTE、双链抗体、DART、TandAb、scDiabody、scDiabody-CH3、三体、微型抗体、微抗体、TriBi微抗体、scFv-CH3KIH、Fab-scFv-Fc KIH、Fab-scFv、scFv-CH-CL-scFv、Fab'、F(ab')2、F(ab')3、F(ab')2-scFv2、scFv、scFv-KIH、Fab-scFv-Fc、四价HCAb、scDiabody-Fc、双链抗体-Fc、串联scFv-Fc或内抗体。编号实施方案55包括如编号实施方案44-54所述的抗tau抗体,其中所述抗tau抗体是IgG1抗体。编号实施方案56包括如编号实施方案44-55所述的抗tau抗体,其中所述抗tau抗体是IgG2抗体。编号实施方案57包括如编号实施方案44-56所述的抗tau抗体,其中所述抗tau抗体是IgG4抗体。编号实施方案58包括如编号实施方案44-57所述的抗tau抗体,其中所述轻链是κ链。编号实施方案59包括如编号实施方案44-58所述的抗tau抗体,其中所述抗tau抗体对于人tau具有约100pM至约3nM的结合亲和力。编号实施方案60包括如编号实施方案44-59所述的抗tau抗体,其中所述抗tau抗体包含由核酸编码的VH结构域,所述核酸与选自SEQ ID NO:52-56的序列具有至少80%、至少85%、至少90%、至少95%序列同一性。编号实施方

案61包括如编号实施方案44-60所述的抗tau抗体,其中所述抗tau抗体包含由核酸编码的VL结构域,所述核酸与选自SEQ ID NO:57-62的序列具有至少80％、至少85％、至少90％、至少95％序列同一性。编号实施方案62包括如编号实施方案44-61所述的抗tau抗体,其中所述抗tau抗体包含由与选自SEQ ID NO:52-56的序列具有至少80％、至少85％、至少90％、至少95％序列同一性的核酸编码的VH结构域和由与选自SEQ ID NO:57-62的序列具有至少80％、至少85％、至少90％、至少95％序列同一性的核酸编码的VL结构域。编号实施方案63包括如编号实施方案44-62所述的抗tau抗体,其中所述抗tau抗体包含由核酸编码的VH结构域,所述核酸包含与SEQ ID NO:52-56相同的序列。编号实施方案64包括如编号实施方案44-63所述的抗tau抗体,其中所述抗tau抗体包含由核酸编码的VL结构域,所述核酸包含与SEQ ID NO:57-62相同的序列。编号实施方案65包括如编号实施方案44-64所述的抗tau抗体,其中所述抗tau抗体包含由包含与SEQ ID NO:52-56相同的序列的核酸编码的VH结构域和由包含与SEQ ID NO:57-62相同的序列的核酸编码的VL结构域。

[0197] 实施例

[0198] 给出以下实施例是出于说明本发明的各种实施方案的目的,并不意味着以任何方式限制本发明。本发明实施例连同本文所述的方法目前代表优选的实施方案,是示例性的,并且不意图作为对本发明范围的限制。本领域技术人员将会想到包含在如权利要求的范围所限定的本发明精神内的其中的变化和其他用途。

[0199] 实施例1:Tau抗体筛选

[0200] 根据制造商的说明书,在Simoa®珠测定中使用2步或3步方案测定来测定检测磷酸化Tau的Tau抗体。参见图1。

[0201] 所测试的抗体是抗体1、抗体2、抗体3、抗体4、抗体5和抗体6。捕获结果可以见于图2A-2D。图2A是来自2步方案测定的数据,其中所有捕获抗体都针对Tau-12检测器(检测N末端的Tau)进行测试。数据表明所有捕获都具有类似的结果,在抗体6的情况下看到改善的灵敏度。图2B是来自2步方案测定的数据,其中所有捕获抗体都针对HT7-BT2检测器(检测中结构域区域的Tau)进行测试。使用了两种生物素化抗体。数据显示与Tau-12检测器相比,背景增加了约10倍。在1000pg/mL下未检测到任何捕获的信号。图2C是来自3步方案测定的数据,其中所有捕获抗体都针对Tau-12检测器进行测试。数据表明与2步方案相比,灵敏度降低,并且在抗体6的情况下看到改善的灵敏度。图2D是来自3步方案测定的数据,其中所有捕获抗体都针对HT7-BT2检测器进行测试。使用了两种生物素化抗体。数据显示与Tau-12检测器相比,背景增加了约10倍。基于所述结果,对2步方案的灵敏度进行进一步优化。

[0202] 实施例2.Tau抗体的药代动力学

[0203] 测试抗体的药代动力学谱。

[0204] 抗体的抗原信息见于表10。

[0205] 表10.

[0206]

描述	抗原名称	SEQ ID NO:	序列
pT217a	WZN-1A	74	RSRTPSLP(pT)PPTREPKC
pT217b	WZN-1B	75	TPSLP(pT)PPTREPKKVAC
T217	WZN-1C	76	RSRTPSLPTPPTREPKKVAC
pT212	WZN-1D	77	RSR(pT)PSLPTPPTREPKC
pS214	WZN-1E	78	RSRTP(pS)LPTPPTREPKC
pT220	WZN-1F	79	RSRTPSLPTPP(pT)REPKKVAC
pT231	WZN-1G	80	KVAVVR(pT)PPKSPSSAC
T231	WZN-1H	81	KVAVVRTPPKSPSSAC

[0207] 生成抗体并进行纯化。使用标准间接ELISA方案测定抗体。简而言之,将对应于SEQ ID NO:74-81的肽抗原在PBS中稀释至1ug/ml,并铺板到Greiner Bio One Microlon 96孔板上。肽抗原由制造商Abcam产生。WZN-1A和WZN-1B充当靶标。WZN-1C、WZN-1D、WZN-1E、WZN-1F、WZN-1G和WZN-1H充当阴性对照。在肽抗原序列中,磷酸化残基通过(pT)指示磷酸化苏氨酸或通过(pS)指示磷酸化丝氨酸。在用在pH 7.4的PBS中的1％ BSA封闭之后,将抗体以1ug/ml的初始浓度连续稀释1至4。在温育之后,用1X TBST洗去未结合的抗体,并且根据制造商的说明书应用HRP标记的山羊抗兔第二抗体。随后,用1X TBST洗去未结合的第二抗体,并且在室温下应用3,3’5,5’-四甲基联苯胺(TMB)5分钟,并在650nm处读取板。数据见于图3。图3示出了不同肽浓度的不同单克隆抗体的筛选数据。

[0208] 实施例3.用于免疫组织化学的Tau抗体

[0209] 在免疫组织化学测定中测试本文所述的Tau抗体。

[0210] 简而言之,使用一系列浓度(0.01-3.00ug/ml)优化所有抗体并使用Leica Bond RX自动IHC平台进行染色:在100℃下进行ER1抗原修复(柠檬酸钠,pH 6)20min;第一抗体在RT下15分钟;在室温下进行IVD级Leica Polymer Refine HRP检测8分钟;DAB发色团在室温下10分钟,并且最后在室温下进行苏木精复染5分钟。通过碱性IHC染色的抗体在碱性磷酸酶(AP)处理(200U/ml,37℃,持续60分钟)后继续进行IHC染色。还采用了仅媒介物对照(不含AP的缓冲液)。阳性抗原对照组织是FFPE正常人大脑皮层和来自阿尔茨海默病患者的大脑皮层。阴性抗原对照组织是FFPE正常人肝脏、骨骼肌和心肌。将所有组织整理成组织微阵列,以简化IHC染色过程。采用阴性试剂(仅检测系统)对照并显示为阴性。与测试抗体一起染色的基准抗体是针对Tau的兔单克隆[EPR22524-95](ab254256,Abcam plc)和针对Tau(磷酸S214)的兔单克隆[EPR1884(2)](ab170892,Abcam plc)。

[0211] 基准抗体证明抗体在阴性对照组织中表现出阴性染色并且在阳性对照组织中表现出阳性染色(数据未示出)。Tau抗体的数据见于图4A-4G(抗体6)、图5A-5G(抗体5)和图6A-6G(抗体2)。抗体5和抗体6显示与基准抗体类似的数据,即在阴性对照组织中观察到阴性染色,所述阴性对照组织包括正常心脏、正常肝脏和正常骨骼肌以及正常大脑皮层,并且在阳性对照组织中观察到阳性染色,所述阳性对照组织包括阿尔茨海默病大脑皮层。抗体

1-4没有表现出与基准抗体类似的数据。

[0212] 实施例4.使用Tau抗体检测Tau肽

[0213] 在ELISA测定中测试本文所述的检测磷酸化Tau的Tau抗体。首先通过间接ELISA测试Tau抗体的pTau 217反应性。图7展示了描绘所使用的间接ELISA测定格式的图。简而言之,将链霉亲和素珠与板结合,并且在允许生物素-链霉亲和素结合的条件下将生物素化的肽添加到板上。生物素化肽是一种合成肽,其包含一部分Tau并在位置217处具有磷酸化苏氨酸残基(pT217)。这是靶肽。在通过生物素-链霉亲和素复合物的形成将合成肽与板结合之后,在允许抗体-靶肽结合的条件下将Tau抗体添加到板上。在结合之后,洗涤板以去除任何未结合的抗体,然后向板添加针对衍生Tau抗体的物种的第二抗体或示踪抗体(与过氧化物酶缀合的山羊抗小鼠抗体或与过氧化物酶缀合的驴抗兔抗体)。在结合之后,洗涤板以去除任何未结合的示踪抗体,接下来向板添加TMB ELISA过氧化物酶发色底物(3,3',5,5'-四甲基联苯胺)以在间接ELISA实验中使抗体反应性可视化。使用ELISA微板读取器对抗体样品结合进行定量。

[0214] 如图7所示,使用这种间接ELISA技术测试了五种抗体检测磷酸化Tau的能力。IBA493 mAb对应于能够与在苏氨酸残基217处磷酸化的Tau(pTau 217)结合的兔抗Tau抗体(Eli Lilly and Company)。PT3对应于小鼠抗磷酸化(T212/T217)Tau选择性抗体(Janssen Biotech Inc.)。30H10L2对应于本文所述的抗体2。71H1L2对应于本文所述的抗体6。62H10L7对应于本文所述的抗体5。在两个单独的ELISA仪器中,以以下浓度测定所有五种抗体与pTau 217的反应性:10^{-2}、10^{-1}、10^{-0}、10^{1}、10^{2}、10^{3}和10^{4}ng/mL/板。如可以在Bio-pt655(磷酸化T217)和Bio-pt660(磷酸化T217)图中所见,IBA493 mAb和PT3均表现出对pTau 217的稳健的浓度依赖性水平的反应性。抗体2对pTau 217表现出更适度的浓度依赖性水平的反应性,在10^{4}ng/mL/板下揭示。抗体5和抗体6在这些测定中没有表现出对pTau217的反应性。图7中显示针对磷酸酶处理的pTau使用五种测试抗体的此ELISA测定的结果的图证明了抗体IBA493 mAb、PT3和抗体2在检测磷酸化Tau方面的特异性。

[0215] 在基于Simoa®的测定中测试本文所述的检测磷酸化Tau的Tau抗体。图8-24展示了Simoa®测定的结果,所述测定被设计来对本文所述的抗体的Tau反应性进行灵敏度测试。在一些方面,与间接ELISA测定中检测相同分析物相比,基于Simoa®的测定可以大约1000X更大的灵敏度检测给定分析物。基于Simoa®的测定的这种升高的灵敏度允许开发和使用先前使用传统测定(诸如间接ELISA)无法生成可检测信号的生物标志物。与常规免疫测定(诸如间接ELISA)相比,基于Simoa®的测定的灵敏度升高是由于以下事实:Simoa®方法能够检测单个靶分子,而传统免疫测定通常需要大的反应体积和数百万个荧光团,或数百万个抗体缀合酶与产色底物反应,然后才可以检测光信号。对于基于Simoa®的测定,平均酶/珠(AEB)代表原始信号输出。

[0216] 在图8中,在基于Simoa®的测定中使用如本文所述的能够检测pTau 217的抗体2,以检测分析物水平/来源于个体的血浆样品。通过Simoa®确定每个样品的信噪(S/N)比并绘制在图中。从个体中取120个血浆样品并进行测定。绘制的S/N比指示,除一个样品外,所有测试样品都产生了预期浓度范围内的信号。当血浆样品以1:3稀释,然后再次测定

时,120个样品中只有3个产生的结果低于空白对照的测量值,并且只有5个样品显示S/N 1.5的测量值,这被确定为检测限值(LOD)。在图8中,对于所测定的120个血浆样品中的每一个,进行计算以确定每个样品的变异系数(CV%),并且将结果针对测量的浓度进行作图。120个样品中的10个样品产生大于20的CV%,并且根据此分析,确定估计的分析定量下限(LLOQ)为0.08pg/mL。此LLOQ值代表可以以可接受的精确性水平定量确定的分析物(在T217处磷酸化的Tau)的最低量。图8中的这些结果指示Simoa®方法使用抗体2检测在T217处磷酸化的Tau的灵敏度。

[0217] 在图9中,使用68个CSF样品和120个血浆样品(使用抗体2将其分到4个板中并且使用ADx神经科学抗体ADx204将其分到4个板中),生成Simoa®pTau-217测定的校准曲线并作图[AEB相对于log(CAL)pg/mL]。在另一个在单独仪器上进行的此测定的图[AEB相对于log(CAL)pg/mL]中,以对数标度绘制的AEB表明,在测量样品时,数据拟合可以实现准确的分析物定量计算。

[0218] 在图10中,使用抗体2,用Simoa® pTau-217测定测量38个配对CSF和EDTA血浆样品。此测定还被称为ALZpath Dx。对结果进行作图,并且用临床诊断(非AD、不确定或AD)来指示样品。这些结果及其统计分析指示,CSF与血浆pTau水平之间有很强的相关性,如使用抗体2的Simoa®pTau-217测定测量(非AD与AD之间双尾T检验的R值为~0.7并且P值<0.0001)。

[0219] 在图11中,用Simoa®pTau-217测定(使用抗体2)和Simoa®pTau-181测定(使用来自Quanterix®(Quanterix®Corp.,项目号103714)的pTau-181抗体)测量42个CSF样品并针对彼此进行绘图。这表明,使用抗体2的Simoa®pTau-217测定显示出与在CSF中检测到的AD相关分析物(pTau-181)的预期关系。统计分析指示双尾T检验的R值为~0.8并且P值<0.0001。

[0220] 在图12中,用Simoa® pTau-217测定(使用抗体2)和Simoa®pTau测定(使用Innotest pTau-181抗体)测量42个CSF样品并针对彼此进行绘图。这表明,使用抗体2的Simoa®pTau-217测定显示出与在CSF中检测到的AD相关分析物(pTau)的预期关系。统计分析指示双尾T检验的R值为~0.77并且P值<0.0001。

[0221] 在图13中,用Simoa®HD-X测定测量42个CSF样本,使用抗体2作为捕获抗体,使用ADx204抗体作为检测器,并且使用肽作为校准器。这表明,使用已知AD生物标志物的Simoa®测定显示出预期的关系。统计分析指示双尾T检验的R值为~0.9并且P值<0.0001。

[0222] 在图14中,使用抗体2,用Simoa®pTau-217测定测量CSF样品和血浆样品并在单独图中进行作图。使用AD的临床诊断或没有AD诊断的对照作为每个样品的分类器。对作图结果的分析指示,对于CSF样品和血浆样品两者,来源于临床AD诊断的个体相对于对照的样品之间存在显著差异。CSF样品的曲线下面积(AUC)计算值为0.94,并且血浆样品为0.86。这些结果指示,使用抗体2的Simoa®pTau-217测定能够区分CSF和血浆中的AD病例。

[0223] 在图15中,4个具有高pTau水平的EDTA血浆样品充当质量对照(标记为QC_L1、QC_

L2、QC_M和QC_H),使用抗体2,用Simoa®pTau-217测定在一式两份测试中进行测量并计算pTau水平。绘制来自重复测试的结果证明了使用抗体2的Simoa®pTau-217测定的精确性和再现性。

[0224] 在图16中,使用抗体2,用Simoa®pTau-217测定测量来自图15的对照样品和额外的测量样品并在两个单独实验中绘图以生成精确性谱。精确性谱是基于测量的样品浓度和4个QC样品的内部运行CV%。根据此实验,确定此测定中pTau-217的功能LLOQ为0.26pg/mL。

[0225] 在图17中,使用抗体2,在Simoa®pTau-217测定中评估并行性。并行性的确定也很重要,因为它显示信号是否是特异性的。并行性确定在稀释之后的标准曲线中,含有高内源性分析物浓度的实际样品在测定中是否提供相同程度的检测。这可以表示抗体对内源性分析物和标准或校准分析物的结合亲和力的差异。这可以确保重组标准物平行于内源性分析物的天然鉴定。在此实验中,从3X的稀释度开始,在5个步骤中用因子2稀释4个血浆样品,每个样品来自不同的供体,具有相对高浓度的检测的pTau-217和加标稀释缓冲液(样品5)。从稀释因子12X开始,所有4个血浆样品的浓度均降至低于LLOD。在log(测量的pg/mL)相对于log[稀释因子(DF)]的图中,血浆测量值与尖峰测量值之间的关系表明,在各种稀释度下,检测呈线性。4个血浆样品中的3个被确定为落在可接受的并行性范围内,样品4正好落在可接收的范围之外。可接受的并行性范围<15%。这些结果表明,对血浆样品使用抗体2的Simoa®pTau-217测定在不同浓度下产生一致且精确的pTau-217水平计算,从而证明了其作为生物标志物测定的实用性。

[0226] 在图18-19中,使用抗体2的Simoa®pTau-217测定进行稀释线性,以证明尖峰浓度为约定量上限(ULOQ)的样品可以稀释至工作范围内的浓度,同时仍能产生可靠的测定结果。在图18中,对三个加标样品(s1、s2和s3)和校准样品进行测定,并将其绘制为log(测量的pg/mL)相对于log(DF),以确定稀释线性。在图19中,对三个加标样品(s1、s2和s3)和校准样品进行测定,并将其绘制为log(测量的pg/mL)相对于log(DF),以确定稀释线性,从s1、s2和s3中省略最高尖峰点,因为它处于50pg/mL的校准范围之外。

[0227] 在图20中,使用抗体2的Simoa®pTau-217测定用于评估从记忆临床队列中采集的样品。测量血浆样品并针对计算的pTau217浓度进行作图。使用AD的临床诊断为分类器。AUC计算为0.916,指示使用抗体2的Simoa®pTau-217测定成功将此队列中的AD+与AD-区分开。同样在图20中,对受试者操作特征(ROC)曲线进行绘图,以说明此二元分类器系统的诊断能力(AD+或AD-),因为分类器之间的判别阈值是不同的。

[0228] 在图21-22中,将使用抗体2的Simoa®pTau-217测定的临床性能与使用抗体P-tau181-Quanterix®的Simoa®pTau-181测定进行比较。在图21中,抗体2能够区分取自AD痴呆个体与对照的血浆样品(抗体2的P值为$1.3e^{-12}$)。在图22中,可商购获得的P-tau181-Quanterix® Simoa®测定(Quanterix®Corp.,项目号103714)也能够区分取自AD痴呆个体与对照的血浆样品(P值为$9.6e^{-08}$)。图21-22中数据的样品所来源于的个体的数据在图22中列出。

[0229] 在图23中,使用P-tau217抗体2和P-tau181-Quanterix®抗体(Quanterix®

Corp.,项目号103714)生成Simoa®测定的精确性图。计算的LLOD分别为0.55pg/mL和0.24pg/mL。没有对浓度进行反计算,并且因此也没有对LLOD值进行反计算。

[0230] 在图24中,针对P-tau181-Quanterix®抗体(Quanterix® Corp.,项目号103714)和P-tau217抗体2绘制ROC曲线。数据分析指示,在将AD痴呆与对照区分开方面,与使用P-tau181-Quanterix®抗体的Simoa®测定相比,P-tau217抗体2表现出更优异的灵敏度和特异性。针对血浆样品进行测试时,此Simoa®方法中抗体2对AD痴呆的诊断准确性为92.5%。针对血浆样品进行测试时,此Simoa®方法中抗体2对AD痴呆的诊断特异性为85%。

[0231] 在图25中,描绘了指示各种蛋白质结构域的相对位置和苏氨酸残基的位置的Tau多肽的示意图,其可以使用本文公开的方法测定磷酸化状态。指示了Tau的P2结构域内pT217的位置。pT181位于P1结构域内,并且pT231位于P2与R1结构域之间的边界附近。

[0232] 在图26中,使用间接ELISA测定各种Tau抗体,并且对对于具有非磷酸化T217(Bio-pt654)的Tau片段和全长Tau(Tau441)的反应性程度进行作图。IBA493 mAB和PT3对于Bio-pt654和Tau441均展示出浓度依赖性反应性。本文所述的抗体2、5和6在此测定中对于Bio-pt654或Tau441均未展示出任何反应性,从而证明了抗体2、5和6的pTau-217检测的精确性和特异性。

[0233] 在图27中,使用间接ELISA测定各种Tau抗体,并且对对于具有磷酸化T181(Bio-pt126)和磷酸化T231(Bio-pt146)的Tau片段的反应性程度进行作图。本文所述的IBA493 mAB和抗体2对于Bio-pt126展示出浓度依赖性反应性。IBA493 mAB是所测试的抗体中对于Bio-pt146展示出浓度依赖性反应性的唯一抗体。这表明基于每种抗体经由间接ELISA与哪些分析物相互作用,本文所述的IBA493mAB、PT3和抗体2各自是可区分的。IBA493 mAB与pTau-217、非磷酸化T217、全长Tau、pTau-181和pTau-231相互作用。PT3与pTau-217、非磷酸化T217和全长Tau相互作用。抗体2与pTau-217和pTau-181相互作用。还显示IBA493 mAB与非磷酸化T217的相互作用明显小于PT3与非磷酸化T217的交互作用。

[0234] 在图28中,描绘了使用抗体2检测特定Tau肽的捕获的测定的图。在此测定中,抗体2与板结合,并且在有利于形成特异性抗体-配体相互作用的条件下将来自板的样品孔经受各种生物素化肽。然后洗涤样品以去除过量的未结合的生物素化肽。链霉亲和素珠与过氧化物酶缀合,然后添加样品中,允许生物素-链霉亲和素复合物在肽结合的抗体上形成。然后添加$TMB_{还原}$底物,并且使用ELISA板读取器测量样品的比色显影。对结果进行绘图,在所测试的各种pTau-217、pTau-231和pTau-181肽中,Adx-pt655产生特定的剂量依赖性反应性。这些结果说明了抗体2对于pTau(即,在苏氨酸217处磷酸化的Tau)的特定特征的特异性。在相同条件下使用相同的Tau肽通过间接ELISA测试的抗体5和抗体6对于所测试的Tau肽没有产生特定的剂量依赖性反应性。

[0235] 还在中尺度发现技术平台上评价对应于在此所述的抗体1、抗体2、抗体3、抗体4、抗体5和抗体5的各种pTau-217抗体,作为直接涂覆到或涂覆到链霉亲和素涂覆板上的捕获抗体。此系统使用非放射性的电化学发光标记,从而赋予与传统ELISA测定相比的显著优势。这些优势包括较低的背景信号、改善的灵敏度和动态检测范围。

[0236] 在图29中,使用蛋白质印迹来评估各种抗体与来自AD患者和对照对象的脑裂解液

样品的结合。在所示的五个蛋白质印迹中,根据图29中所示的相同样品密钥加载样品。在泳道1和10中运行蛋白质梯。在泳道2中运行以0.05ug的量加载的磷酸酶处理的pTau。在泳道3中运行以0.05ug的量加载的全长Tau(Tau411)。泳道4-6含有来自不同对照对象的稀释因子为5的样品。泳道7-9含有来自不同AD对象的稀释因子为5的样品。结果指示,IBA394 mAb和PT3均与对照样品和AD样品中不同长度的Tau亚型结合并使其免疫沉淀,并且在AD样品中使显著更多的Tau免疫沉淀,同时与合成的全长Tau或磷酸酶处理的pTau没有显示相互作用。抗体2(30H2L10)与AD样品中不同长度的Tau亚型结合并使其免疫沉淀,但在来自对照个体的样品中没有使显著量的Tau免疫沉淀。抗体5和抗体6在此测定中没有产生可检测的蛋白质印迹信号。

[0237] 虽然本文已经示出并描述了本公开的优选实施方案,但对于本领域技术人员显而易见的是,此类实施方案仅以示例的方式提供。在不脱离本公开的情况下,本领域技术人员将会想到许多变化、改变和替换。应理解,在实践本公开时可以采用本文所述的本公开的实施方案的各种替代方案。旨在以所附权利要求书限定本公开的范围,并且由此涵盖这些权利要求范围内的方法和结构及其等同方案。

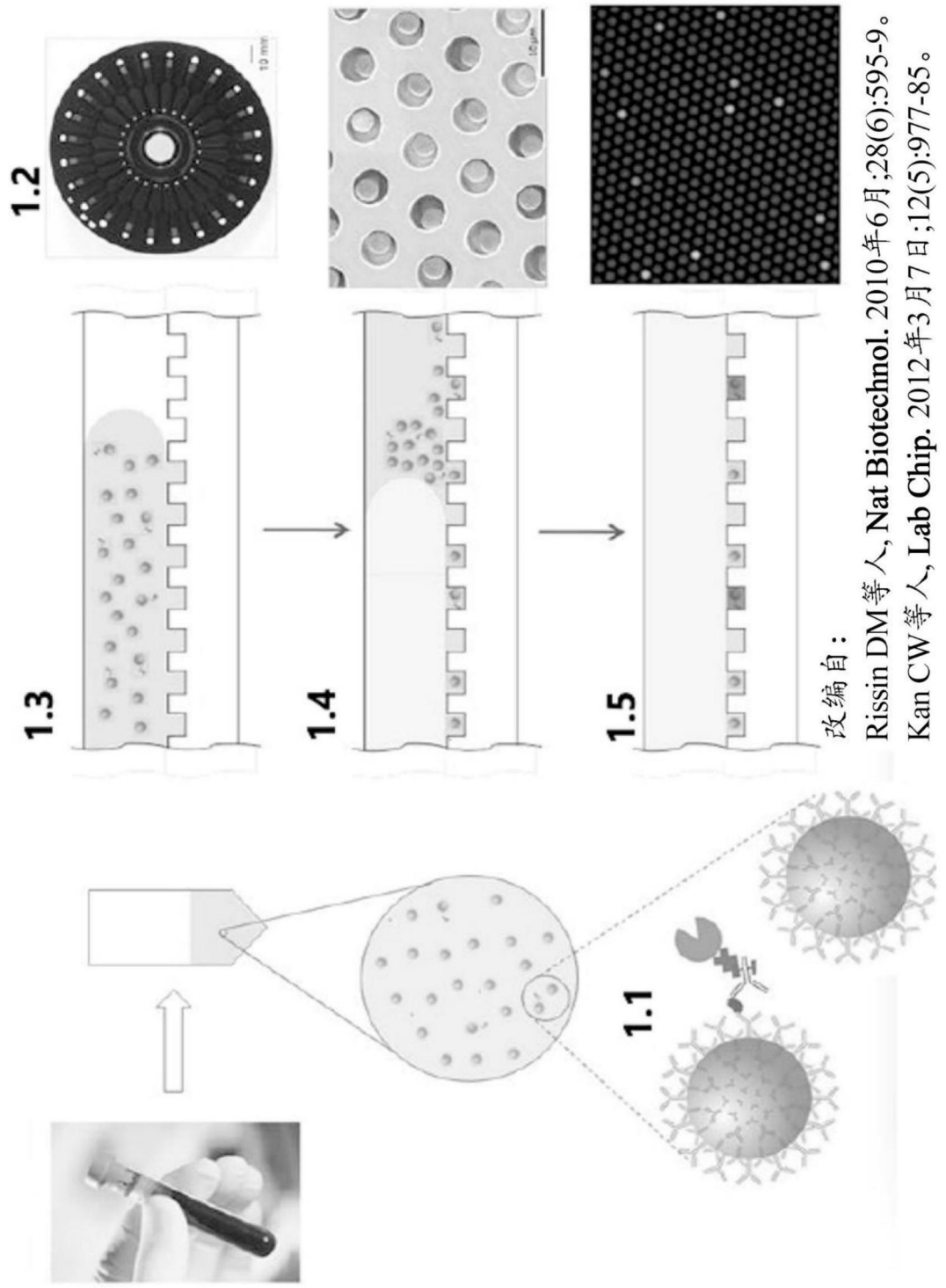

1.2
1.3
1.4
1.5
1.1
改编自：
Rissin DM等人, Nat Biotechnol. 2010年6月;28(6):595-9。
Kan CW等人, Lab Chip. 2012年3月7日;12(5):977-85。

图1

捕获	pTau (pg/mL)	平均AEB	S/B	LOD (pg/mL)
H7L4	0	0.0059	-	6.8
	1	0.0051	0.9	
	10	0.0087	1.5	
	100	0.0357	6.0	
	1000	0.2782	47	
H3L12	0	0.0065	-	5.0
	1	0.0078	1.2	
	10	0.0092	1.4	
	100	0.0369	5.7	
	1000	0.2622	41	
H1L2	0	0.0034	-	2.6
	1	0.0036	1.1	
	10	0.0069	2.0	
	100	0.0326	9.5	
	1000	0.2832	83	

图2A

捕获	pTau (pg/mL)	平均AEB	S/B
H7L4	0	0.0491	-
	1	0.0487	1.0
	10	0.0433	0.9
	100	0.0436	0.9
	1000	0.0441	0.9
H3L12	0	0.0660	-
	1	0.0669	1.0
	10	0.0640	1.0
	100	0.0660	1.0
	1000	0.0595	0.9
H1L2	0	0.0364	-
	1	0.0344	0.9
	10	0.0318	0.9
	100	0.0302	0.8
	1000	0.0323	0.9

图2B

捕获	pTau (pg/mL)	平均AEB	S/B	LOD (pg/mL)
H7L4	0	0.0034	-	14.7
	1	0.0035	1.0	
	10	0.0045	1.3	
	100	0.0078	2.3	
	1000	0.0447	13	
H3L12	0	0.0056	-	88.1
	1	0.0052	0.9	
	10	0.0046	0.8	
	100	0.0106	1.9	
	1000	0.0474	8.4	
H1L2	0	0.0026	-	9.1
	1	0.0029	1.1	
	10	0.0036	1.4	
	100	0.0069	2.7	
	1000	0.0331	13	

图2C

捕获	pTau (pg/mL)	平均AEB	S/B
H7L4	0	0.0204	-
	1	0.0218	1.1
	10	0.0210	1.0
	100	0.0227	1.1
	1000	0.0247	1.2
H3L12	0	0.0348	-
	1	0.0356	1.0
	10	0.0352	1.0
	100	0.0366	1.1
	1000	0.0347	1.0
H1L2	0	0.0123	-
	1	0.0125	1.0
	10	0.0157	1.3
	100	0.0141	1.2
	1000	0.0157	1.3

图2D

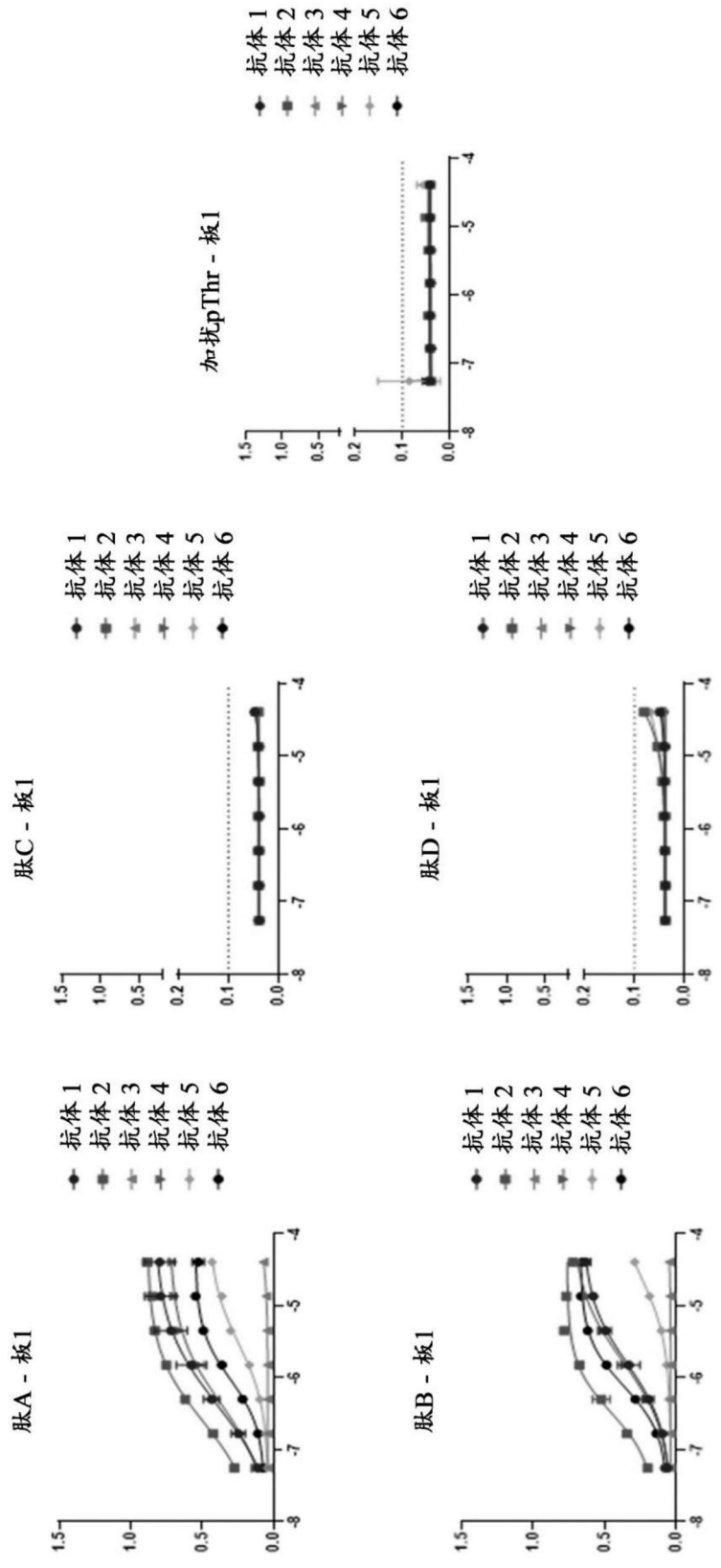
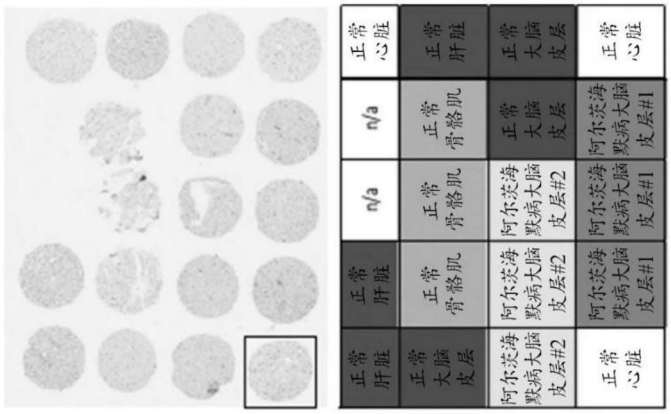


图3


正常肝脏
正常大脑皮层
阿尔茨海默病大脑皮层#2
正常肝脏
正常骨骼肌
阿尔茨海默病大脑皮层#2
阿尔茨海默病大脑皮层#1
正常心脏
n/a
正常骨骼肌
阿尔茨海默病大脑皮层#2
阿尔茨海默病大脑皮层#1
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正常心脏
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正常大脑皮层
正常心脏

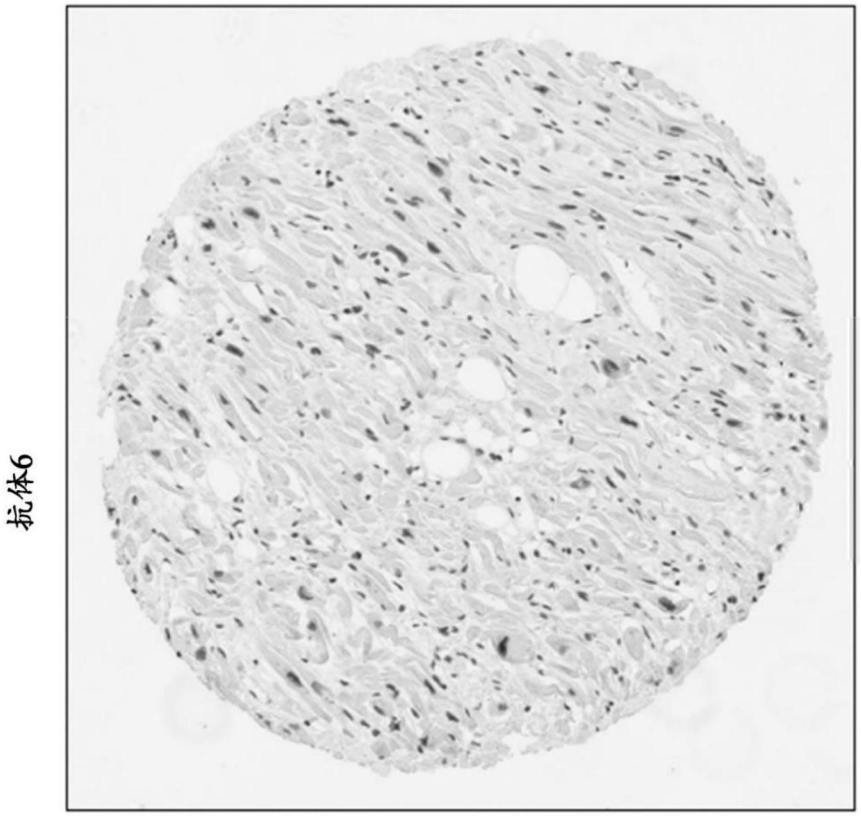
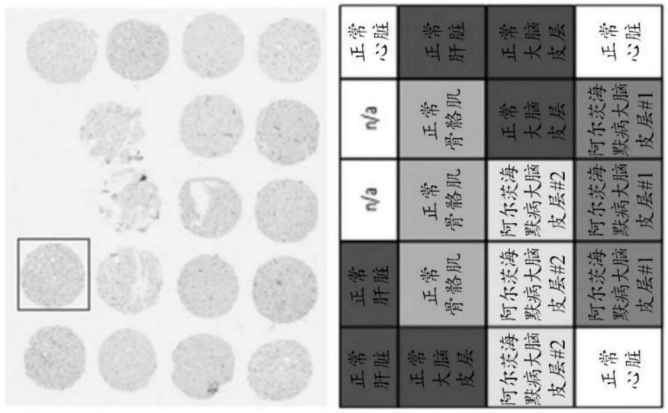

抗体6
正常心脏：
没有染色

图4A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正常大脑皮层
阿尔茨海默病大脑皮层#2
正常肝脏
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阿尔茨海默病大脑皮层#2
n/a
阿尔茨海默病大脑皮层#1
n/a
正常骨骼肌
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阿尔茨海默病大脑皮层#1
正常心脏
正常心脏
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阿尔茨海默病大脑皮层#2
正常心脏

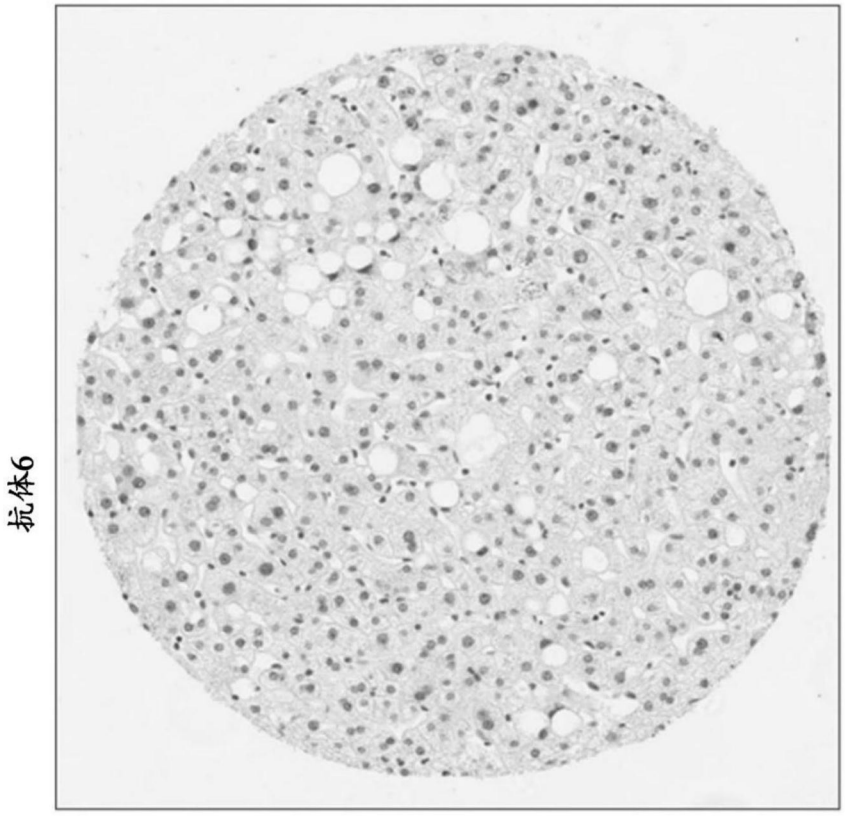
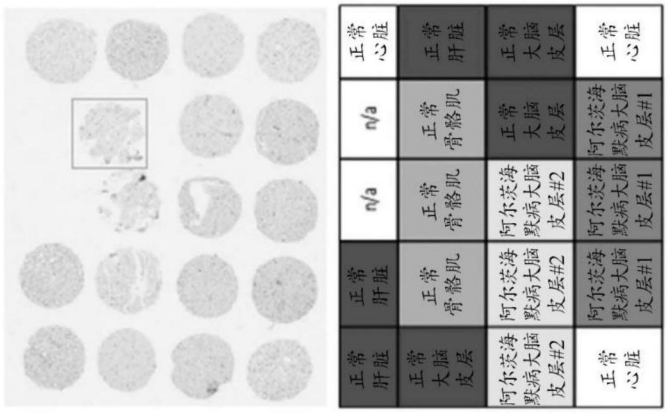
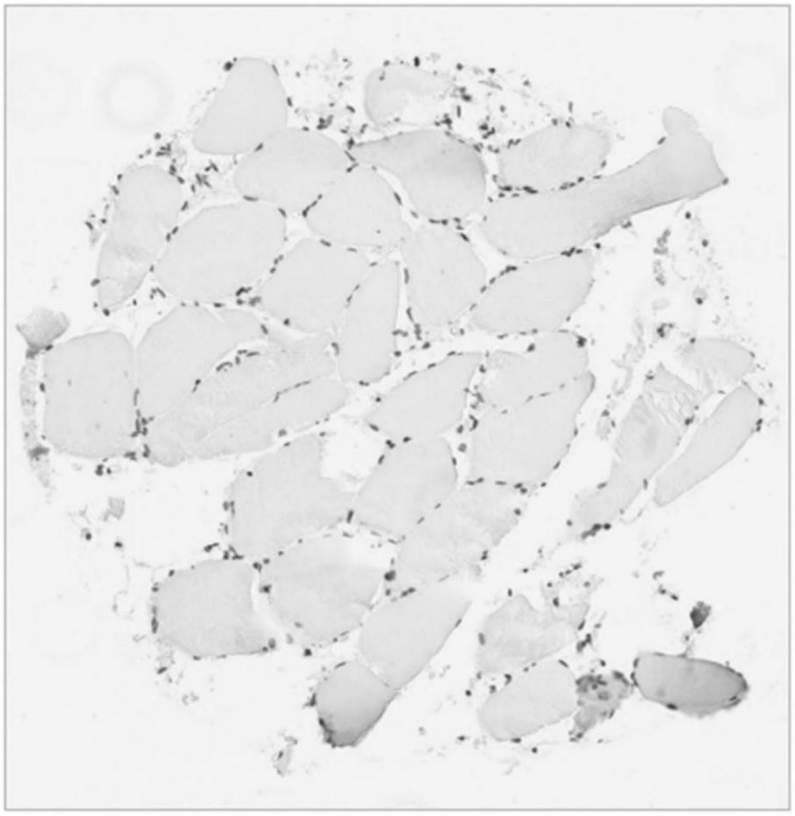

抗体6
正常肝脏：
没有染色

图4B

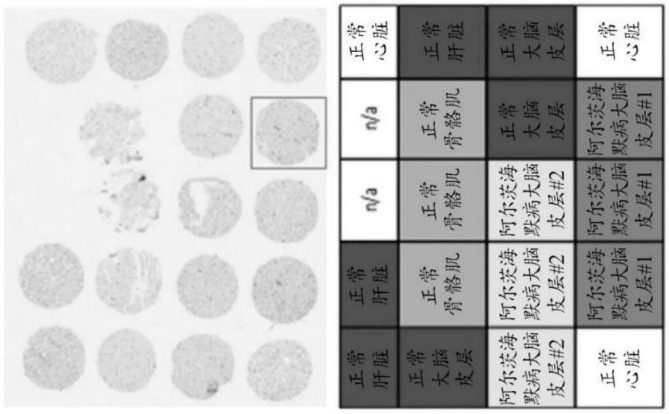


抗体6



正常骨骼肌：没有染色

图4C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n/a
阿尔茨海默病大脑皮层#1
n/a
正常骨骼肌
正常大脑皮层
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正常心脏

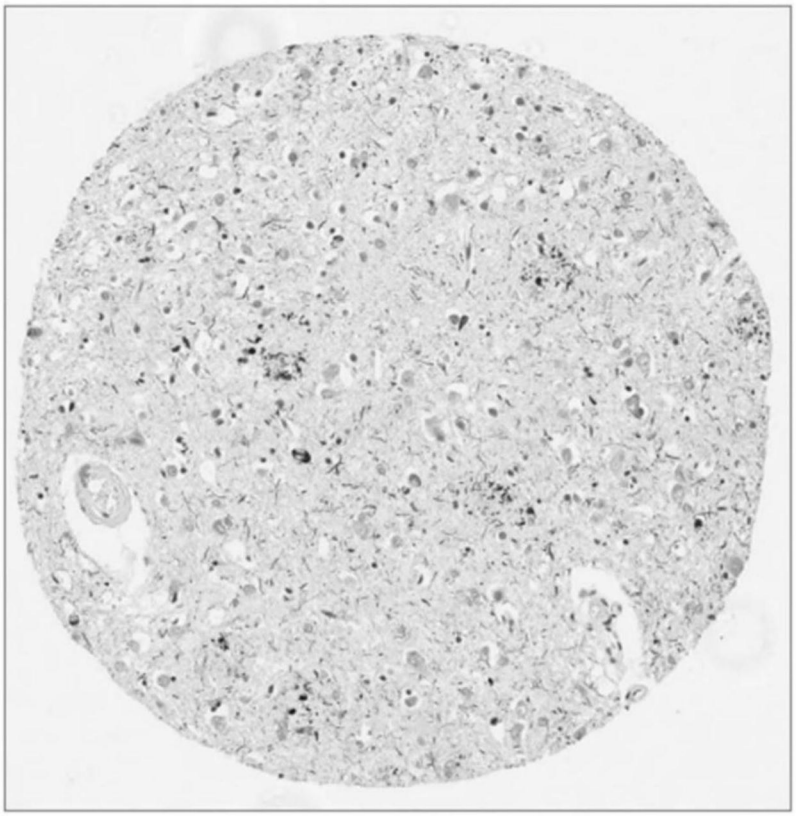
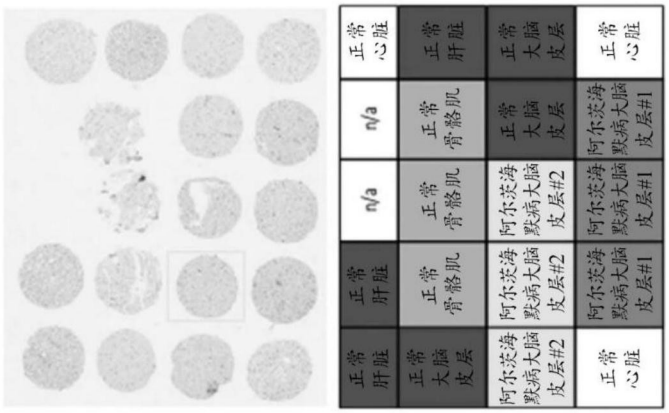

抗体6
阿尔茨海默病大脑皮层#1

图4D


正常肝脏
正常大脑皮层
阿尔茨海默病大脑皮层#2
正常肝脏
正常骨骼肌
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n/a
正常骨骼肌
阿尔茨海默病大脑皮层#1
n/a
正常大脑皮层
阿尔茨海默病大脑皮层#1
正常心脏
正常心脏
正常肝脏
正常大脑皮层
阿尔茨海默病大脑皮层#1
正常心脏

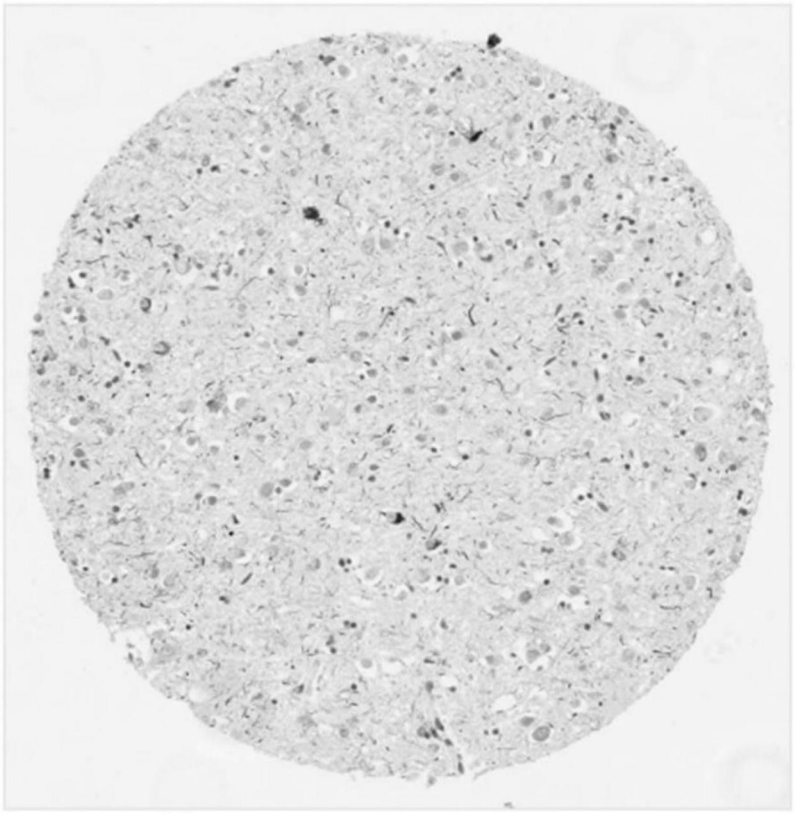
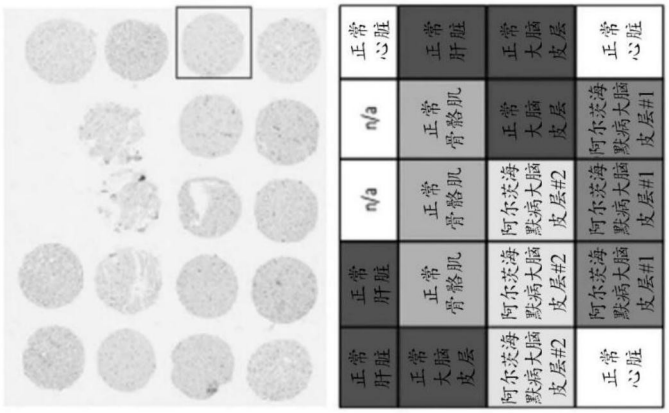

抗体6
阿尔茨海默病大脑皮层#2

图4E


正常肝脏
正常大脑皮层
阿尔茨海默病大脑皮层#2
正常肝脏
正常骨骼肌
阿尔茨海默病大脑皮层#1
n/a
n/a
正常心脏
正常大脑皮层
阿尔茨海默病大脑皮层#2
正常心脏
正常骨骼肌
阿尔茨海默病大脑皮层#2
阿尔茨海默病大脑皮层#1
正常大脑皮层
阿尔茨海默病大脑皮层#1
正常心脏
正常肝脏

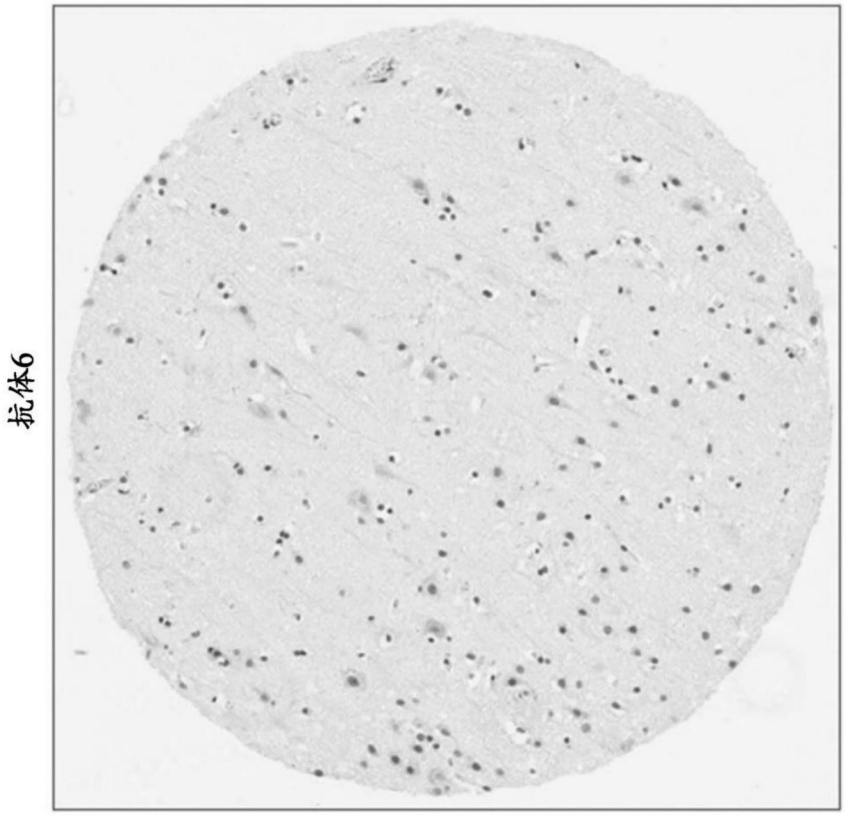

抗体6
正常
大脑皮层

图4F

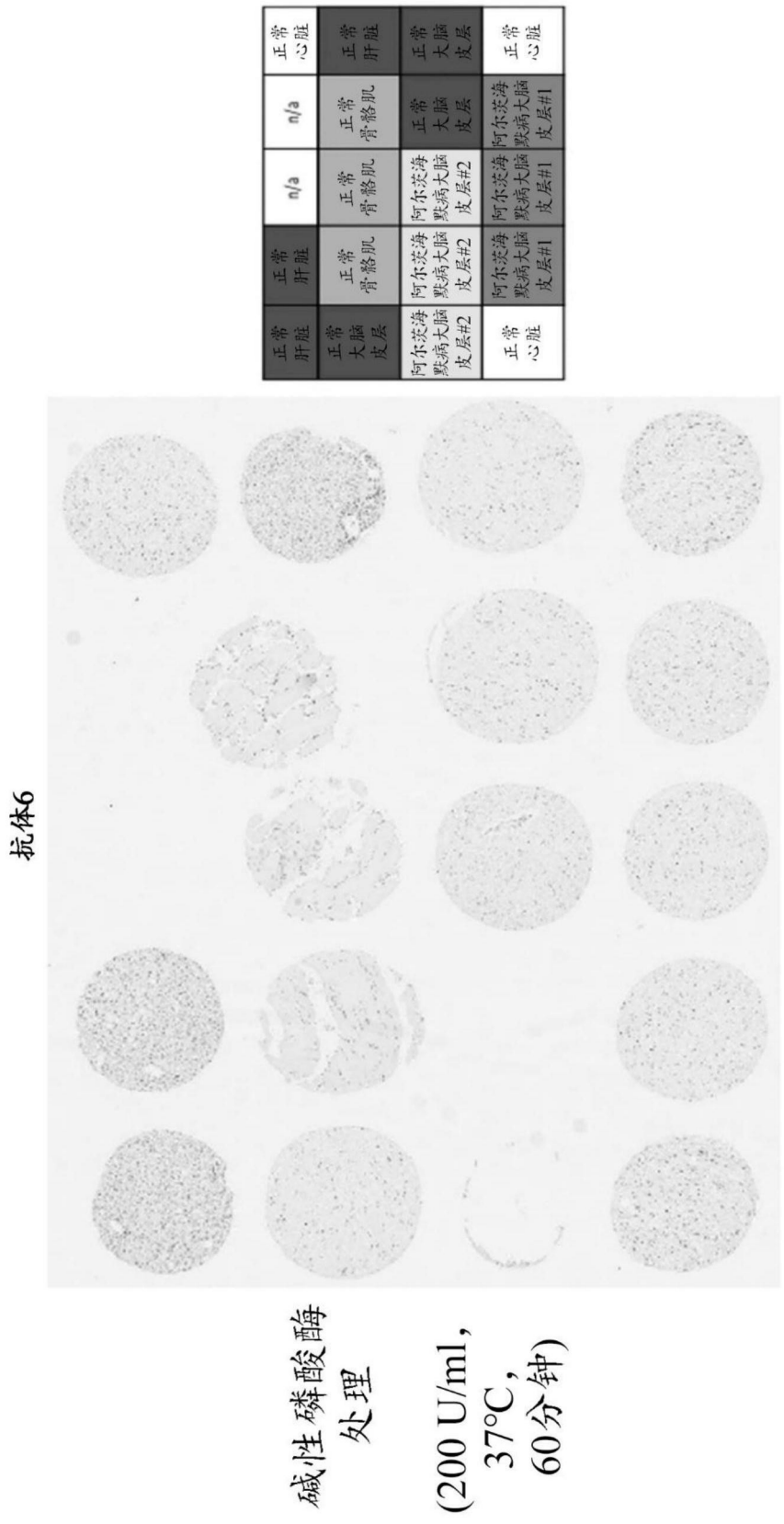
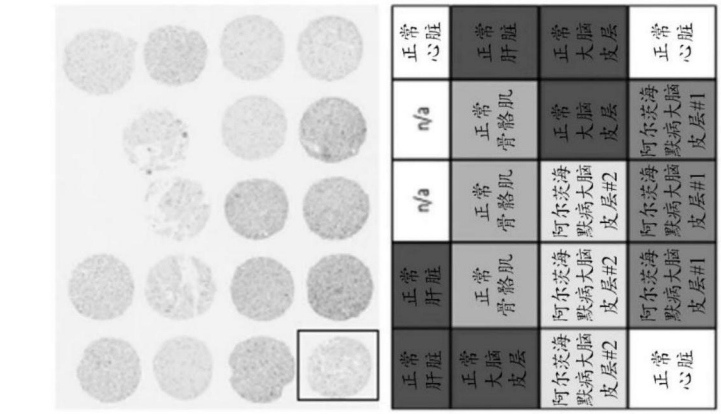

抗体6
碱性磷酸酶处理
(200 U/ml, 37°C, 60分钟)
正常肝脏
正常大脑皮层
阿尔茨海默病大脑皮层#2
正常肝脏
正常胃骼肌
阿尔茨海默病大脑皮层#2
n/a
正常胃骼肌
阿尔茨海默病大脑皮层#1
n/a
正常大脑皮层
正常心脏
阿尔茨海默病大脑皮层#1
正常心脏
正常肝脏
正常大脑皮层
阿尔茨海默病大脑皮层#2
正常胃骼肌
阿尔茨海默病大脑皮层#1
正常大脑皮层
正常心脏

图4G


正常肝脏
正常大脑皮层
阿尔茨海默病大脑皮层#2
正常肝脏
正常骨骼肌
阿尔茨海默病大脑皮层#1
n/a
正常骨骼肌
阿尔茨海默病大脑皮层#2
n/a
阿尔茨海默病大脑皮层#1
正常心脏
正常心脏
正常肝脏
正常大脑皮层
阿尔茨海默病大脑皮层#2
正常心脏
阿尔茨海默病大脑皮层#1

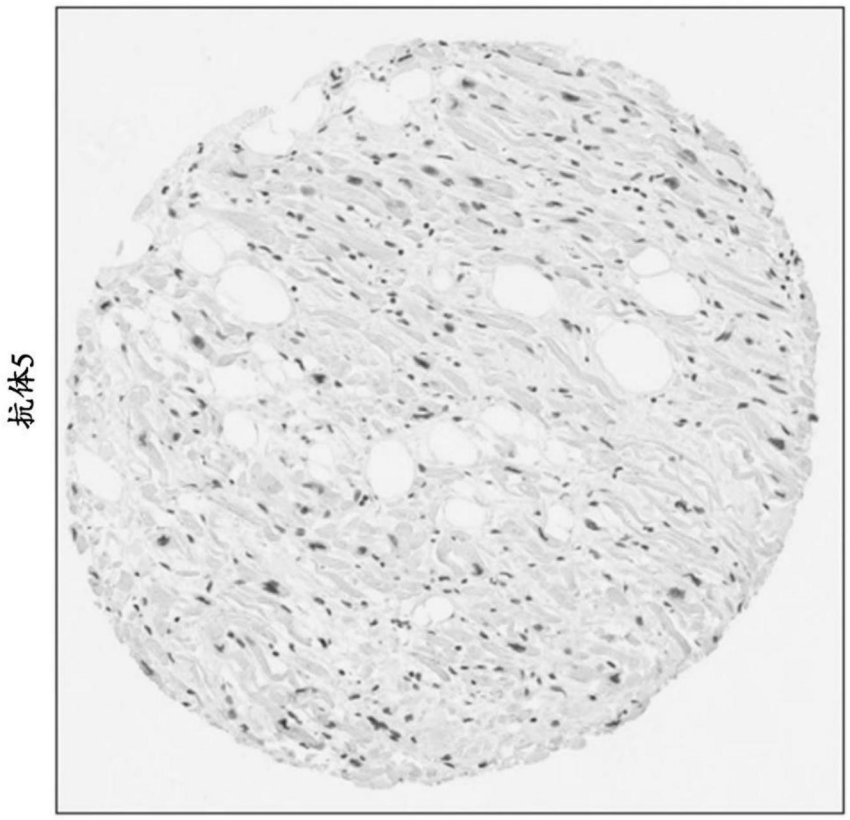
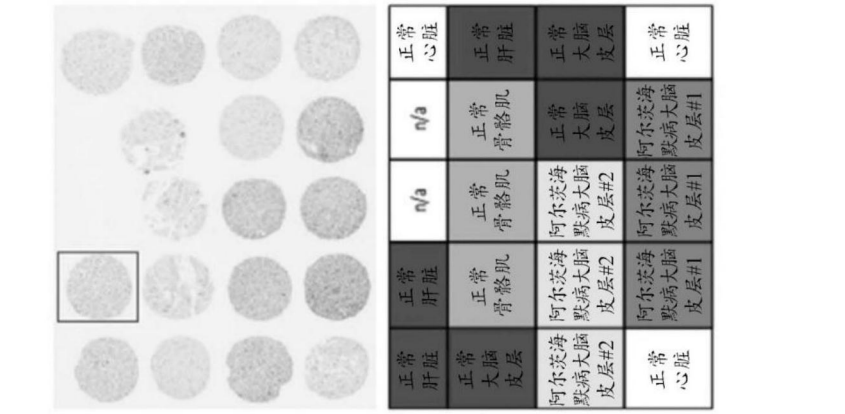

抗体5
正常心脏：
没有染色

图5A


正常肝脏
正常大脑皮层
阿尔茨海默病大脑皮层#2
正常肝脏
正常骨骼肌
阿尔茨海默病大脑皮层#2
阿尔茨海默病大脑皮层#1
正常心脏
n/a
正常骨骼肌
阿尔茨海默病大脑皮层#2
正常大脑皮层
阿尔茨海默病大脑皮层#1
n/a
正常肝脏
阿尔茨海默病大脑皮层#1
阿尔茨海默病大脑皮层#1
正常大脑皮层
正常心脏
正常肝脏
正常心脏

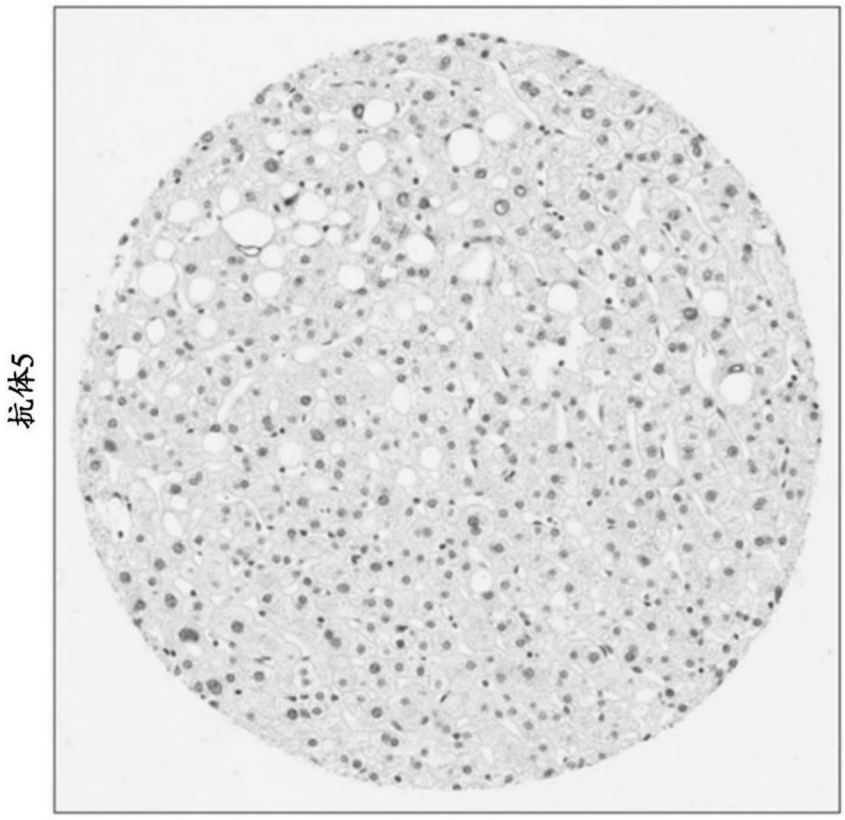
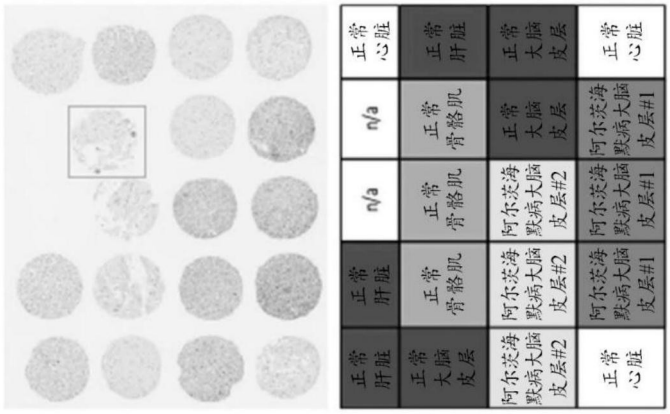

抗体5
正常肝脏：
没有染色

图5B


正常肝脏
正常大脑皮层
阿尔茨海默病大脑皮层#2
正常心脏
正常肝脏
正常骨骼肌
阿尔茨海默病大脑皮层#2
阿尔茨海默病大脑皮层#1
n/a
n/a
正常大脑皮层
正常大脑皮层
阿尔茨海默病大脑皮层#1
正常心脏
正常骨骼肌
阿尔茨海默病大脑皮层#1
正常心脏
正常肝脏
正常大脑皮层
正常心脏

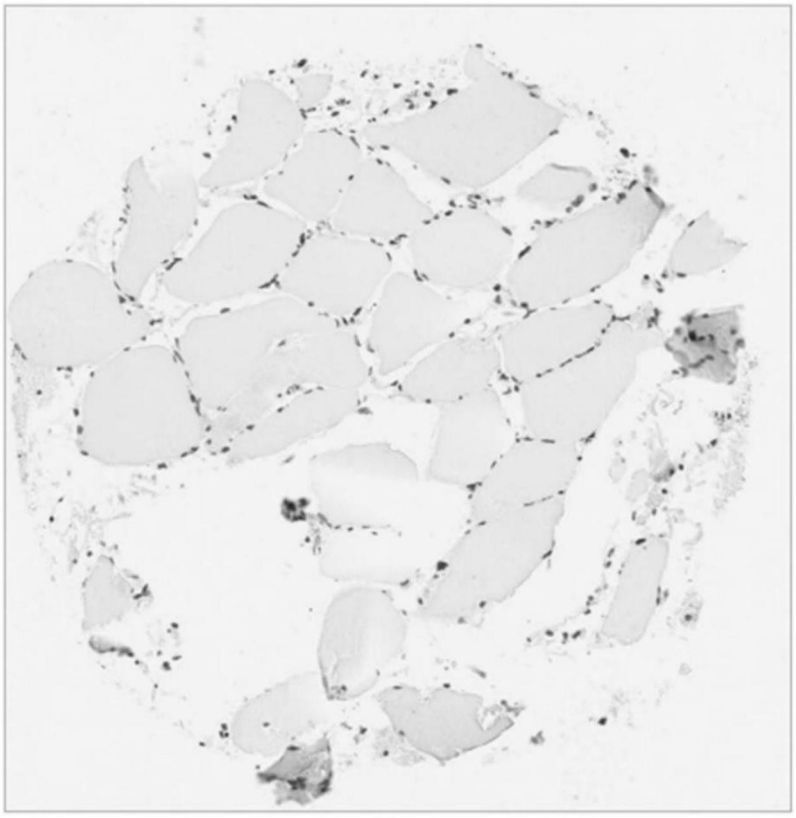
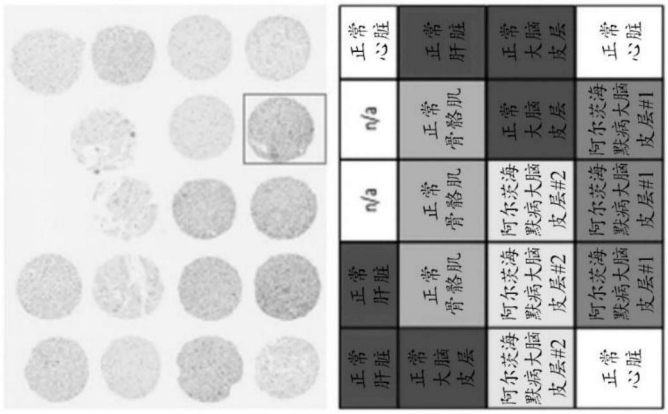

抗体5
正常骨骼肌：
没有染色

图5C


正常肝脏
正常大脑皮层
阿尔茨海默病大脑皮层#2
正常肝脏
正常骨骼肌
阿尔茨海默病大脑皮层#2
n/a
正常骨骼肌
阿尔茨海默病大脑皮层#1
n/a
正常心脏
阿尔茨海默病大脑皮层#1
正常心脏

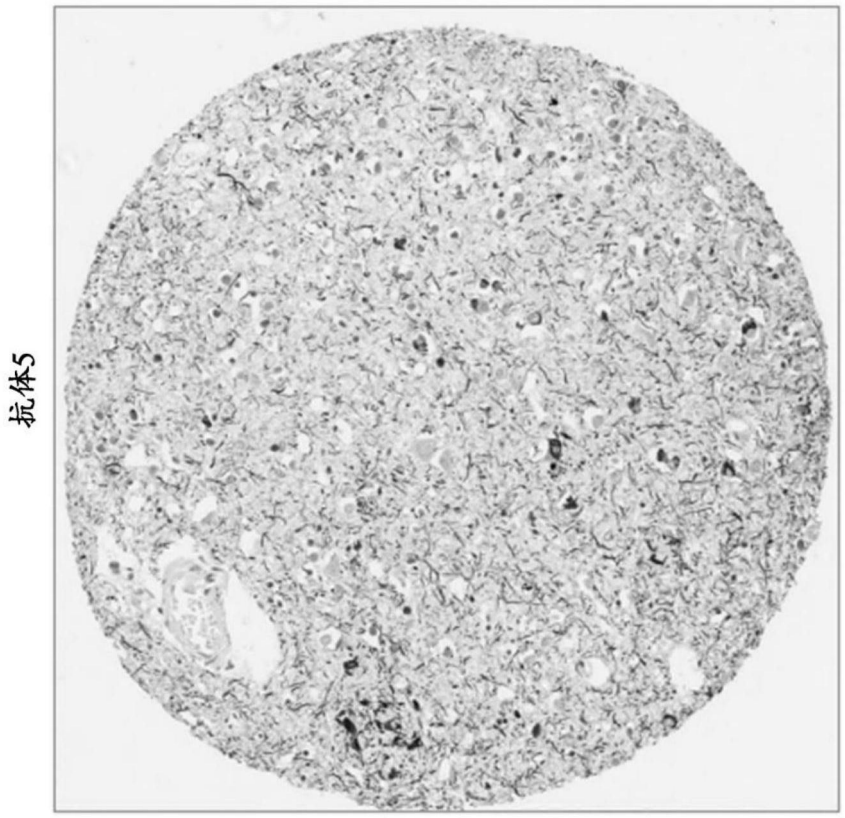
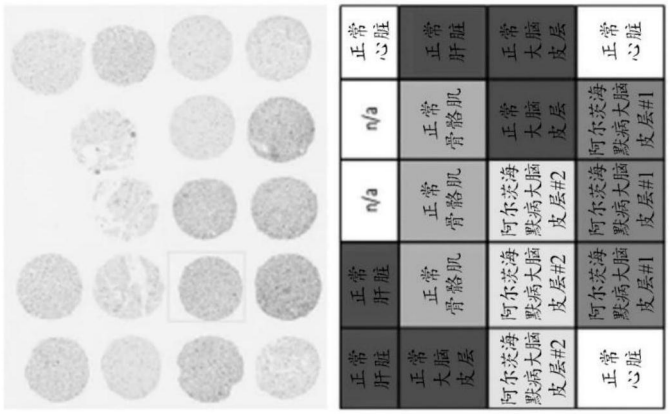

抗体5
阿尔茨海默病大脑皮层#1

图5D


正常肝脏
正常大脑皮层
阿尔茨海默病大脑皮层#2
正常肝脏
正常骨骼肌
阿尔茨海默病大脑皮层#2
阿尔茨海默病大脑皮层#1
n/a
正常骨骼肌
阿尔茨海默病大脑皮层#1
n/a
正常心脏
正常肝脏
正常大脑皮层
阿尔茨海默病大脑皮层#1
正常心脏

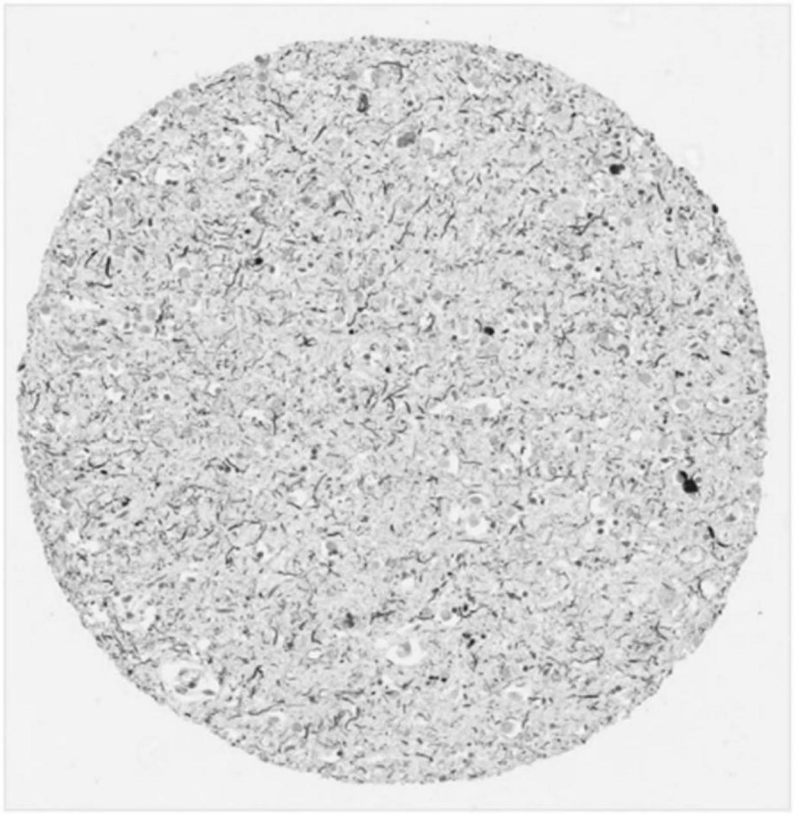
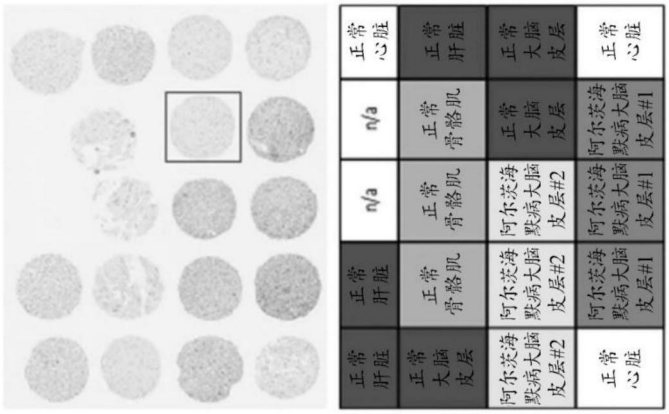

抗体5
阿尔茨海默病大脑皮层#2

图5E


正常肝脏
正常大脑皮层
阿尔茨海默病大脑皮层#2
正常肝脏
正常骨骼肌
阿尔茨海默病大脑皮层#2
阿尔茨海默病大脑皮层#1
n/a
正常骨骼肌
正常大脑皮层
阿尔茨海默病大脑皮层#2
阿尔茨海默病大脑皮层#1
n/a
正常大脑皮层
阿尔茨海默病大脑皮层#1
正常心脏
正常心脏

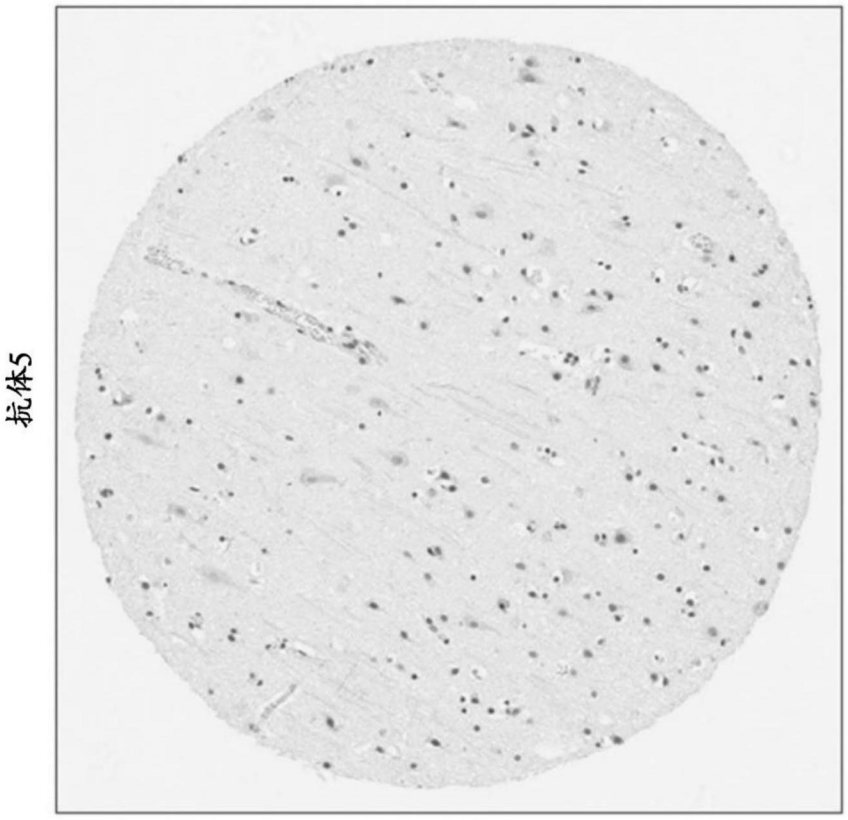

抗体5
正常
大脑皮层

图5F

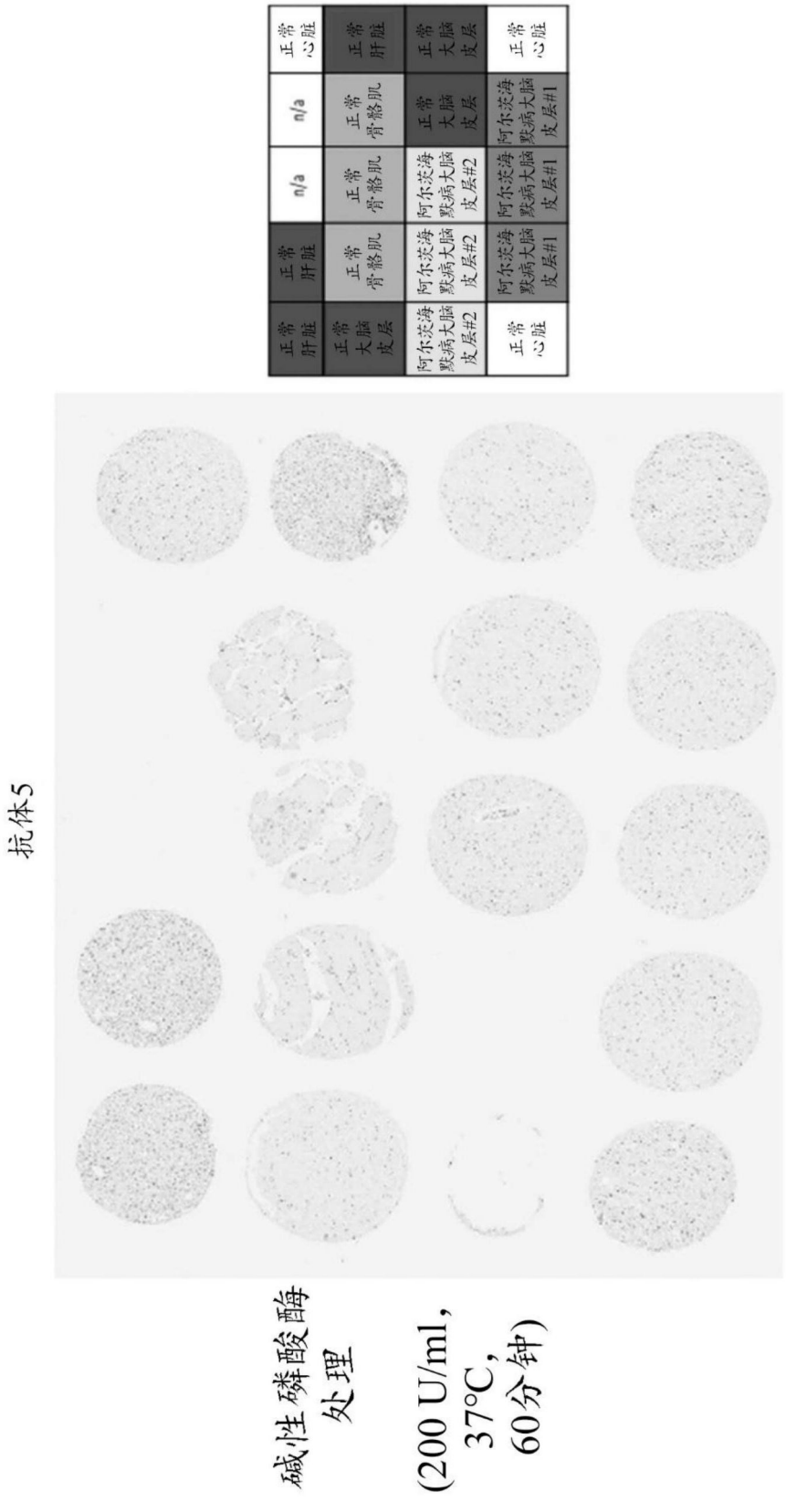
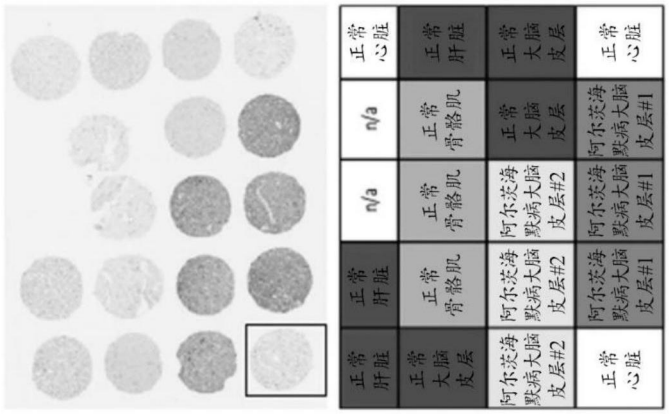

抗体5
碱性磷酸酶处理
(200 U/ml, 37°C, 60分钟)
正常肝脏
正常大脑皮层
阿尔茨海默病大脑皮层#2
正常肝脏
正常骨骼肌
阿尔茨海默病大脑皮层#2
n/a
正常骨骼肌
阿尔茨海默病大脑皮层#1
n/a
正常大脑皮层
阿尔茨海默病大脑皮层#1
正常心脏
正常心脏

图5G


正常肝脏
正常大脑皮层
阿尔茨海默病大脑皮层#2
正常肝脏
正常骨骼肌
阿尔茨海默病大脑皮层#2
n/a
正常骨骼肌
阿尔茨海默病大脑皮层#1
n/a
正常心脏
阿尔茨海默病大脑皮层#1
正常心脏
正常大脑皮层
阿尔茨海默病大脑皮层#2
正常肝脏
正常大脑皮层
阿尔茨海默病大脑皮层#1
正常心脏

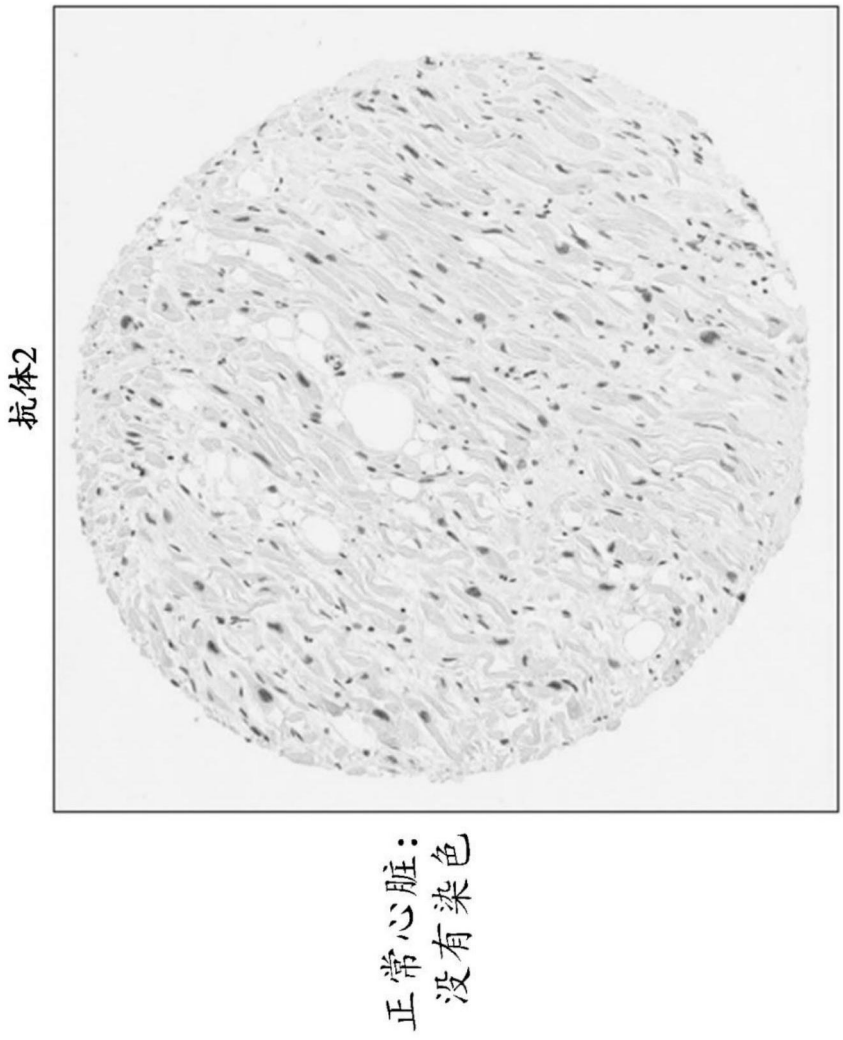
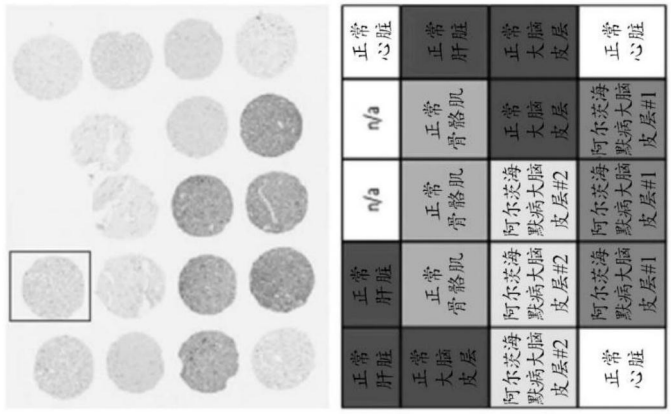

抗体2
正常心脏：
没有染色

图6A


正常肝脏
正常大脑皮层
阿尔茨海默病大脑皮层#2
正常肝脏
正常骨骼肌
阿尔茨海默病大脑皮层#2
n/a
阿尔茨海默病大脑皮层#1
n/a
正常骨骼肌
正常大脑皮层
阿尔茨海默病大脑皮层#2
阿尔茨海默病大脑皮层#1
正常心脏
正常大脑皮层
阿尔茨海默病大脑皮层#1
正常心脏
正常肝脏

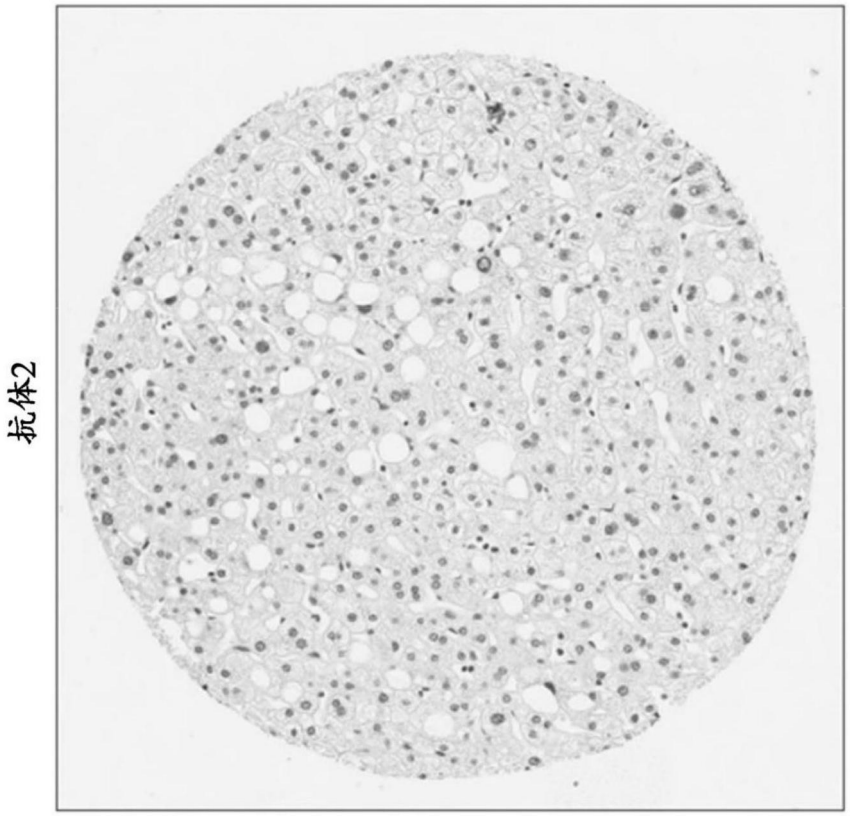
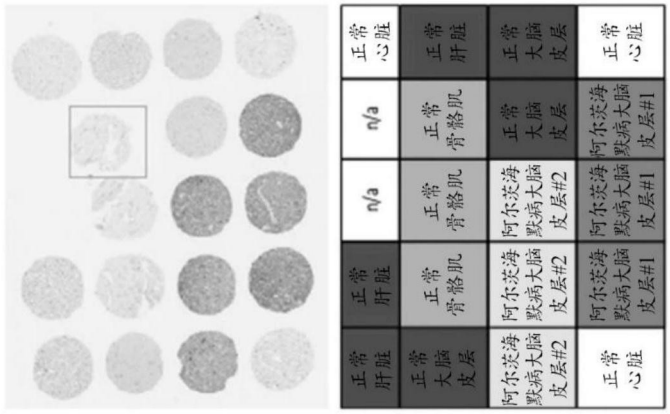

抗体2
正常肝脏：
没有染色

图6B


正常肝脏
正常大脑皮层
阿尔茨海默病大脑皮层#2
正常心脏
正常肝脏
正常骨骼肌
阿尔茨海默病大脑皮层#2
阿尔茨海默病大脑皮层#1
n/a
n/a
正常骨骼肌
阿尔茨海默病大脑皮层#1
正常大脑皮层
阿尔茨海默病大脑皮层#2
正常大脑皮层
正常心脏

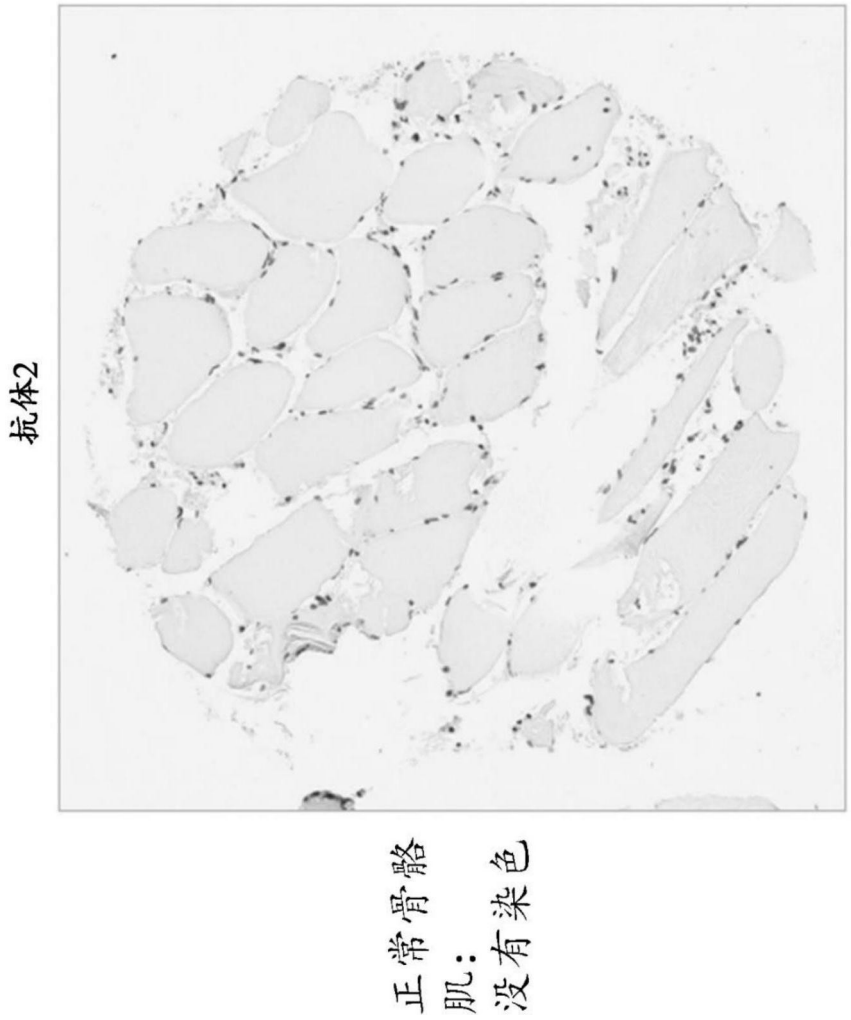
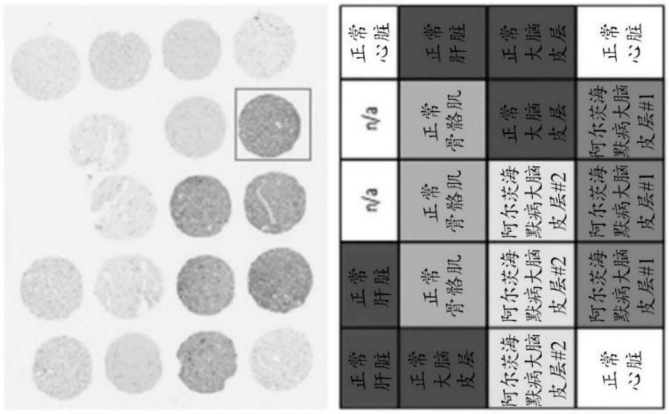

抗体2
正常骨骼肌：没有染色

图6C


正常肝脏
正常大脑皮层
阿尔茨海默病大脑皮层#2
正常心脏
正常肝脏
正常胥骼肌
阿尔茨海默病大脑皮层#2
阿尔茨海默病大脑皮层#1
n/a
正常胥骼肌
正常大脑皮层
阿尔茨海默病大脑皮层#1
n/a
正常大脑皮层
正常心脏

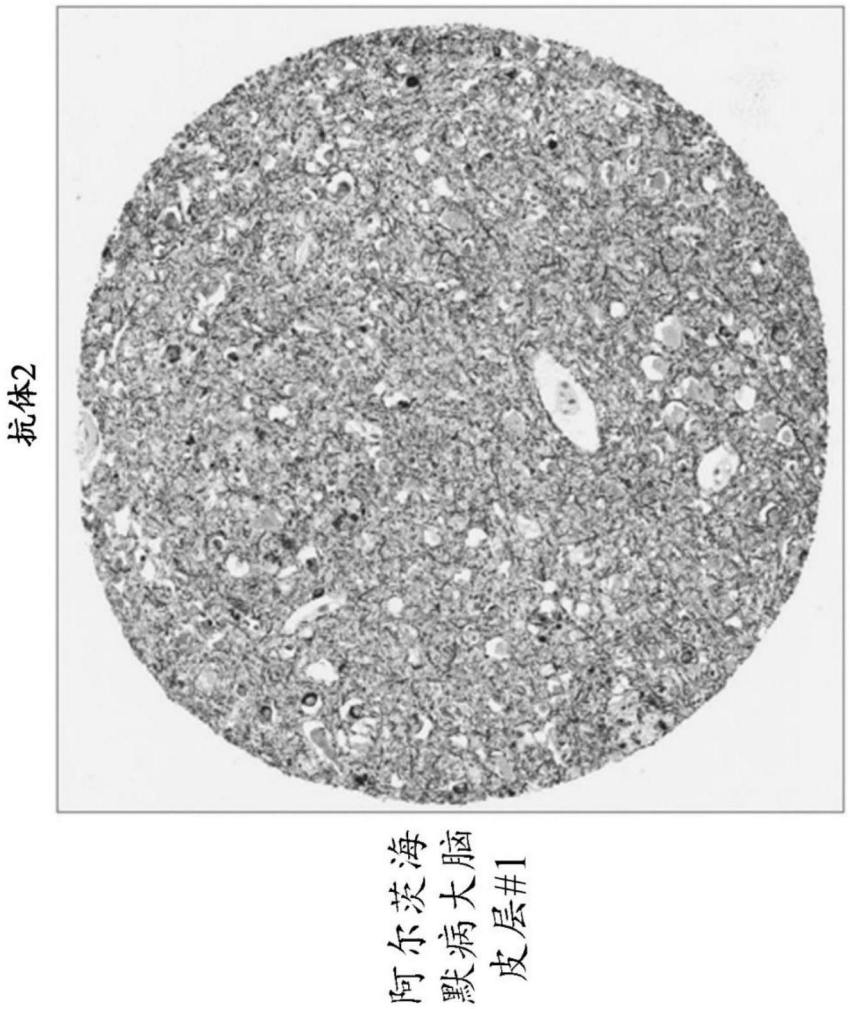
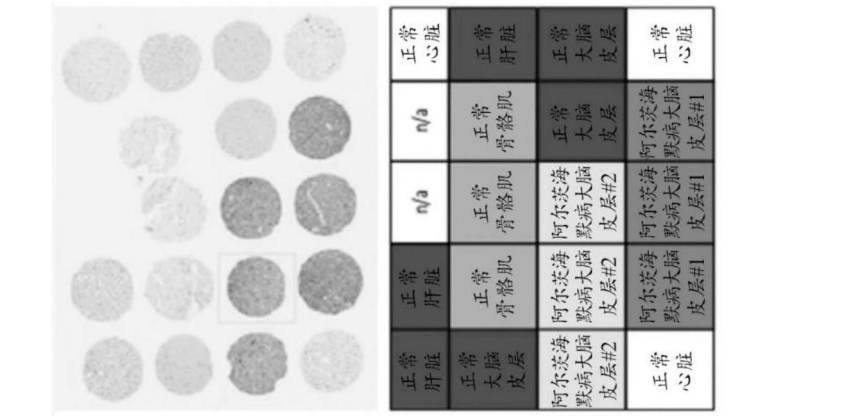

抗体2
阿尔茨海默病大脑皮层#1

图6D


正常肝脏
正常大脑皮层
阿尔茨海默病大脑皮层#2
正常心脏
正常肝脏
正常骨骼肌
阿尔茨海默病大脑皮层#2
n/a
正常骨骼肌
正常大脑皮层
阿尔茨海默病大脑皮层#2
n/a
阿尔茨海默病大脑皮层#1
正常大脑皮层
阿尔茨海默病大脑皮层#1
正常心脏
正常心脏
正常肝脏
正常大脑皮层
阿尔茨海默病大脑皮层#1

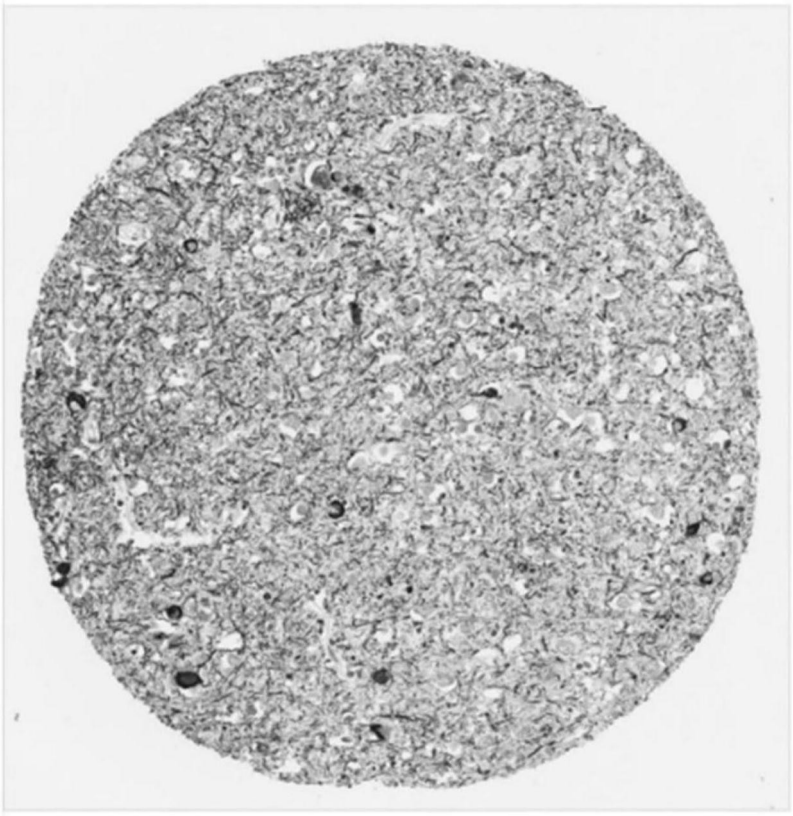
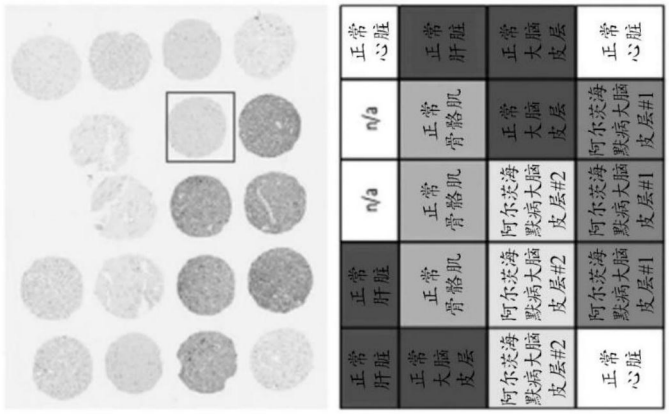

抗体2
阿尔茨海默病大脑皮层#2

图6E


正常肝脏
正常大脑皮层
阿尔茨海默病大脑皮层#2
正常肝脏
正常骨骼肌
阿尔茨海默病大脑皮层#1
n/a
正常骨骼肌
阿尔茨海默病大脑皮层#2
n/a
正常大脑皮层
阿尔茨海默病大脑皮层#1
正常心脏
正常心脏

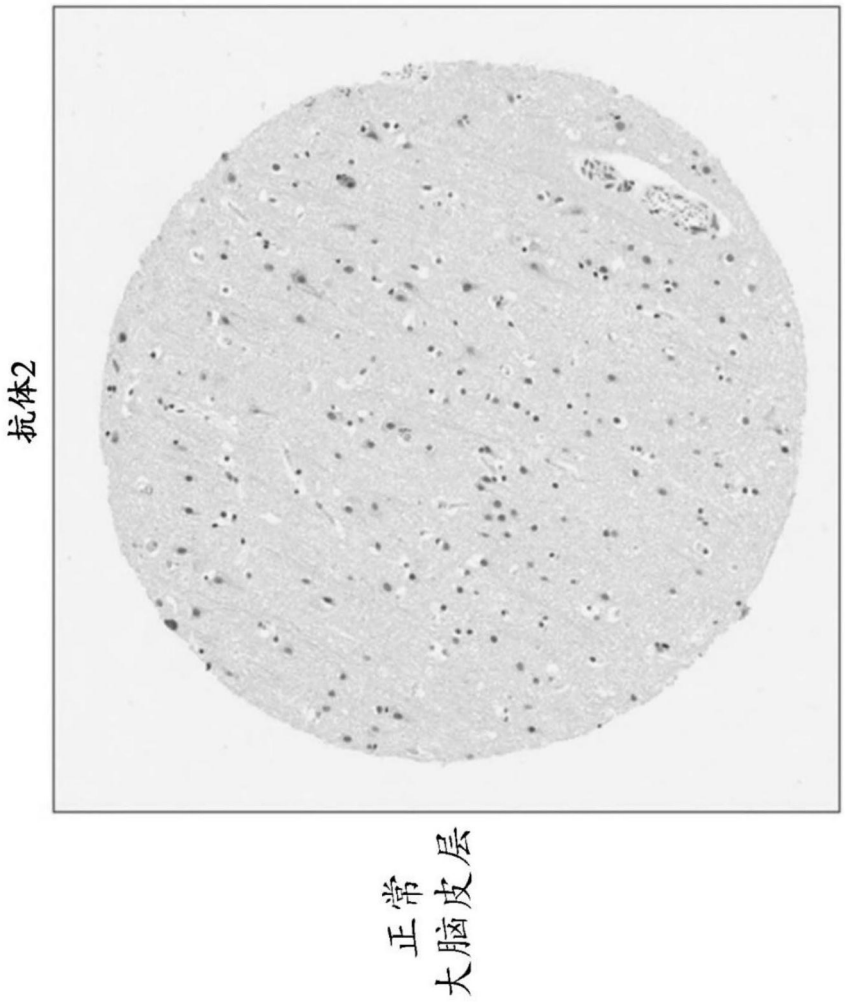

抗体2
正常
大脑皮层

图6F

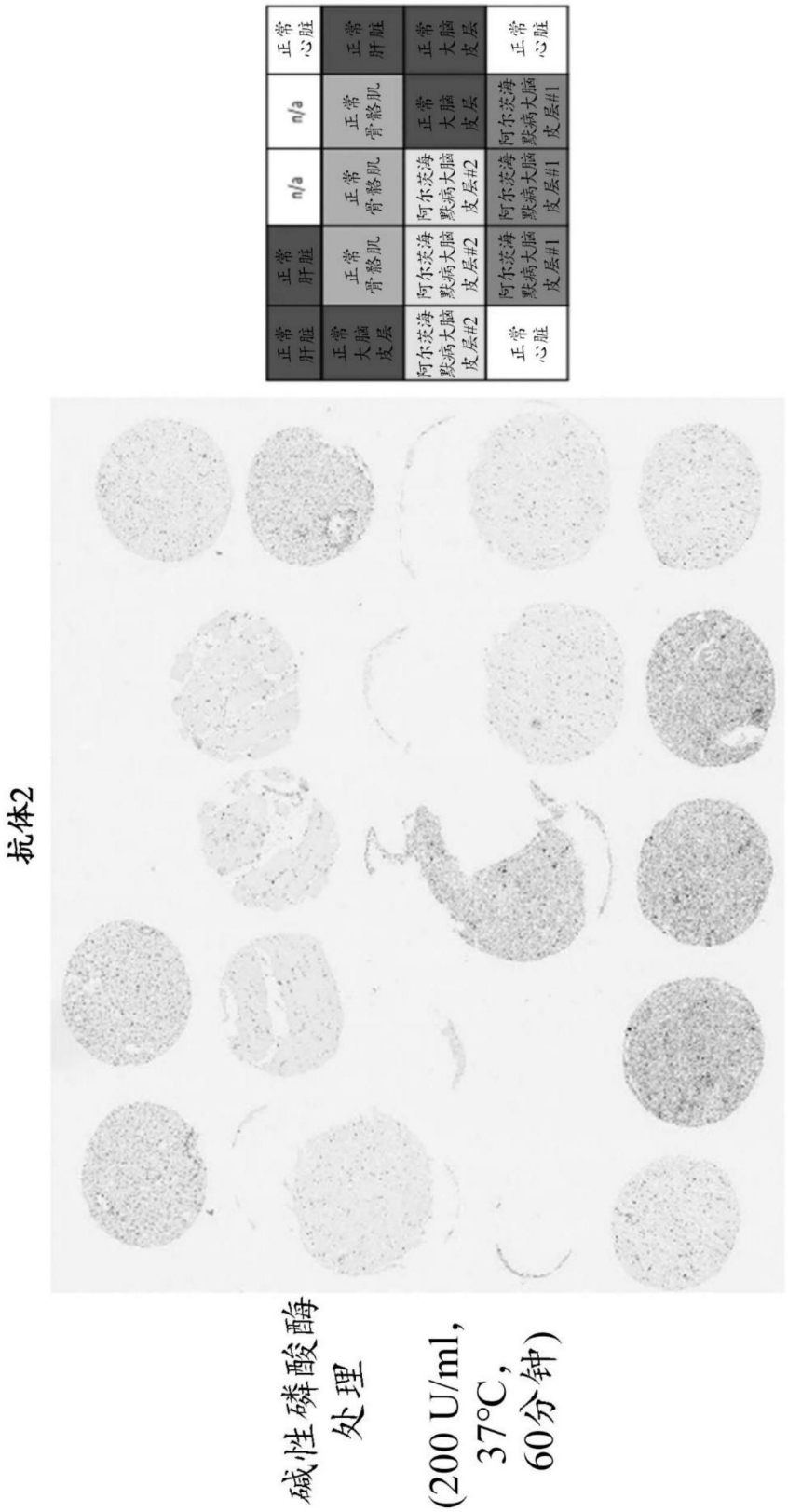

抗体2
碱性磷酸酶处理
(200 U/ml, 37°C, 60分钟)
正常肝脏
正常大脑皮层
阿尔茨海默病大脑皮层#2
正常肝脏
正常骨骼肌
阿尔茨海默病大脑皮层#2
阿尔茨海默病大脑皮层#1
n/a
n/a
正常骨骼肌
正常大脑皮层
阿尔茨海默病大脑皮层#2
阿尔茨海默病大脑皮层#1
正常心脏
正常肝脏
正常大脑皮层
阿尔茨海默病大脑皮层#1
正常心脏

图6G

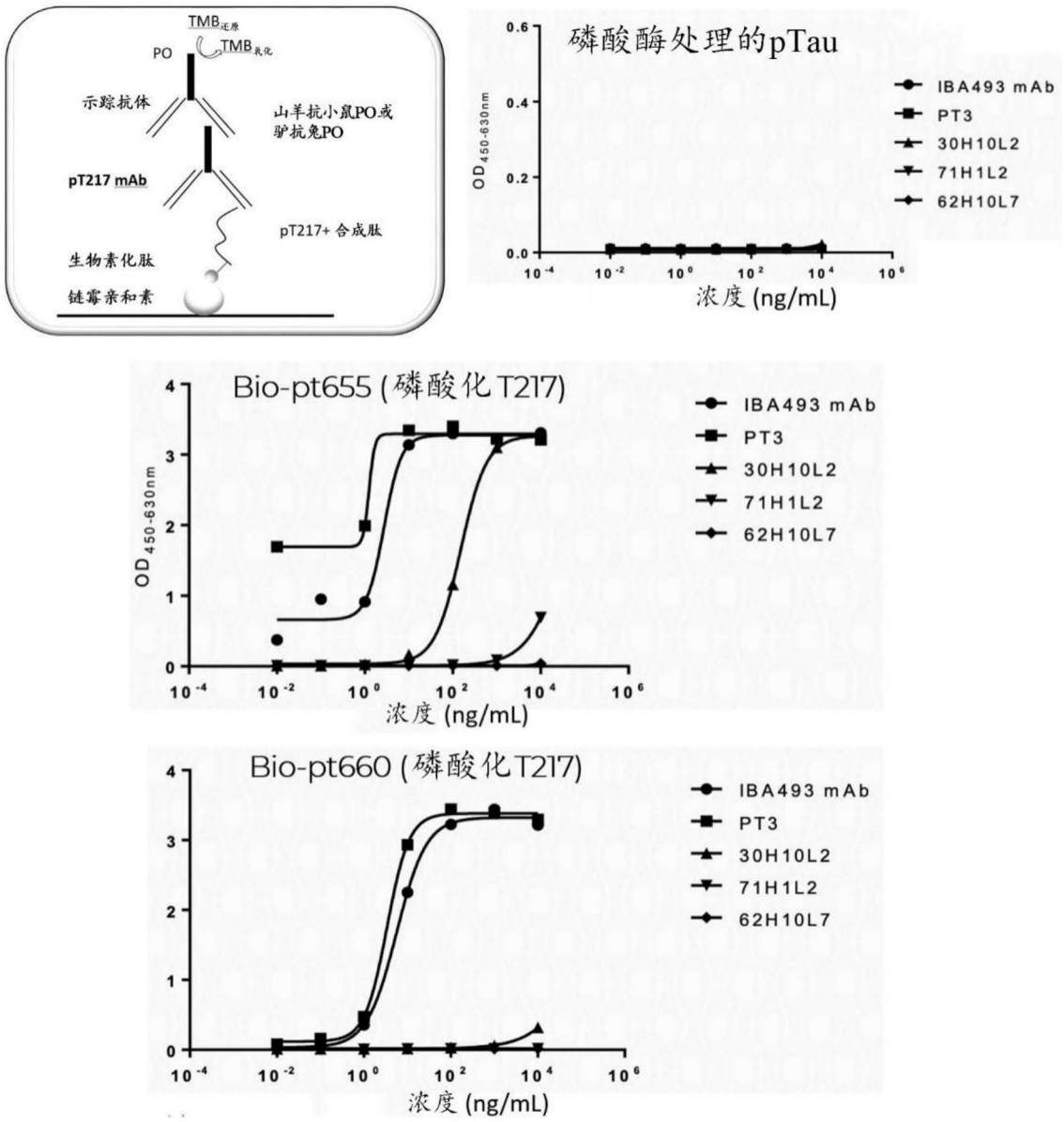

TMB还原
PO
TMB氧化
示踪抗体
山羊抗小鼠PO或
驴抗兔PO
pT217 mAb
pT217+ 合成肽
生物素化肽
链霉亲和素
磷酸酶处理的pTau
OD450-630nm
浓度 (ng/mL)
IBA493 mAb
PT3
30H10L2
71H1L2
62H10L7
Bio-pt655 (磷酸化T217)
Bio-pt660 (磷酸化T217)

图7

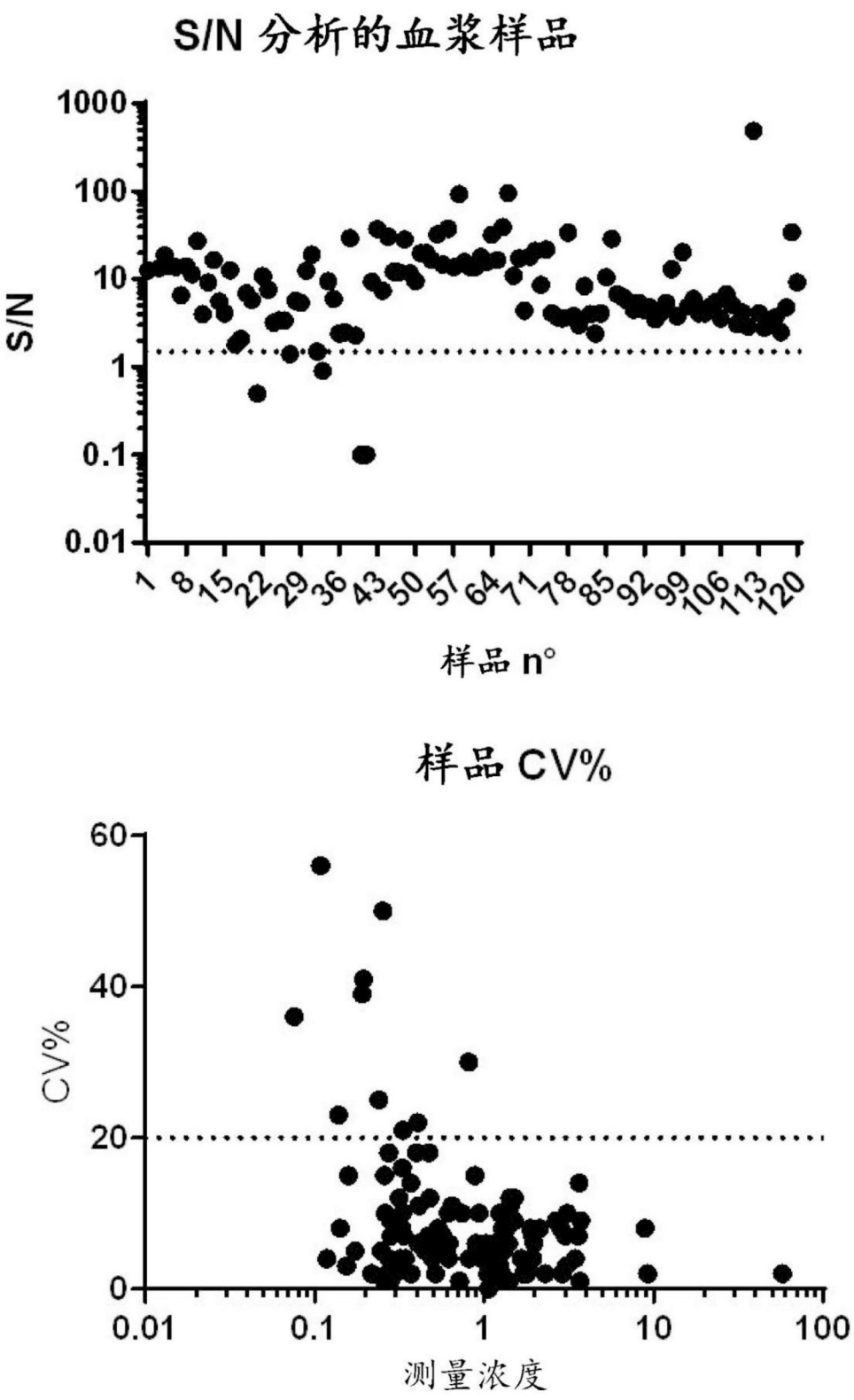

S/N 分析的血浆样品
S/N
1000
100
10
1
0.1
0.01
1
8
15
22
29
36
43
50
57
64
71
78
85
92
99
106
113
120
样品 n°
样品 CV%
CV%
60
40
20
0
0.01
0.1
1
10
100
测量浓度

图8

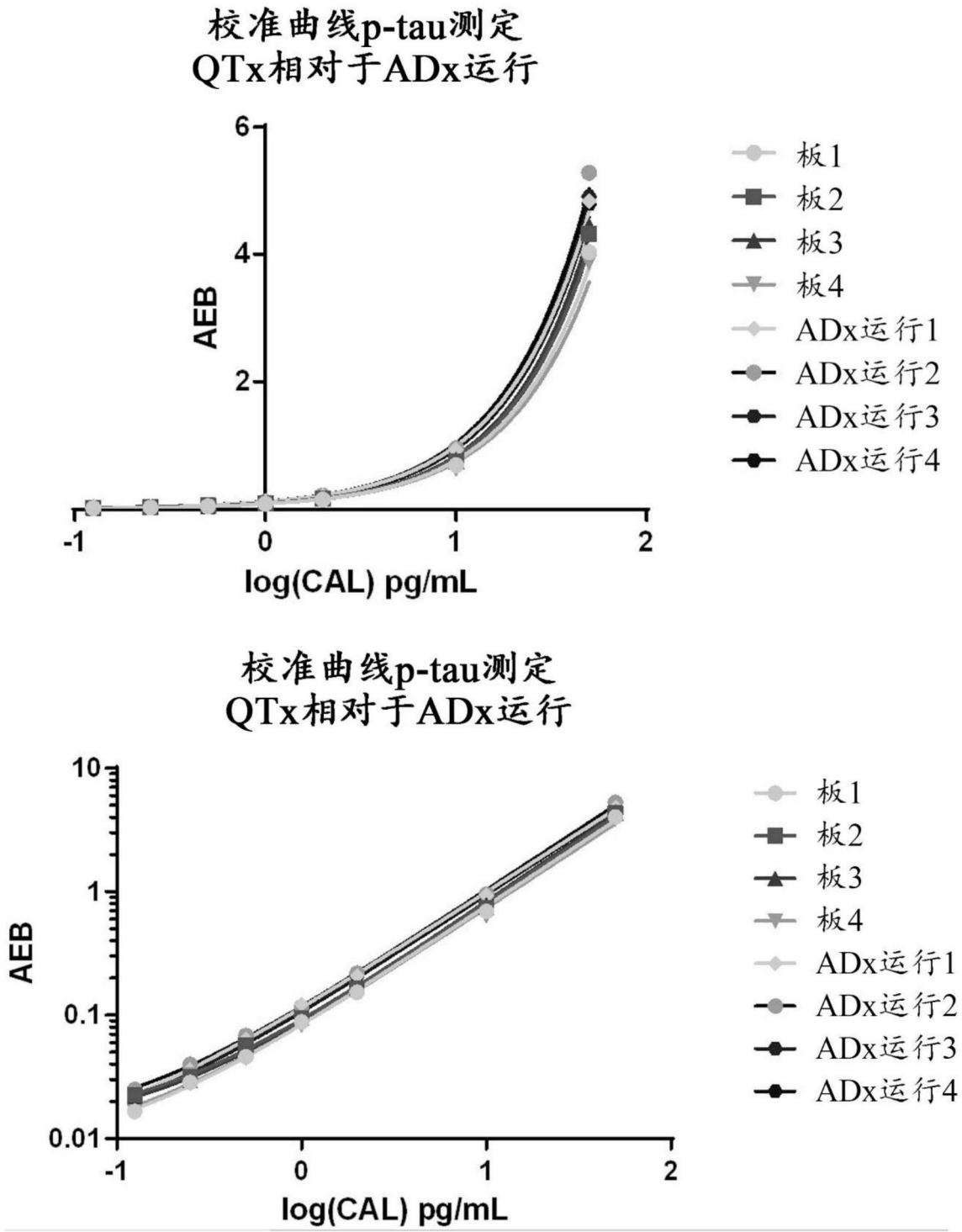
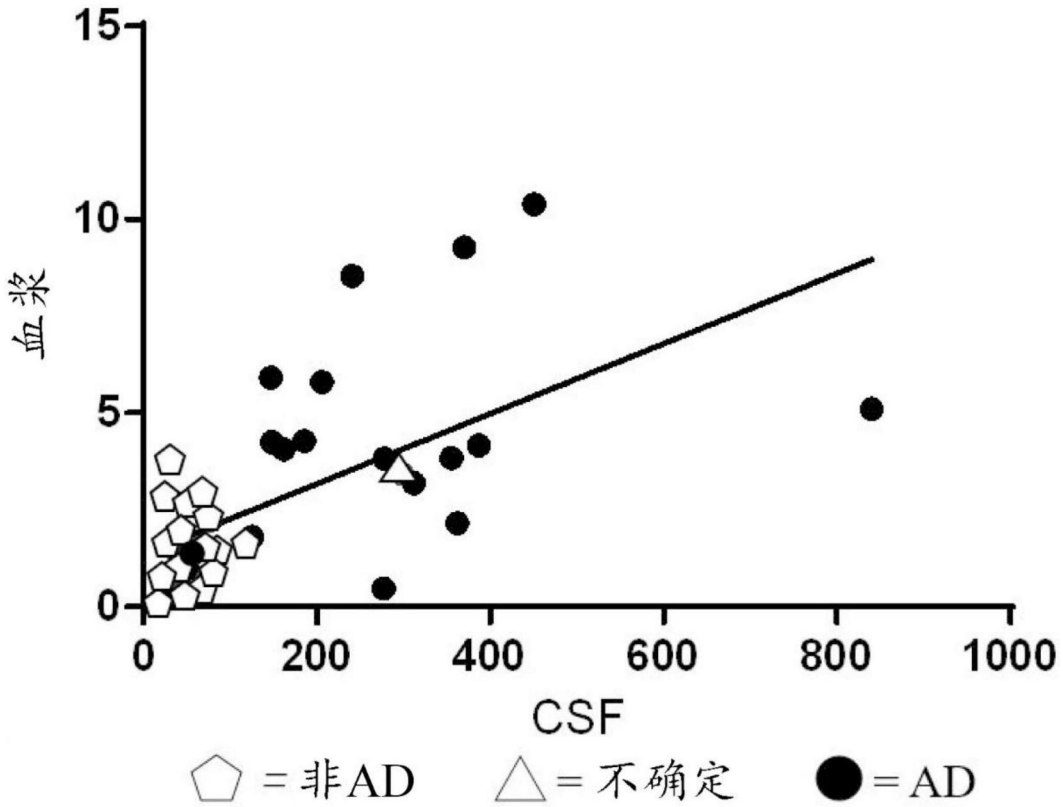
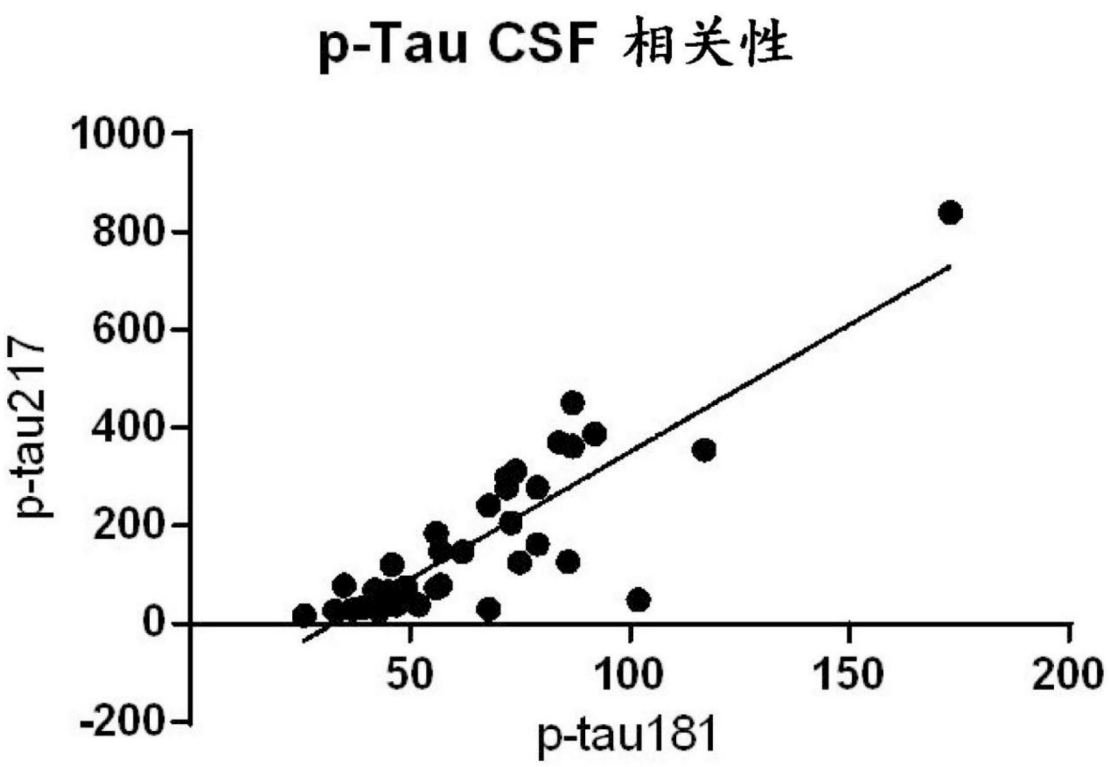

校准曲线p-tau测定
QTx相对于ADx运行
AEB
log(CAL) pg/mL
板1
板2
板3
板4
ADx运行1
ADx运行2
ADx运行3
ADx运行4
校准曲线p-tau测定
QTx相对于ADx运行
AEB
log(CAL) pg/mL
板1
板2
板3
板4
ADx运行1
ADx运行2
ADx运行3
ADx运行4

图9



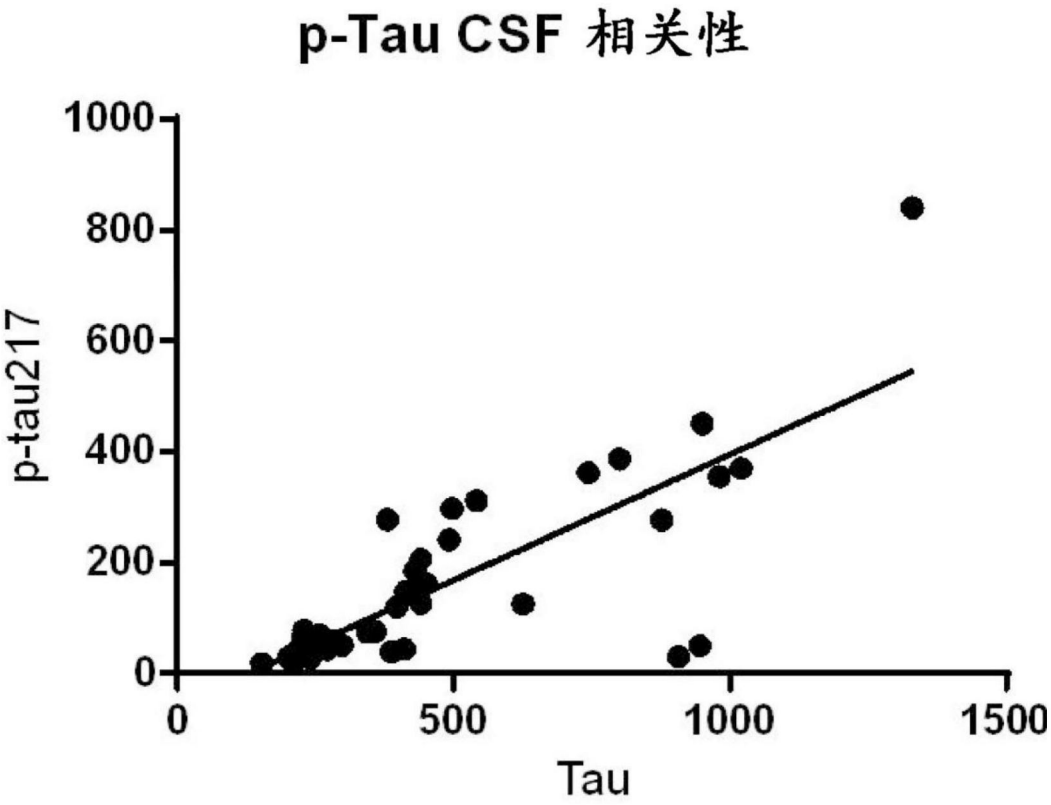
	CSF 相对于 血浆
Spearman r	
r	0.6965
95%置信区间	0.4772 至 0.8341
P值	
P(双尾)	< 0.0001
P值总结	****
精确或近似的P值?	近似
显著?(α = 0.05)	是
XY对的数量	38

图10



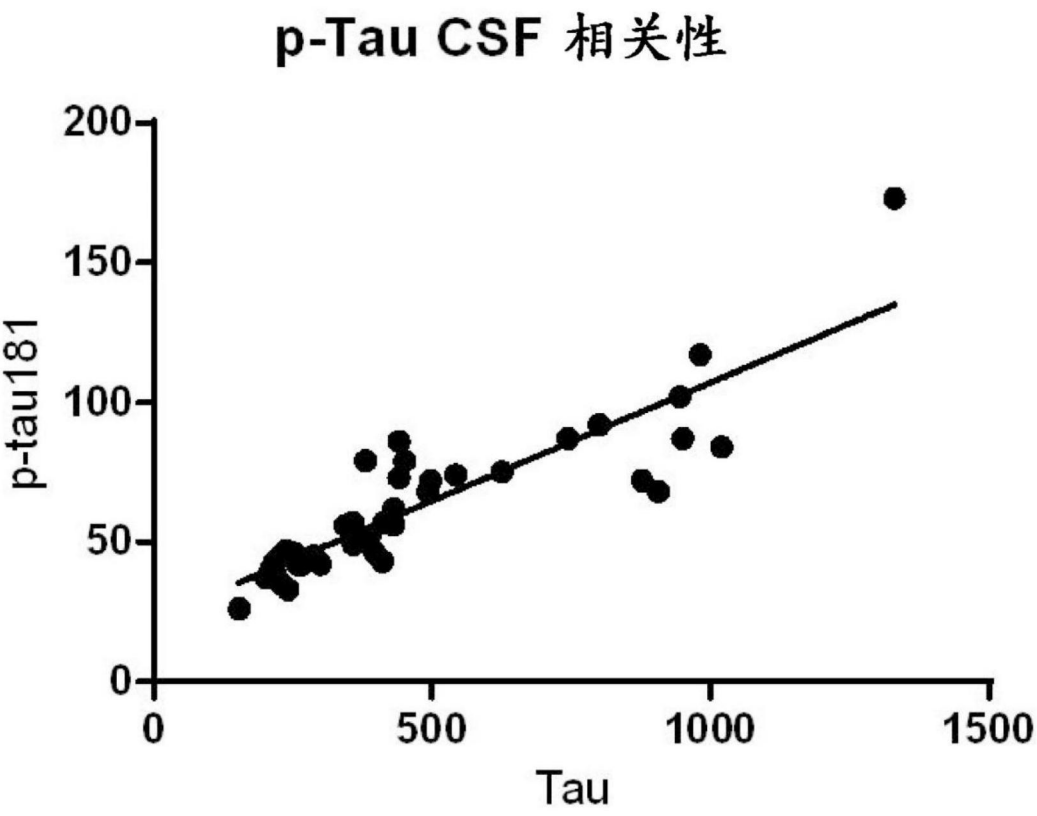
	p-tau181 相对于 p-tau217
Spearman r	
r	0.7952
95%置信区间	0.6424 至 0.8872
P值	
P(双尾)	< 0.0001
P值总结	****
精确或近似的P值?	近似
显著?(α = 0.05)	是
XY对的数量	42

图11



	Tau 相对于 p-tau217
Spearman r	
r	0.7729
95%置信区间	0.6071 至 0.8742
P值	
P(双尾)	< 0.0001
P值总结	****
精确或近似的P值?	近似
显著?(α = 0.05)	是
XY对的数量	42

图12



	Tau 相对于 p-tau181
Spearman r	
r	0.8978
95%置信区间	0.8136 至 0.9451
P值	
P(双尾)	< 0.0001
P值总结	****
精确或近似的P值?	近似
显著?(α = 0.05)	是
XY对的数量	42

图13

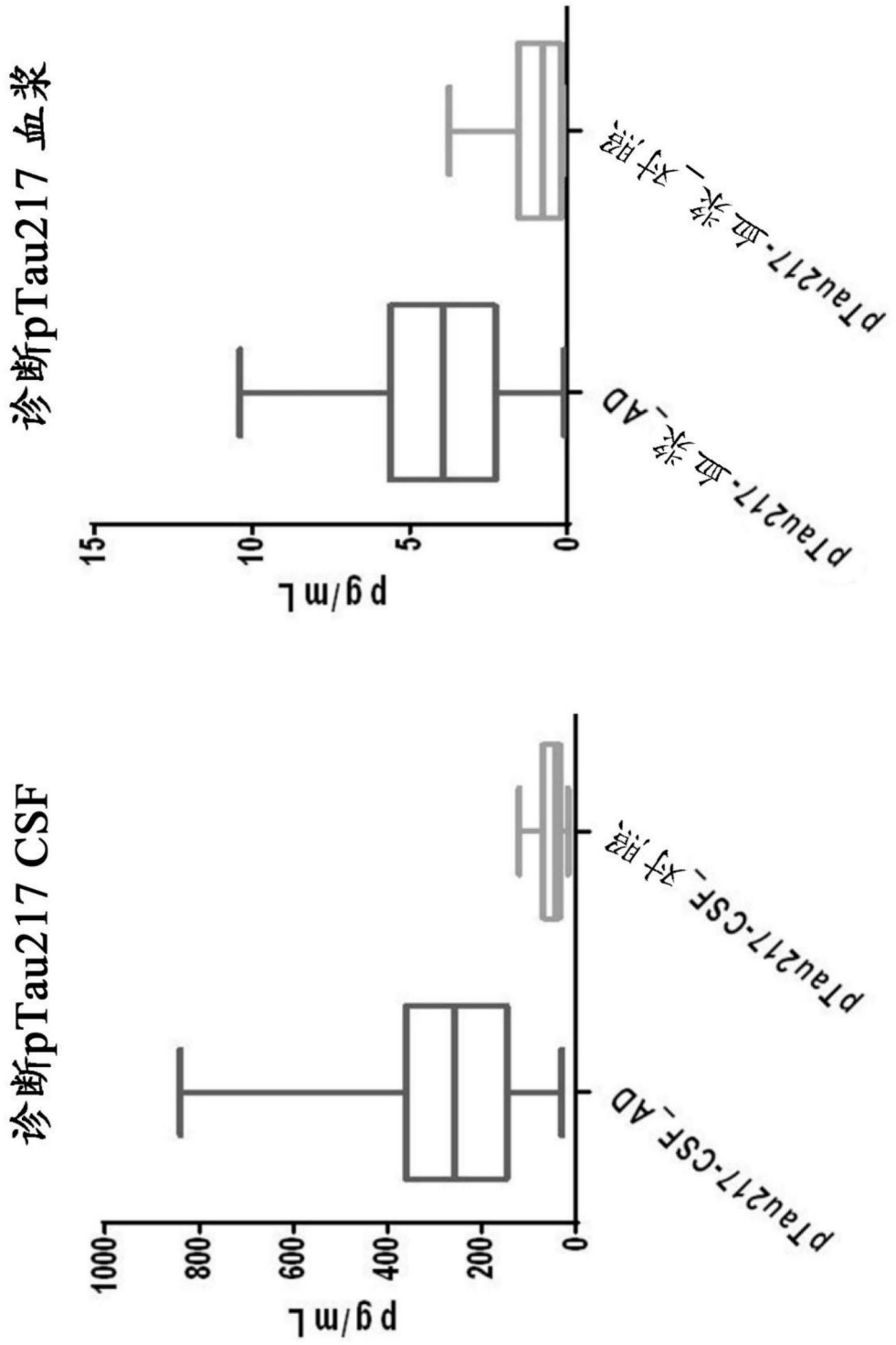

诊断pTau217 血浆
pTau217-血浆_对照
pTau217-血浆_AD
pg/mL
0
5
10
15
诊断pTau217 CSF
pTau217-CSF_对照
pTau217-CSF_AD
pg/mL
0
200
400
600
800
1000

图14

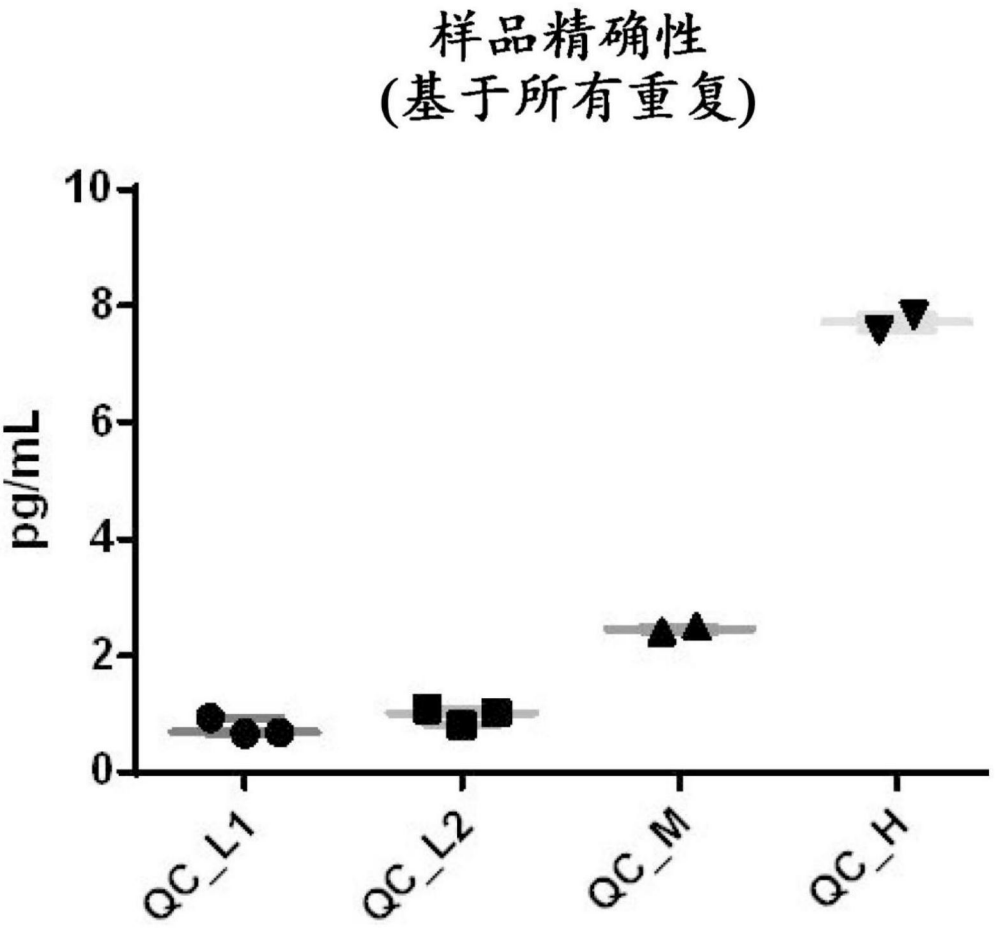
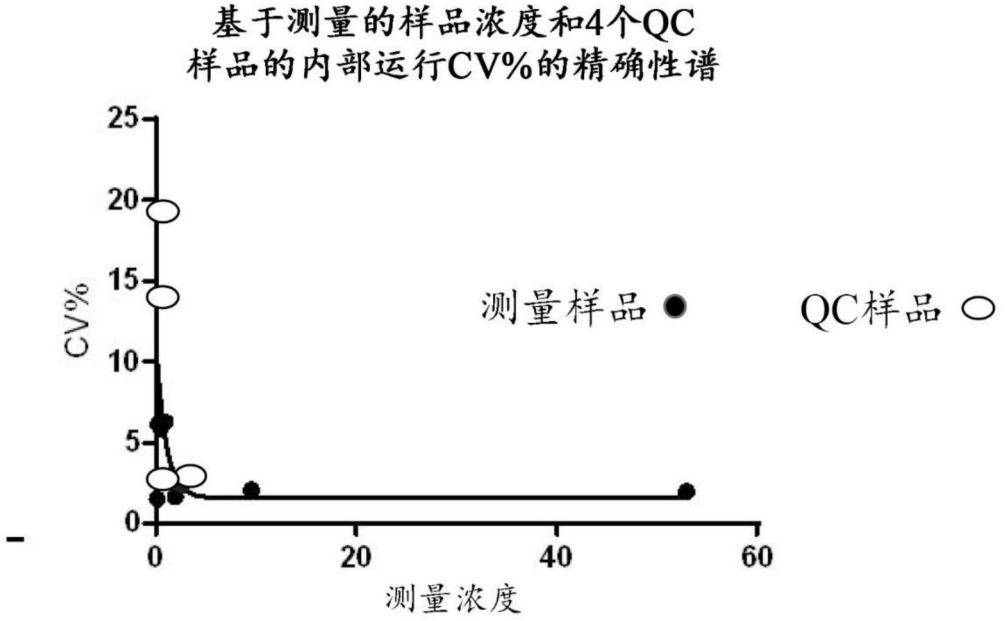


图15



	QC_L1	QC_L2	QC_M	QC_H
值的数量	3	3	2	2
变异系数	19.42%	14.00%	2.70%	2.36%

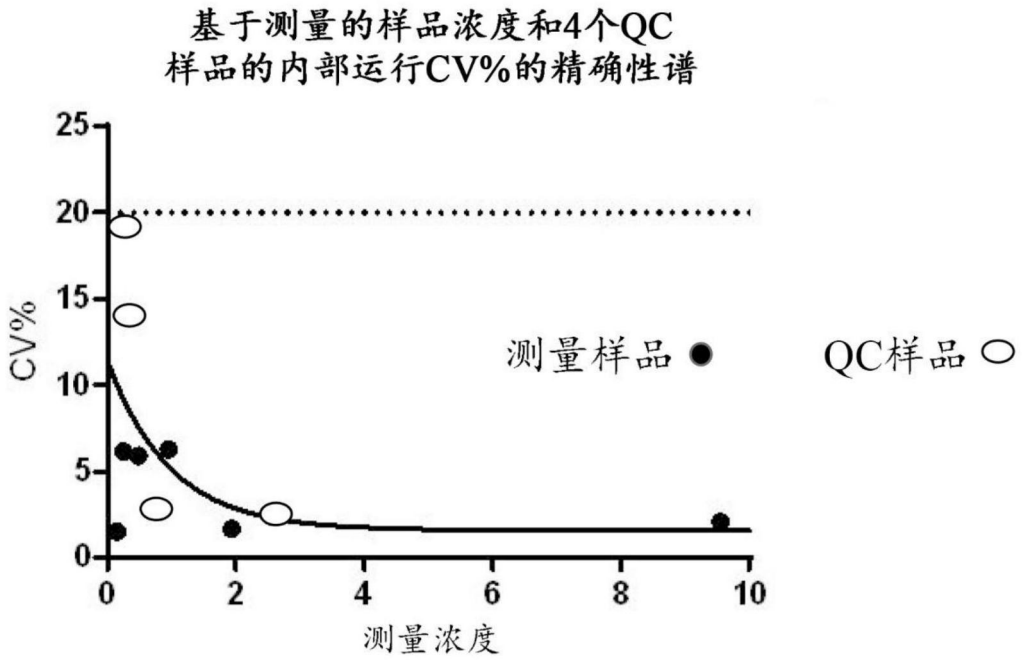
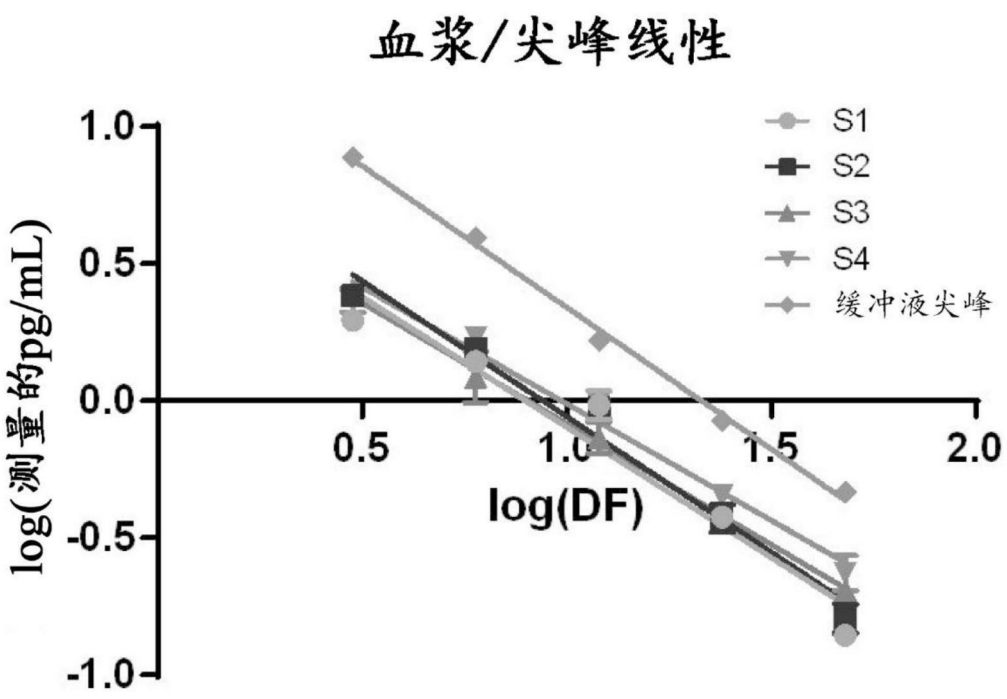


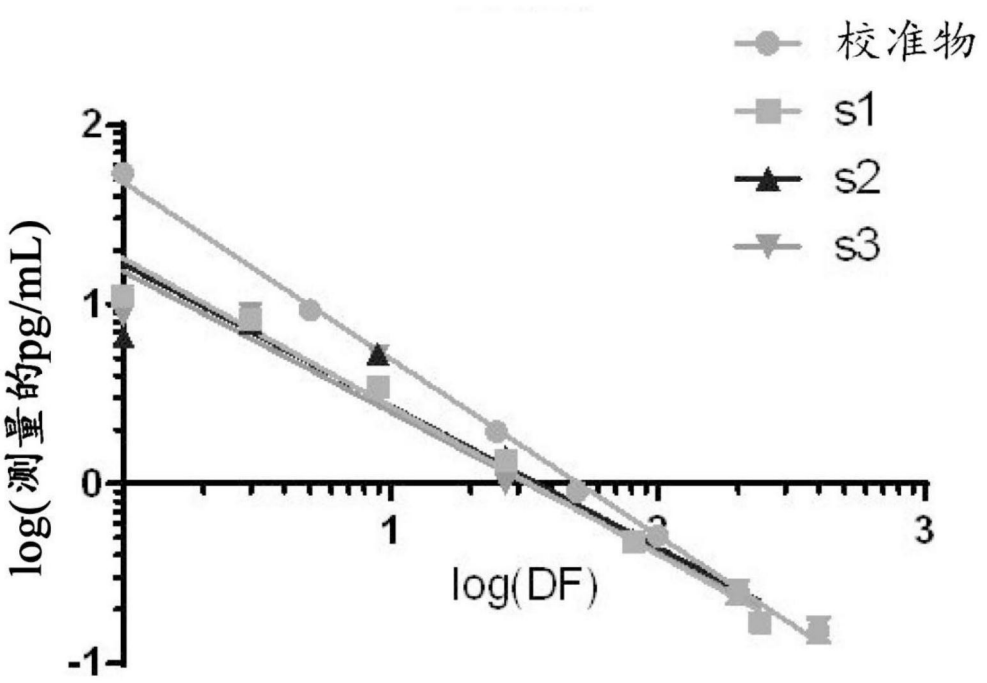
图16



	S1	S2	S3	S4	缓冲液尖峰
Y截距	0.86	0.93	0.80	0.84	1.38
斜率	-0.95	-0.99	-0.88	-0.85	-1.03
差值%	92%	96%	85%	82%	100%

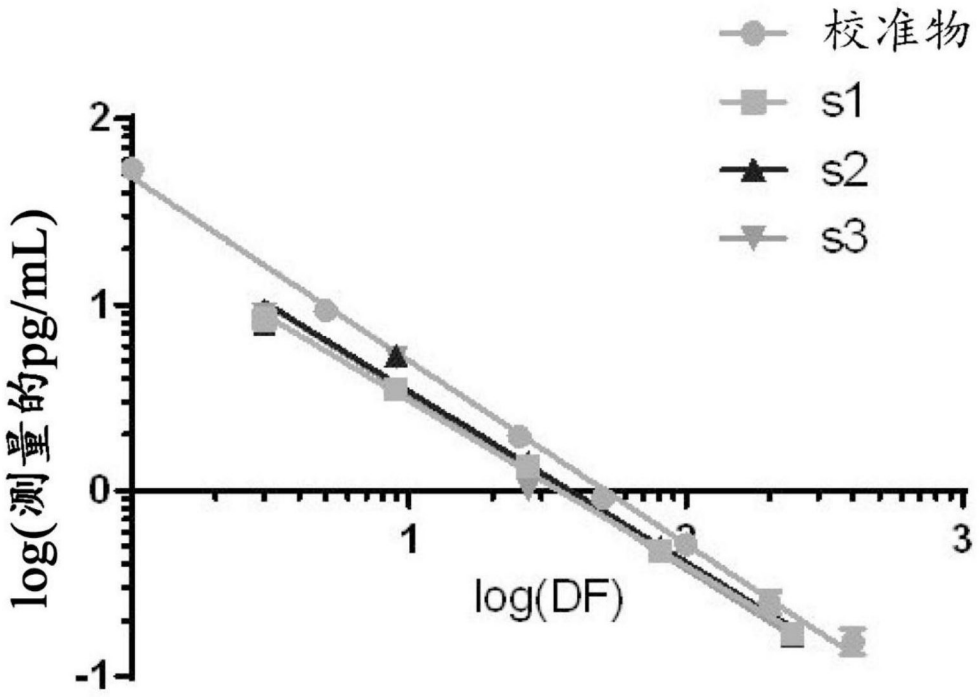
表4. 并行性	校准器 (在以下 段落中表示 为'reeks 1')	样品1	样品2	样品3	样品4	样品5 (加标稀释 缓冲液)
名称标志物	Df (x)	Df (x)	Df (x)	Df (x)	Df (x)	Df (x)
pTAU217	1	3	3	3	3	3
	5	6	6	6	6	6
	25	12	12	12	12	12
	50	24	24	24	24	24
	100	48	48	48	48	48
	200					
斜率(a)	0,8669	0,7865	0,8272	0,7459	0,7239	0,9167
在范围内(%)	85-115	91	95	86	84	106
Df=测定中使用的样品的稀释因子						

图17



	校准物	s1	s2	s3
Y截距	1.68	1.26	1.23	1.19
斜率	-0.99	-0.83	-0.79	-0.78
差值%		84%	80%	79%

图18



	校准物	s1	s2	s3
Y截距	1.68	1.38	1.45	1.46
斜率	-0.99	-0.89	-0.92	-0.94
差值%		90%	93%	95%

图19

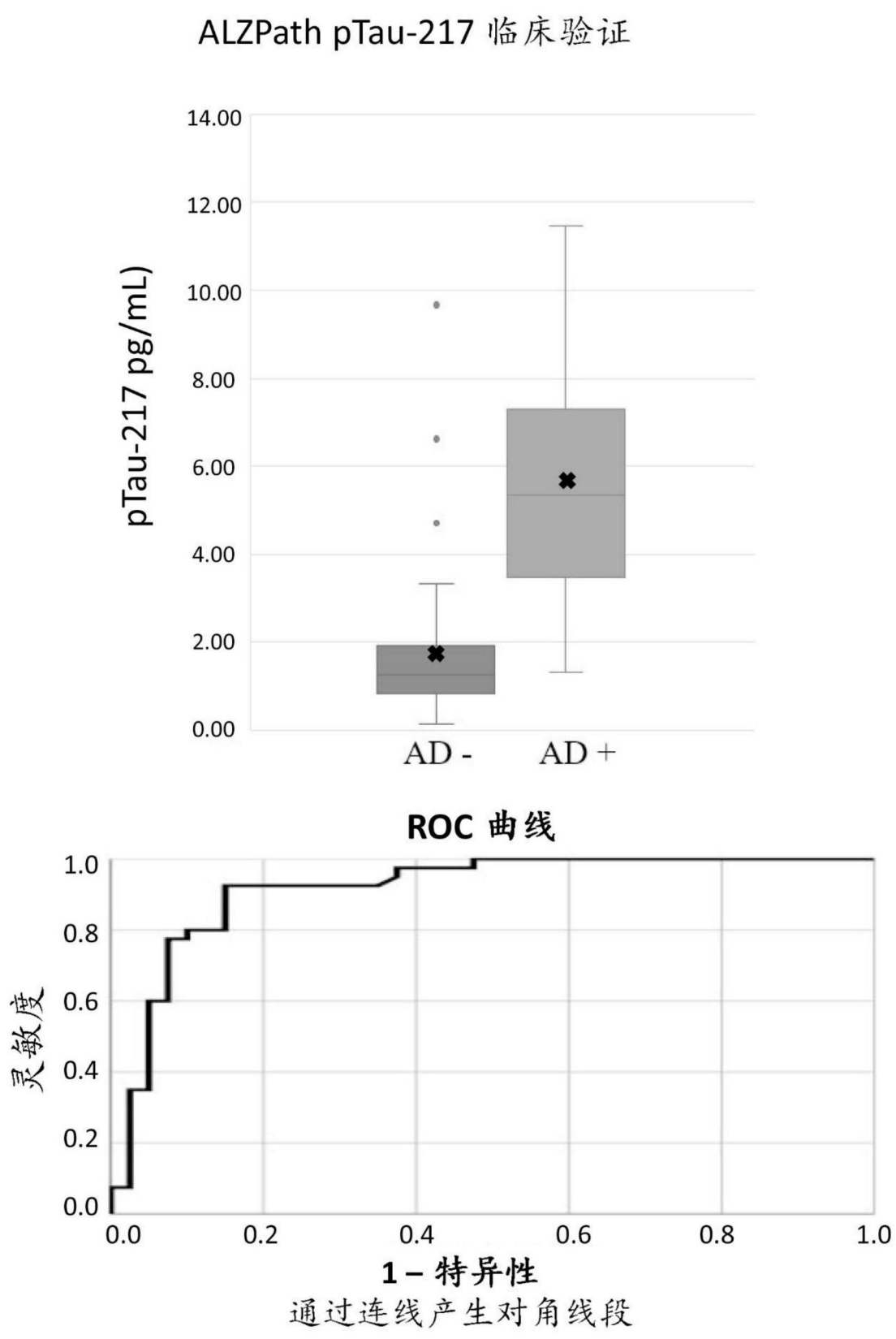

ALZPath pTau-217 临床验证
pTau-217 pg/mL)
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
AD -
AD +
ROC 曲线
灵敏度
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.2
0.4
0.6
0.8
1.0
1 – 特异性
通过连线产生对角线段

图20

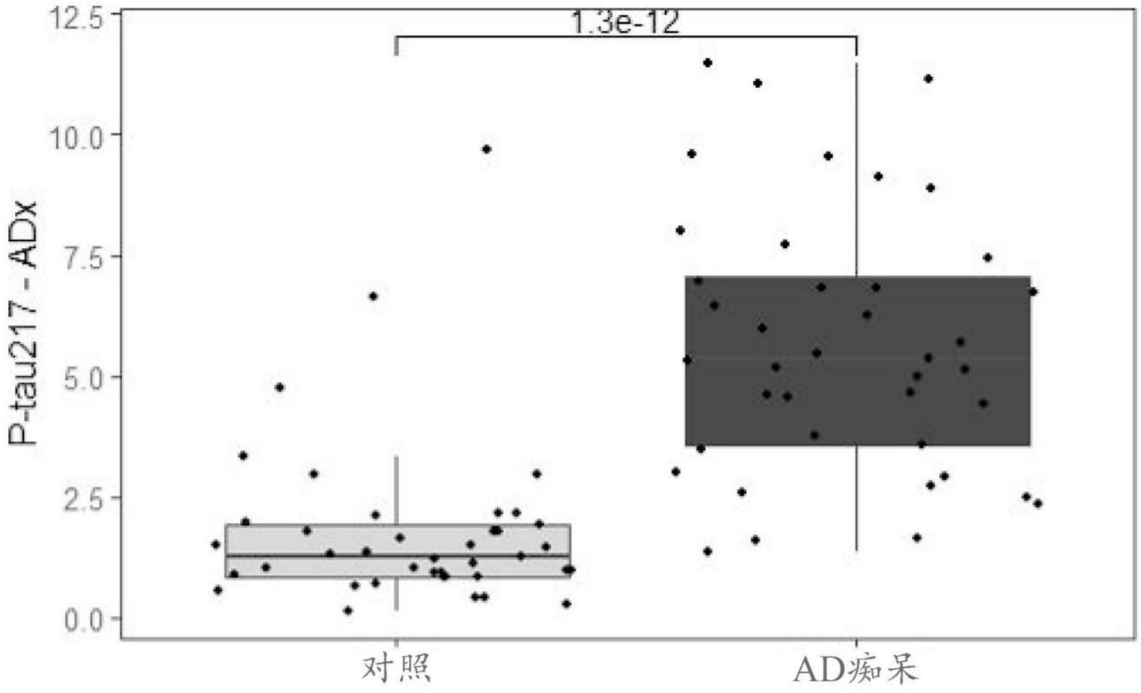
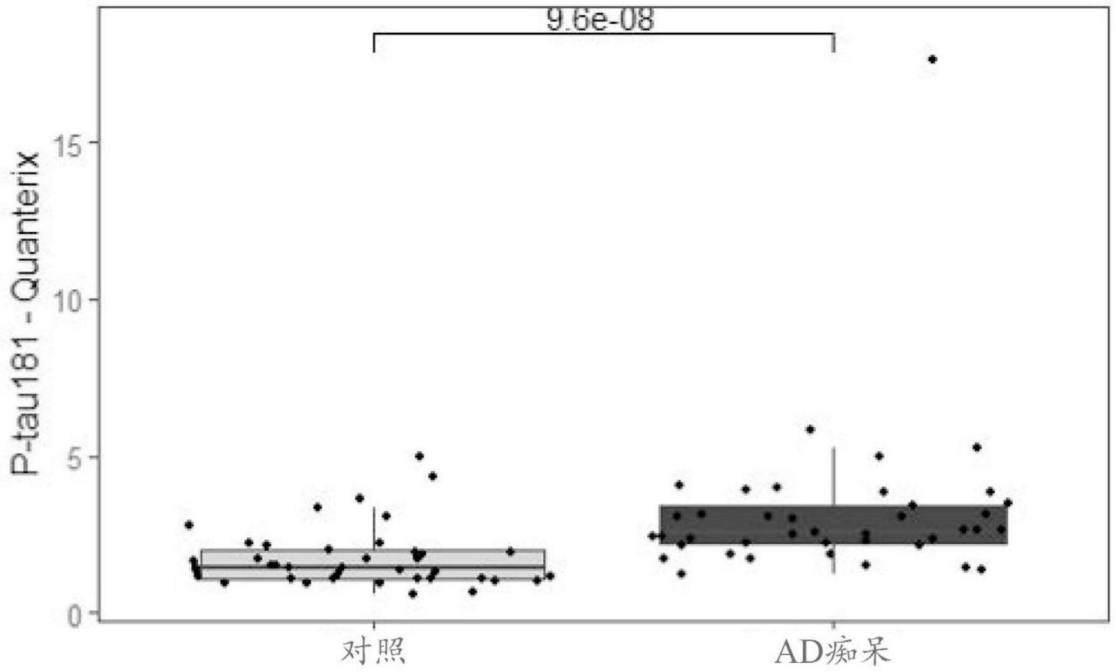
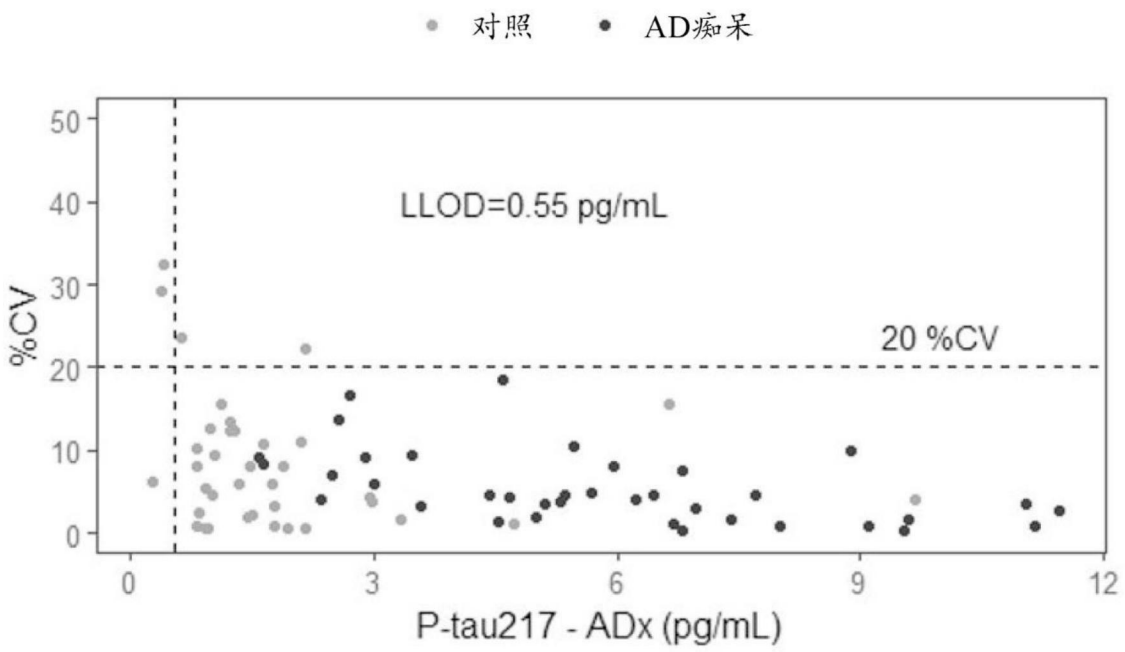


图21



		针对诊断分层			
		对照		AD痴呆	
	总计	女性	男性	女性	男性
总计,n	80	28	12	28	12
女性性别(%)	28 (70%)	28 (70%)	12 (30%)	28 (70%)	12 (30%)
年龄 (中值[范围])	64 [54-83]	63 [54-81]	66 [58-83]	65 [57-71]	66.5 [61-72]

图22


对照
AD痴呆
LLOD=0.55 pg/mL
20 %CV
%CV
P-tau217 - ADx (pg/mL)

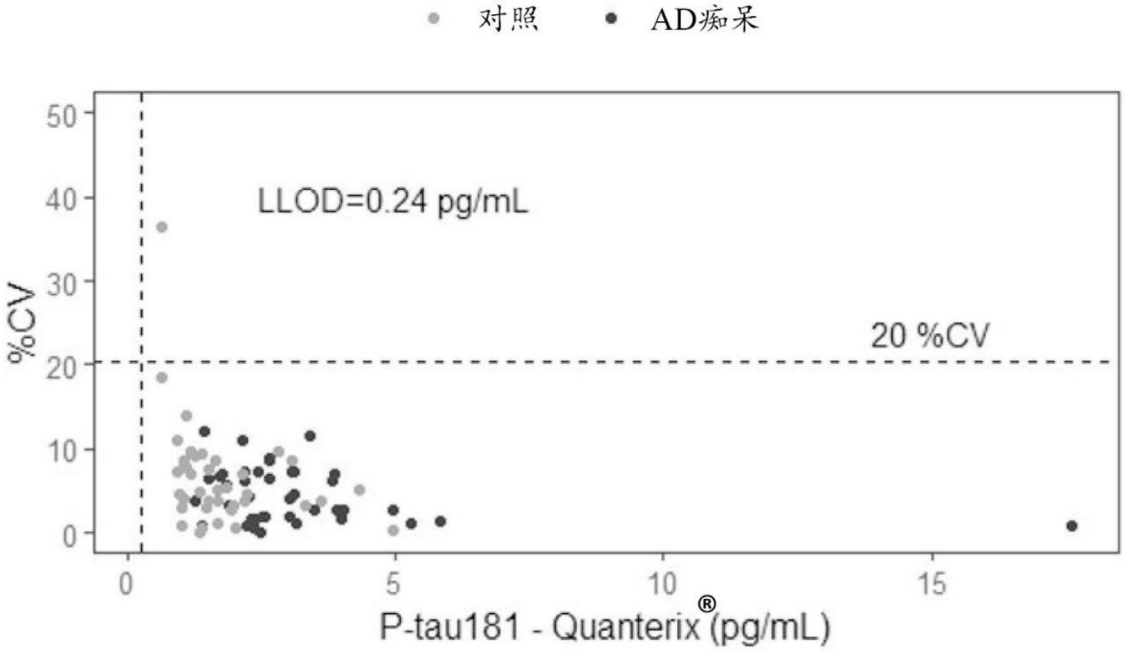
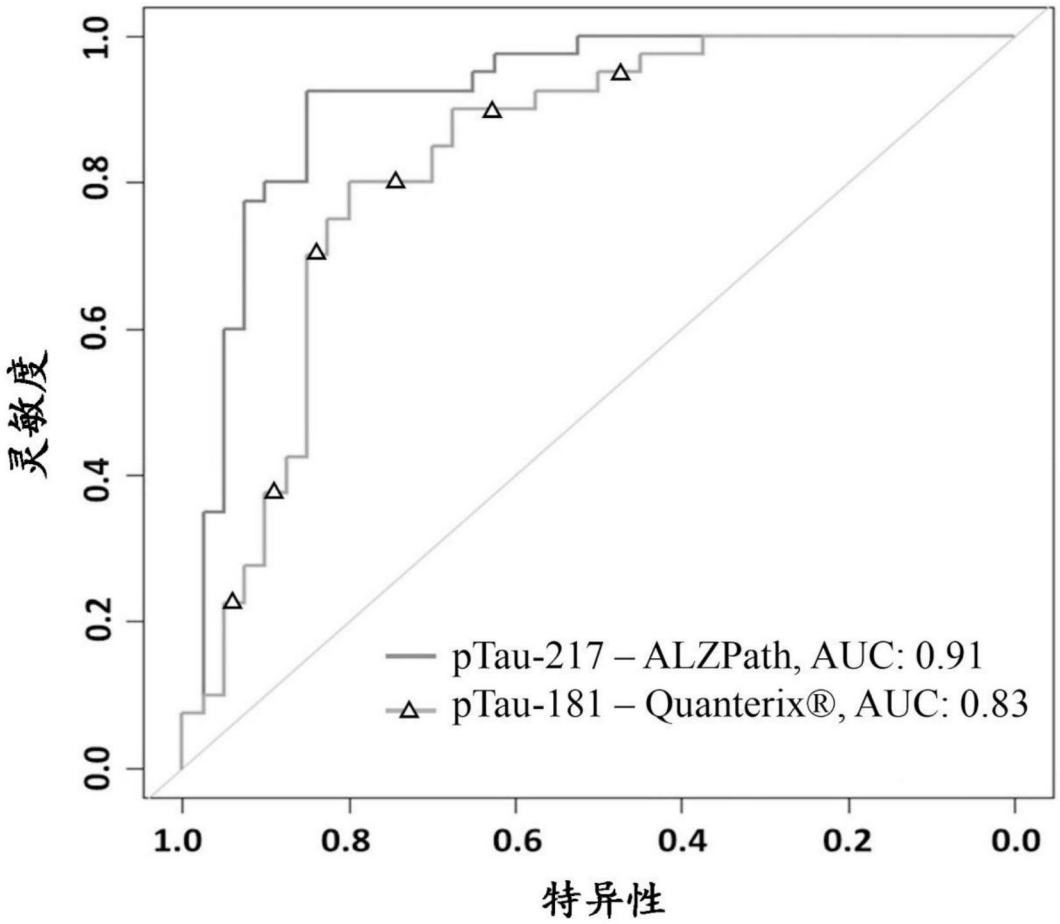

对照
AD痴呆
LLOD=0.24 pg/mL
20 %CV
%CV
P-tau181 - Quanterix® (pg/mL)

图23



	AD-痴呆	对照		微分	AD-痴呆相对于对照		
	中值(IQR)	中值	倍数变化	AUC (95% CI)	截止值	灵敏度%	特异性%
pTau-217 ALZPath	5.2 (3.5 - 6.8)	1.3 (0.8 – 1.9)	4.2	0.91 (0.85 – 0.98)	2.26	92.5	85
pTau-181 Quanterix®	2.5 (2.2 - 3.3)	1.4 (1.1 – 1.9)	1.8	0.83 (0.74 – 0.920	2.14	80	80

图24

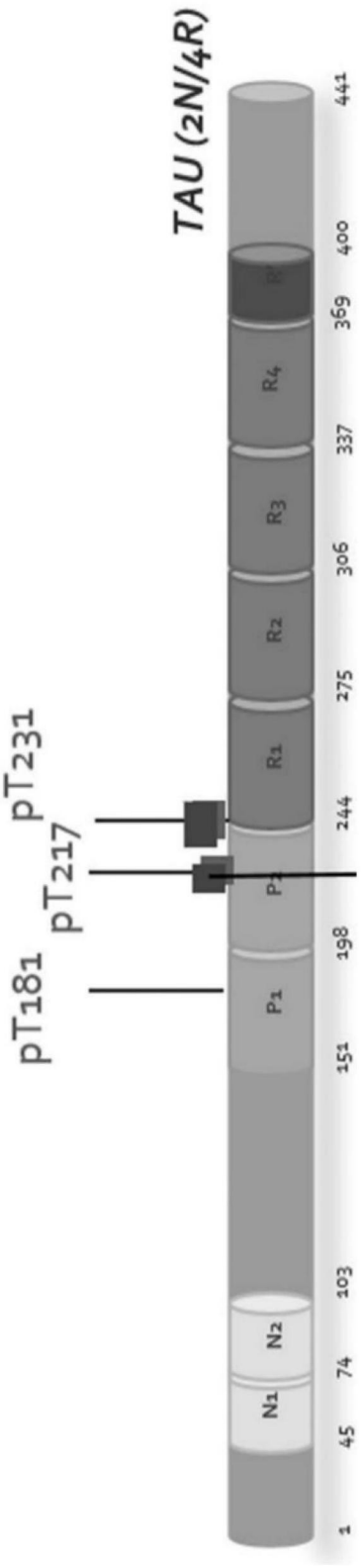

TAU (2N/4R)
pT231
pT217
pT181
R4
R3
R2
R1
P2
P1
N2
N1
441
400
369
337
306
275
244
198
151
103
74
45
1

图25

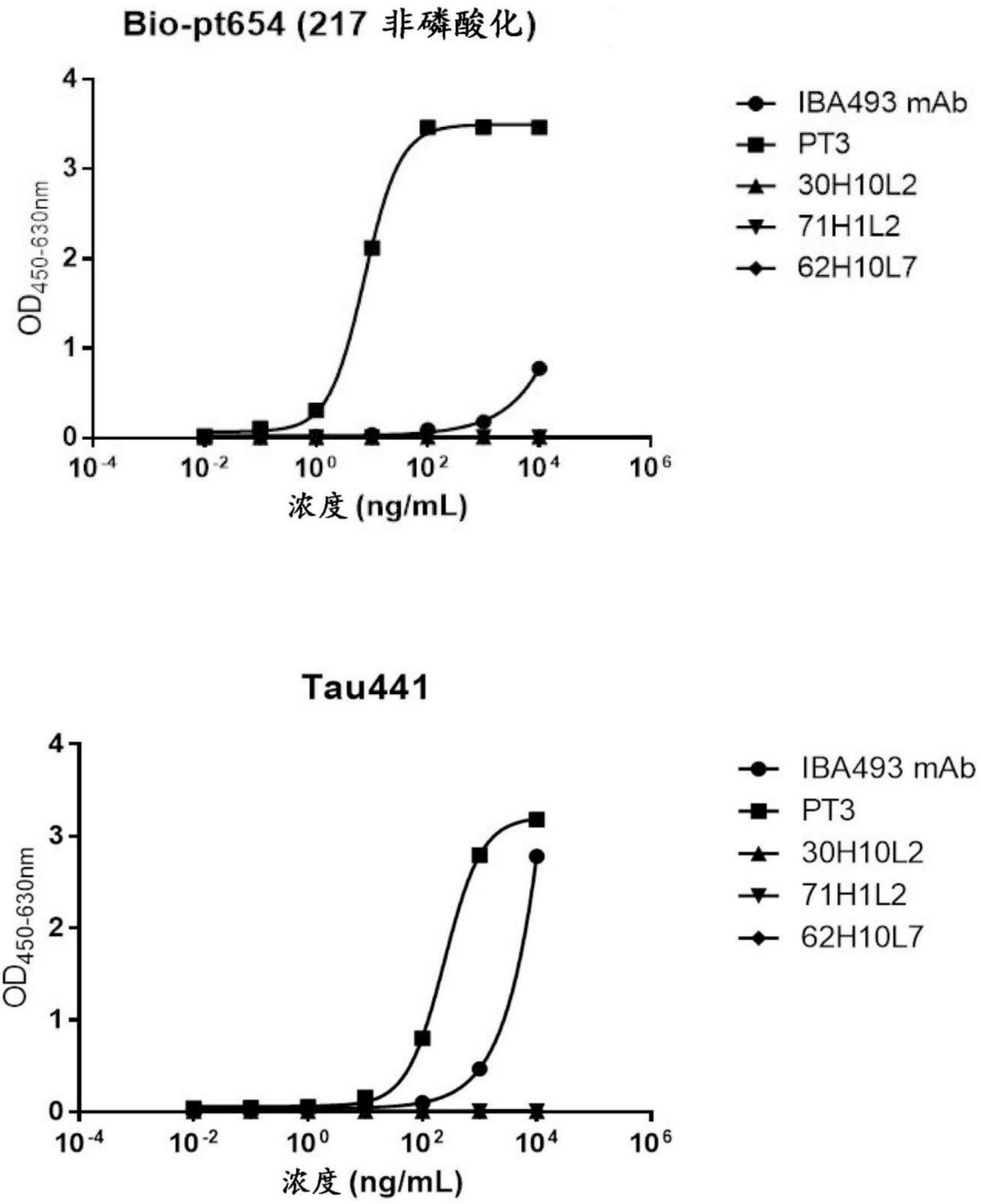

Bio-pt654 (217 非磷酸化)
OD450-630nm
浓度 (ng/mL)
IBA493 mAb
PT3
30H10L2
71H1L2
62H10L7
Tau441
OD450-630nm
浓度 (ng/mL)
IBA493 mAb
PT3
30H10L2
71H1L2
62H10L7

图26

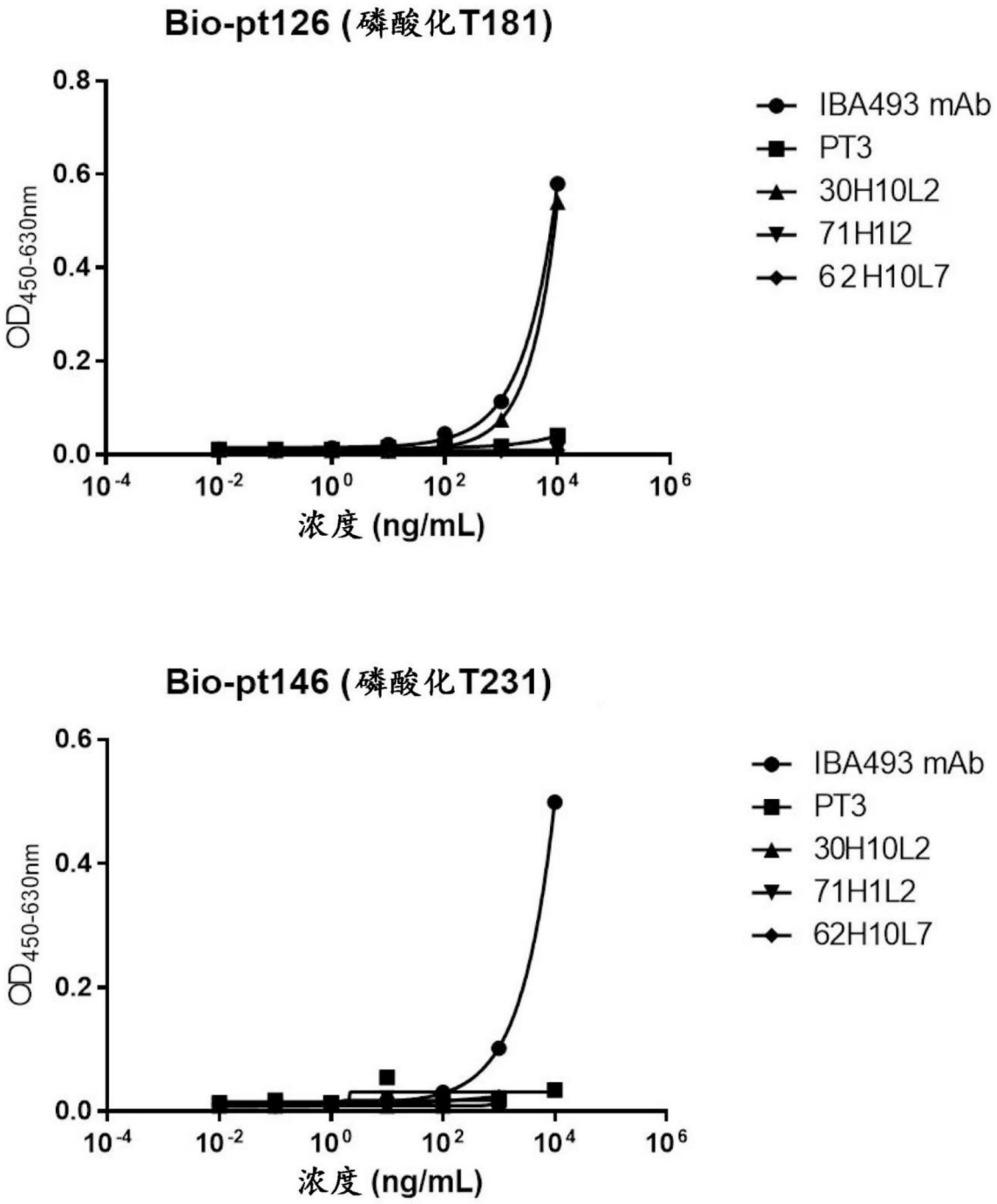
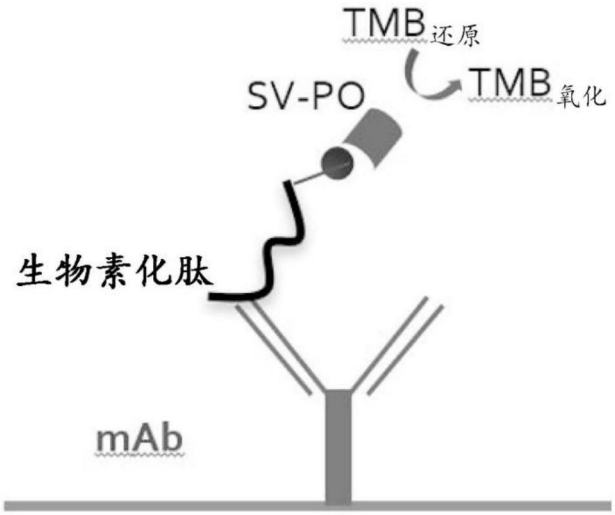

Bio-pt126 (磷酸化T181)
OD450-630nm
0.8
0.6
0.4
0.2
0.0
10-4
10-2
100
102
104
106
浓度 (ng/mL)
IBA493 mAb
PT3
30H10L2
71H1l2
62H10L7
Bio-pt146 (磷酸化T231)
OD450-630nm
0.6
0.4
0.2
0.0
10-4
10-2
100
102
104
106
浓度 (ng/mL)
IBA493 mAb
PT3
30H10L2
71H1L2
62H10L7

图27


TMB还原
TMB氧化
SV-PO
生物素化肽
mAb

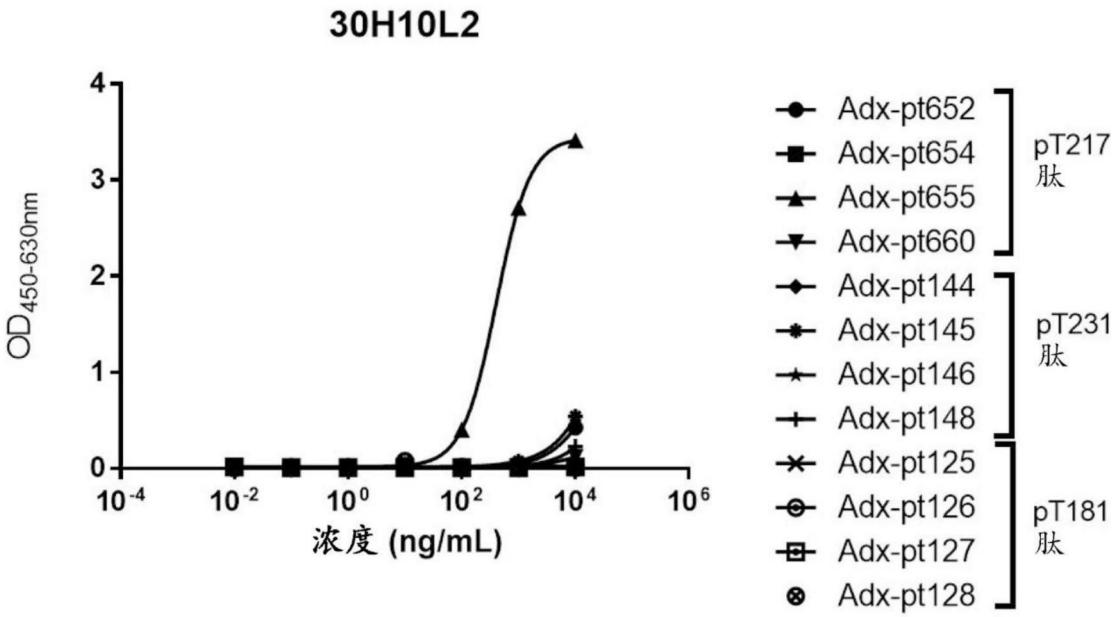
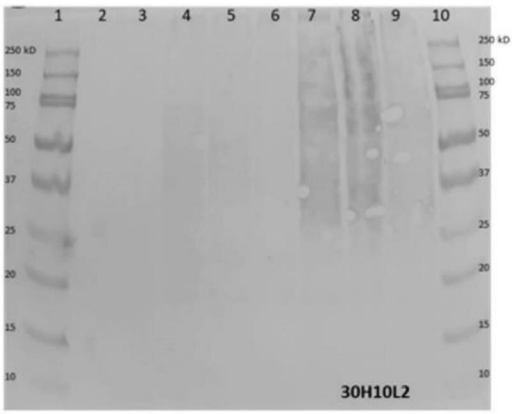
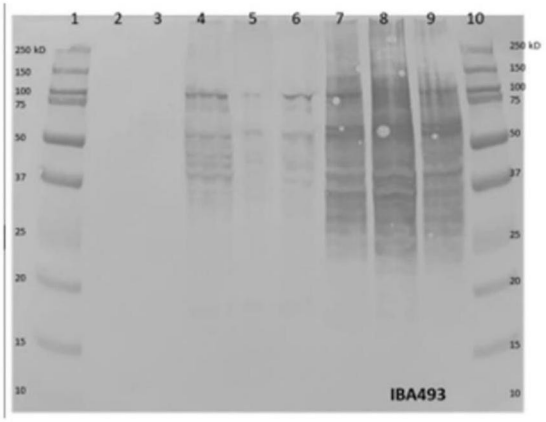
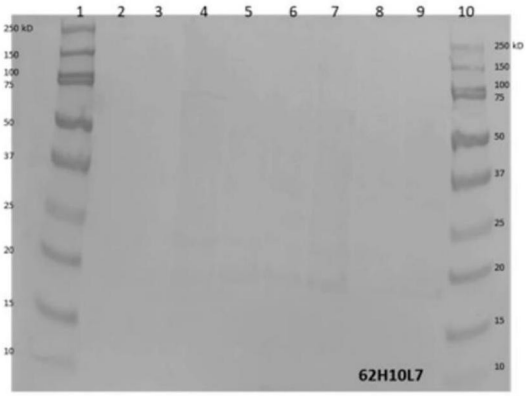

30H10L2
OD450-630nm
浓度 (ng/mL)
Adx-pt652
Adx-pt654
Adx-pt655
Adx-pt660
pT217肽
Adx-pt144
Adx-pt145
Adx-pt146
Adx-pt148
pT231肽
Adx-pt125
Adx-pt126
Adx-pt127
Adx-pt128
pT181肽

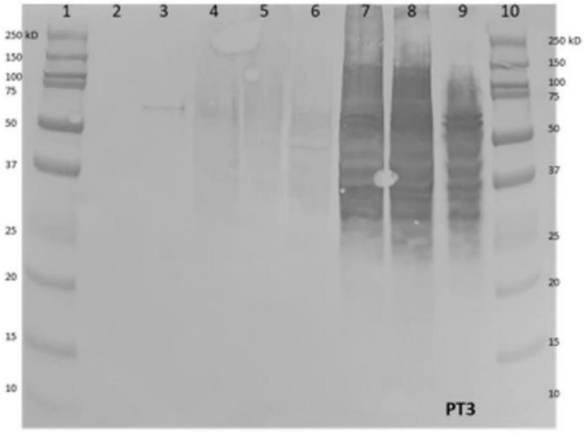
图28

泳道	生物材料	加载的	病理学	批次#
1	PL			
2	磷酸酶处理的pTau	0,05 µg		ADR21051
3	Tau441	0,05 µg		ADIP20939
4	BS18022	DF 5	对照	ADIP19534
5	BS18024	DF 5	对照	ADIP19534
6	BS18025	DF 5	对照	ADIP19534
7	BS18027	DF 5	AD	ADIP19534
8	BS18028	DF 5	AD	ADIP19534
9	BS18029	DF 5	AD	ADIP19534
10	PL			









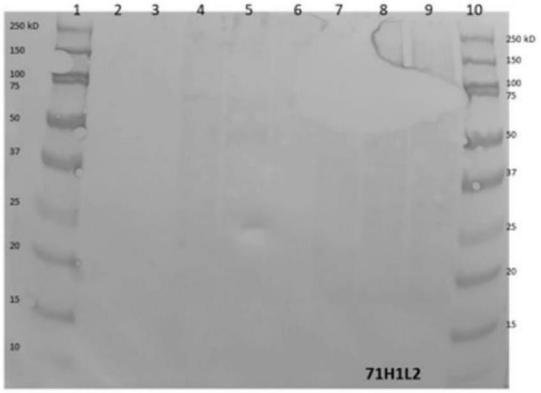


图29