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(54) Title: ANGPTL8 ASSAY AND USES THEREOF

(57) Abstract: The present invention provides ELISA-based methods for detecting and/or quantifying ANGPTL8 in biological samples using anti-ANGPTL8 antibodies.

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ANGPTL8 ASSAY AND USES THEREOF

[0001] This application is being filed on December 18, 2018 as a PCT International Patent Application and claims the benefit of priority to US provisional application No. 62/599,911, filed on December 18, 2017, the disclosure of which is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0002] The present invention relates to diagnostic tests and assay methods for detecting angiopoietin-like protein (ANGPTL) 8 levels in a subject and for identifying capture and detection monoclonal antibodies and/or fragments thereof that specifically bind ANGPTL8.

BACKGROUND

[0003] ANGPTL8 (alternatively called TD26, RIFL, Lipasin, C19orf80 and Betatrophin) is an ANGPTL family member that has been implicated in both triglyceride (TG) and glucose metabolism. It is a circulating protein that is expressed primarily in liver and adipose tissue. Unlike ANGPTL3 and ANGPTL4, ANGPTL8 lacks a fibrinogen-like domain at the C-terminus, but contains an N-terminal coiled-coil domain, much like other ANGPTL family members. Phylogenetic analysis reveals that ANGPTL8 shares common ancestors with ANGPTL3 and ANGPTL4 (Fu, Z. *et. al.*, (2013), Biochem. Biophys. Res. Commun. 430:1126-1131).

[0004] Hepatic overexpression of ANGPTL8 is associated with hypertriglyceridemia, whereas inactivation of *Angptl8* causes a reduction in plasma TG levels (Quagliarini, F. *et. al.* (2012), Proc. Natl. Acad. Sci. USA 109(48):19751-19756; Wang, Y. *et. al.* (2013), Proc. Natl. Acad. Sci. USA 110:16109-16114). Despite the consensus that ANGPTL8 is involved in lipid regulation, the mechanism responsible for this process is still under debate. One proposed mechanism is that ANGPTL8 inhibits lipoprotein lipase (LPL) activity, resulting in reduced triglyceride hydrolysis and clearance (Zhang, R. *et.al.*, (2012), Biochem. Biophys. Res. Commun. 424:786-792).

[0005] ANGPTL8 has also been reported to play a role in beta cell proliferation and beta cell mass in mice, where insulin resistance was induced by an insulin receptor antagonist, S961 (Yi, P. *et. al.* (2013), Cell 153:747-758). However, subsequent studies revealed that ANGPTL8 is not required for beta cell function, or the beta cell growth response to insulin resistance. Furthermore, overexpression of ANGPTL8 does not increase beta cell area or

improve glycemic control (Gusarova, V. *et. al.* (2014) Cell 159:691-696). Hepatic overexpression of ANGPTL8 is associated with hypertriglyceridemia.

[0006] Given the role of ANGPTL8 in hypertriglyceridemia and other disorders or conditions associated with elevated triglyceride and lipid levels, it could be beneficial to be able to assess the presence and/or level of ANGPTL8 in a subject in order to determine a need for treatment or to monitor the amount of ANGPTL8 during treatment and/or at the time of completion of treatment. Such a test or assay method would provide information as to whether a patient needs or is responding to treatment, and, as such, it may also allow for a determination as to whether the patient will be adequately protected by the therapy. Such a test or assay method would also aid in the determination of when a patient can initiate or terminate maintenance therapy. An unmet need exists in the art for the development of such a diagnostic test that is specific to and effective for ANGPTL8 and ANGPTL8-associated disorders, or an assay method for determining the safety, efficacy, or outcome of therapy.

[0007] Such a test or assay method would provide information as to the level of ANGPTL8 in a subject and could also provide information as to the strength and specificity of binding of an ANGPTL8 antagonist.

BRIEF SUMMARY OF THE INVENTION

[0008] There is a need for a commercial ELISA for the evaluation of ANGPTL8 levels in humans. ANGPTL8 levels correlate with many diseases, including, without limitation, obesity, Type 2 diabetes, metabolic syndrome, non-alcoholic steatohepatitis (NASH), and the like. However, most known ELISAs do not yield accurate or reproducible results for ANGPTL8 measurement. Disclosed herein is a method for evaluating ANGPTL8 level(s) in different disease populations, including in previously sequenced cohorts, and for conducting an analysis of humans from these populations to determine whether or not ANGPTL8 level(s) indeed correlate(s) with specific diseases, as well as to evaluate whether or not ANGPTL8 is a or the factor causing the disease and/or can be used as a circulating biomarker to predict the development or presence of the disease.

[0009] In one aspect, the invention provides a method for detecting and/or quantifying human Angiopoietin-like protein 8 (hANGPTL8) in a sample, the method comprising:

Obtaining a sample from a subject;

Adding the sample to the wells of an Enzyme Linked Immunosorbent Assay (ELISA) plate coated with a capture antibody, wherein the capture antibody is a first anti-hANGPTL8 antibody;

Adding a detection antibody to the wells of the plate, wherein the detection antibody is a second anti-hANGPTL8 antibody bound to a tag;

Adding an agent that binds the tag, wherein the agent is conjugated to an enzyme;

Adding a substrate for the enzyme; and

Measuring the amount of product of the enzyme-substrate reaction.

[00010] In one embodiment, the measuring comprises reading the absorbance with a spectrophotometer.

[00011] In one embodiment, the agent is avidin or streptavidin. In another embodiment, the enzyme is horseradish peroxidase (HRP) or alkaline phosphatase. In still another embodiment, the enzyme is HRP.

[00012] In one embodiment, the tag is selected from the group consisting of a fluorescence label, a radiolabel, an enzyme label, a luminescent label, an electrochemical, or a visual label. In another embodiment, the tag is biotin.

[00013] In one embodiment, the inventive method detects and/or quantifies human Angiopoietin-like protein 8 (hANGPTL8) in a sample, but does not detect (and/or quantify) non-human ANGPTL8. In another embodiment, the inventive method detects and/or quantifies human Angiopoietin-like protein 8 (hANGPTL8) in a sample, but does not detect (and/or quantify) hANGPTL3 nor hANGPTL4.

[00014] In one embodiment, the subject is human. In another embodiment, the sample is plasma or serum. In further embodiments, the sample is diluted 1:5, 1:10, or 1:50 in PBS or some other known appropriate diluent. In a particular embodiment, the sample is diluted 1:10 in PBS.

[00015] In one embodiment, the substrate is selected from a chromogenic substrate, a chemiluminescent substrate, and a chemifluorescent substrate. In another embodiment, the substrate is a chromogenic substrate. In one embodiment, the chromogenic substrate is selected from 2,2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt (ABTS), 3,3'-Diaminobenzidine (DAB), *o*-phenylenediamine dihydrochloride (OPD), and 3,3',5,5'-tetramethylbenzidine(TMB). In another embodiment, the chromogenic substrate is p-Nitrophenyl Phosphate, disodium salt (pNPP).

[00016] In one embodiment, the capture or detection antibody is selected from the antibodies listed in Table 1. In another embodiment, the capture or detection antibody is

selected from H4H15318P, H4H15347P, H4H15361P2, and H4H15341P. In still another embodiment, the capture antibody is H4H15347P or H4H15361P2. In still another embodiment, the detection antibody is H4H15318P.

[00017] In another aspect, the invention provides a method for assessing the efficacy or outcome of treating an ANGPTL8-associated disorder in a subject, the method comprising:

Obtaining a sample from a subject;

Adding the sample to the wells of an Enzyme Linked Immunosorbent Assay (ELISA) plate coated with a capture antibody, wherein the capture antibody is a first anti-hANGPTL8 antibody;

Adding a detection antibody to the wells of the plate, wherein the detection antibody is a second anti-hANGPTL8 antibody bound to a tag;

Adding an agent that binds the tag, wherein the agent is conjugated to an enzyme;

Adding a substrate for the enzyme; and

Measuring the amount of product of the enzyme-substrate reaction.

[00018] In one embodiment, a decrease in the level of ANGPTL8 in the sample indicates efficacy and/or a positive outcome of the treatment. In another embodiment, the level of ANGPTL8 in the sample assists the clinician in determining whether or not further treatment is warranted.

[00019] In another aspect, the invention provides a method for determining whether a subject is at risk for developing an ANGPTL8-associated disorder, the method comprising:

Obtaining a sample from a subject;

Adding the sample to the wells of an Enzyme Linked Immunosorbent Assay (ELISA) plate coated with a capture antibody, wherein the capture antibody is a first anti-hANGPTL8 antibody;

Adding a detection antibody to the wells of the plate, wherein the detection antibody is a second anti-hANGPTL8 antibody bound to a tag;

Adding an agent that binds the tag, wherein the agent is conjugated to an enzyme;

Adding a substrate for the enzyme; and

Measuring the amount of product of the enzyme-substrate reaction.

[00020] In one embodiment, an augmented level of ANGPTL8 in the sample indicates risk for developing an ANGPTL8-associated disorder.

[00021] In another aspect, the invention provides a method for treating an ANGPTL8-associated disorder in a subject, the method comprising, before, during, and/or after treatment, respectively:

a) Obtaining a sample from a subject;
b) Adding the sample to the wells of an Enzyme Linked Immunosorbent Assay (ELISA) plate coated with a capture antibody, wherein the capture antibody is a first anti-hANGPTL8 antibody;
c) Adding a detection antibody to the wells of the plate, wherein the detection antibody is a second anti-hANGPTL8 antibody bound to a tag;
d) Adding an agent that binds the tag, wherein the agent is conjugated to an enzyme;
e) Adding a substrate for the enzyme; and
f) Measuring the amount of product of the enzyme-substrate reaction;

the method further comprising comparing the amounts measured before, during, and/or after treatment with one another, and continuing or discontinuing treatment based on the comparison. In additional embodiments, a standard curve with known concentrations of hANGPTL8 is generated each time the ELISA is run, and the concentration of hANGPTL8 in the sample is determined using this curve. As a result, the amount of product (measured as optical density) at the end of the ELISA directly correlates to the hANGPTL8 level in the sample.

[00022] In one embodiment, a decrease in the level of ANGPTL8 in the sample indicates efficacy and/or a positive outcome of the treatment.

[00023] In one aspect, the invention provides a kit for determining if a patient is responsive to treatment of an ANGPTL8-associated disorder/condition, the kit comprising: an Enzyme Linked Immunosorbent Assay (ELISA) plate coated with a capture antibody, wherein the capture antibody is a first anti-hANGPTL8 antibody; a detection antibody, wherein the detection antibody is a second anti-hANGPTL8 antibody bound to a tag, an agent conjugated to an enzyme, a substrate for the enzyme, instructions for obtaining a sample from a subject, and instructions for measuring the amount of ANGPTL8 in the sample.

[00024] In another aspect, the invention provides a chromogenic sandwich ELISA assay for detecting and/or determining the level of ANGTPL8, in particular, human ANGPTL8 in a sample, wherein the assay plate wells are coated with a capture antibody (a first anti-hANGPTL8 antibody) before adding the sample (preferably diluted) to the wells; wherein a detection antibody (a second anti-hANGPTL8 antibody bound to a tag) is then added to

the wells, followed by an agent conjugated to an enzyme, and then a substrate for the enzyme, allowing for subsequent measurement of the amount of product of the enzyme-substrate reaction. That amount reflects the amount of ANGPTL8 in the sample.

[00025] In certain embodiments of a method or kit according to the invention, the capture antibody is H4H15347P, and the detection antibody is H4H15318P.

[00026] Other embodiments will become apparent from a review of the ensuing detailed description.

BRIEF DESCRIPTION OF THE FIGURES

[00027] **Figure 1** schematically depicts the basic principle of a chromogenic sandwich ELISA system, as applied to human ANGPTL8.

[00028] **Figure 2** shows an immunoblot providing the results of the immunoprecipitations that tested the ability of each instantly purified human ANGPTL8 mAb to pull down human ANGPTL8 protein from cell-conditioned media.

[00029] **Figure 3A** graphically depicts the results of the first mAb pair screen, using H4H15318P as the capture mAb with biotinylated-H4H15341P, -H4H15347P, and -H4H15361P2 as the detection mAbs. The upper line (blue in color figure) corresponds to hANGPTL8.mFc. **Figure 3B** graphically depicts the results of the second mAb pair screen, using H4H15341P as the capture mAb with biotinylated-H4H15318P, -H4H15347P, and -H4H15361P2 as the detection mAbs. The upper line (blue in color figure) (except in lower right-hand corner graph, where it is the lower line) corresponds to hANGPTL8.mFc. **Figure 3C** graphically depicts the results of the third mAb pair screen, using H4H15347P as the capture mAb with biotinylated-H4H15318P, and -H4H15341P as the detection mAbs. The upper line (blue in color figure) corresponds to hANGPTL8.mFc. **Figure 3D** graphically depicts the results of the fourth mAb pair screen, using H4H15361P2 as the capture mAb with biotinylated-H4H15318P, -H4H15341P, and -H4H15347P as the detection mAbs. The upper line (blue in color figure) (except in lower three graphs, where it is the lower line) corresponds to hANGPTL8.mFc.

[00030] **Figure 4** shows, in bar graph form, the results of applying the ELISA system described herein to test serum samples from wild type, knockout, and ANGPTL8 humanized mice.

[00031] **Figure 5** graphically depicts the results of testing various forms of human ANGPTL8 proteins to use as the standard protein in the ELISA system described herein.

[00032] **Figure 6** graphically depicts the results of serially diluting human ANGPTL8 standard protein in various matrices to generate standard curves, in order to verify that the presence of serum cofactors do not impact the results of the ELISA system described herein.

[00033] **Figure 7** graphically depicts the results of serially diluting various ANGPTL proteins in standard curves in order to confirm that the ELISA system described herein is specific for the target antigen, and not for similar proteins.

[00034] **Figure 8A** shows, in bar graph form, the results of measuring the human ANGPTL8 protein concentrations in human male and female serum samples, based on diluting the serum 1:10 in PBS, and interpolating the raw optical densities from the standard curve, and accounting for the dilution factor. **Figure 8B** shows, in bar graph form, the results of measuring the human ANGPTL8 protein concentrations in human male and female plasma samples, based on diluting the plasma 1:10 in PBS, and interpolating the raw optical densities from the standard curve, and accounting for the dilution factor.

DETAILED DESCRIPTION

[00035] Before the present invention is described, it is to be understood that this invention is not limited to particular methods and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[00036] 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 this invention belongs. As used herein, the term "about," when used in reference to a particular recited numerical value, means that the value may vary from the recited value by no more than 1%. For example, as used herein, the expression "about 100" includes 99 and 101 and all values in between (*e.g.*, 99.1, 99.2, 99.3, 99.4, etc.).

[00037] Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All patents, applications and non-patent publications mentioned in this specification are incorporated herein by reference in their entireties.

Definitions

[00038] "Angiopoietin-like protein 8" or, "ANGPTL8," is a member of the angiopoietin family of proteins, and is sometimes referred to as TD26, RIFL, Lipasin, C19orf80 and Betatrophin. "ANGPTL8", as used herein, refers to human ANGPTL8 comprising the amino acid sequence as set forth in amino acid residues 1-177 of SEQ ID NO: 340. The full-length human ANGPTL8 amino acid sequence, including the signal sequence, can also be found in GenBank accession number NP_061157.3, while the full-length nucleic acid sequence encoding human ANGPTL8 can be found in GenBank accession number NM_018687.6. The N-terminal coiled-coil domain of human ANGPTL8 spans amino acid residues 1-39 of SEQ ID NO: 340 and is also depicted as SEQ ID NO: 337. All references to proteins, polypeptides and protein fragments herein are intended to refer to the human version of the respective protein, polypeptide or protein fragment unless explicitly specified as being from a non-human species. Thus, the expression "ANGPTL8" means human ANGPTL8 unless specified as being from a non-human species, *e.g.*, "mouse ANGPTL8," "monkey ANGPTL8," etc.

[00039] The term "human angiopoietin-like protein 3" or "hANGPTL3", as used herein, refers to ANGPTL3 having the nucleic acid sequence shown in SEQ ID NO:343 and the amino acid sequence of SEQ ID NO:342, or a biologically active fragment thereof. The N-terminal coiled-coil domain of human ANGPTL3 is depicted as SEQ ID NO: 338.

[00040] The term "human angiopoietin-like protein 4" or "hANGPTL4", as used herein, refers to ANGPTL4 having the nucleic acid sequence shown in SEQ ID NO:345 and the amino acid sequence of SEQ ID NO:344, or a biologically active fragment thereof. The N-terminal coiled-coil domain of human ANGPTL4 is depicted as SEQ ID NO: 339.

[00041] As used herein, the expression "anti-ANGPTL8 antibody" includes both monovalent antibodies with a single specificity, as well as bispecific antibodies comprising a first arm that binds ANGPTL8 and a second arm that binds a second (target) antigen, wherein the anti-ANGPTL8 arm comprises any of the HCVR/LCVR or CDR sequences as set forth in Table 1 herein.

[00042] The term "antibody", as used herein, means any antigen-binding molecule or molecular complex comprising at least one complementarity determining region (CDR) that specifically binds to or interacts with a particular antigen (*e.g.*, ANGPTL8). The term "antibody" includes immunoglobulin molecules comprising four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, as well as multimers thereof (*e.g.*, IgM). Each heavy chain comprises a heavy chain variable region

(abbreviated herein as HCVR or V_H) and a heavy chain constant region. The heavy chain constant region comprises three domains, C_H1, C_H2 and C_H3. Each light chain comprises a light chain variable region (abbreviated herein as LCVR or V_L) and a light chain constant region. The light chain constant region comprises one domain (C_L1). The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. In different embodiments of the invention, the FRs of the anti-ANGPTL8 antibody (or antigen-binding portion thereof) may be identical to the human germline sequences, or may be naturally or artificially modified. An amino acid consensus sequence may be defined based on a side-by-side analysis of two or more CDRs.

[00043] The term "antibody", as used herein, also includes antigen-binding fragments of full antibody molecules. The terms "antigen-binding portion" of an antibody, "antigen-binding fragment" of an antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. Antigen-binding fragments of an antibody may be derived, *e.g.*, from full antibody molecules using any suitable standard techniques such as proteolytic digestion or recombinant genetic engineering techniques involving the manipulation and expression of DNA encoding antibody variable and optionally constant domains. Such DNA is known and/or is readily available from, *e.g.*, commercial sources, DNA libraries (including, *e.g.*, phage-antibody libraries), or can be synthesized. The DNA may be sequenced and manipulated chemically or by using molecular biology techniques, for example, to arrange one or more variable and/or constant domains into a suitable configuration, or to introduce codons, create cysteine residues, modify, add or delete amino acids, etc.

[00044] Non-limiting examples of antigen-binding fragments include: (i) Fab fragments; (ii) F(ab')2 fragments; (iii) Fd fragments; (iv) Fv fragments; (v) single-chain Fv (scFv) molecules; (vi) dAb fragments; and (vii) minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (*e.g.*, an isolated complementarity determining region (CDR) such as a CDR3 peptide), or a constrained FR3-CDR3-FR4 peptide. Other engineered molecules, such as domain-specific antibodies, single domain antibodies, domain-deleted antibodies, chimeric antibodies,

CDR-grafted antibodies, diabodies, triabodies, tetrabodies, minibodies, nanobodies (*e.g.* monovalent nanobodies, bivalent nanobodies, etc.), small modular immunopharmaceuticals (SMIPs), and shark variable IgNAR domains, are also encompassed within the expression "antigen-binding fragment," as used herein.

[00045] An antigen-binding fragment of an antibody will typically comprise at least one variable domain. The variable domain may be of any size or amino acid composition and will generally comprise at least one CDR, which is adjacent to or in frame with one or more framework sequences. In antigen-binding fragments having a V_H domain associated with a V_L domain, the V_H and V_L domains may be situated relative to one another in any suitable arrangement. For example, the variable region may be dimeric and contain V_H-V_H, V_H-V_L or V_L-V_L dimers. Alternatively, the antigen-binding fragment of an antibody may contain a monomeric V_H or V_L domain.

[00046] In certain embodiments, an antigen-binding fragment of an antibody may contain at least one variable domain covalently linked to at least one constant domain. Non-limiting, exemplary configurations of variable and constant domains that may be found within an antigen-binding fragment of an antibody of the present invention include: (i) V_H-C_H1; (ii) V_H-C_H2; (iii) V_H-C_H3; (iv) V_H-C_H1-C_H2; (v) V_H-C_H1-C_H2-C_H3; (vi) V_H-C_H2-C_H3; (vii) V_H-C_L; (viii) V_L-C_H1; (ix) V_L-C_H2; (x) V_L-C_H3; (xi) V_L-C_H1-C_H2; (xii) V_L-C_H1-C_H2-C_H3; (xiii) V_L-C_H2-C_H3; and (xiv) V_L-C_L. In any configuration of variable and constant domains, including any of the exemplary configurations listed above, the variable and constant domains may be either directly linked to one another or may be linked by a full or partial hinge or linker region. A hinge region may consist of at least 2 (*e.g.*, 5, 10, 15, 20, 40, 60 or more) amino acids which result in a flexible or semi-flexible linkage between adjacent variable and/or constant domains in a single polypeptide molecule. Moreover, an antigen-binding fragment of an antibody of the present invention may comprise a homo-dimer or hetero-dimer (or other multimer) of any of the variable and constant domain configurations listed above in non-covalent association with one another and/or with one or more monomeric V_H or V_L domain (*e.g.*, by disulfide bond(s)).

[00047] As with full antibody molecules, antigen-binding fragments may be monospecific or multispecific (*e.g.*, bispecific). A multispecific antigen-binding fragment of an antibody will typically comprise at least two different variable domains, wherein each variable domain is capable of specifically binding to a separate antigen or to a different epitope on the same antigen. Any multispecific antibody format, including the exemplary bispecific antibody formats disclosed herein, may be adapted for use in the context of an

antigen-binding fragment of an antibody of the present invention using routine techniques available in the art.

[00048] The term "monoclonal antibody (mAb)", as used herein, is intended to include an antibody specific for a target antigen that is engineered by identical immune cells that are clones from a parent cell.

[00049] The term "human antibody", as used herein, is intended to include non-naturally occurring human antibodies. The term includes antibodies that are recombinantly produced in a non-human mammal, or in cells of a non-human mammal. The term is not intended to include antibodies isolated from or generated in a human subject.

[00050] The antibodies described herein may, in some embodiments, be recombinant human antibodies. The term "recombinant human antibody", as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell (described further below), antibodies isolated from a recombinant, combinatorial human antibody library (described further below), antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes (see e.g., Taylor et al. (1992) Nucl. Acids Res. 20:6287-6295) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. In certain embodiments, such recombinant human antibodies are subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the V_H and V_L regions of the recombinant antibodies are sequences that, while related to human germline V_H and V_L sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

[00051] Human antibodies can exist in two forms that are associated with hinge heterogeneity. In one form, an immunoglobulin molecule comprises a stable four chain construct of approximately 150-160 kDa in which the dimers are held together by an interchain heavy chain disulfide bond. In a second form, the dimers are not linked via inter-chain disulfide bonds and a molecule of about 75-80 kDa is formed composed of a covalently coupled light and heavy chain (half-antibody). These forms have been extremely difficult to separate, even after affinity purification.

[00052] The frequency of appearance of the second form in various intact IgG isotypes is due to, but not limited to, structural differences associated with the hinge region isotype of the antibody. A single amino acid substitution in the hinge region of the human IgG4

hinge can significantly reduce the appearance of the second form (Angal et al. (1993) Molecular Immunology 30:105) to levels typically observed using a human IgG1 hinge. The instant invention encompasses antibodies having one or more mutations in the hinge, C_H2 or C_H3 region, which may be desirable, for example, in production, to improve the yield of the desired antibody form.

[00053] The antibodies described herein may be isolated antibodies. An "isolated antibody," as used herein, means an antibody that has been identified and separated and/or recovered from at least one component of its natural environment. For example, an antibody that has been separated or removed from at least one component of an organism, or from a tissue or cell in which the antibody naturally exists or is naturally produced, is an "isolated antibody" for purposes of the present invention. An isolated antibody also includes an antibody *in situ* within a recombinant cell. Isolated antibodies are antibodies that have been subjected to at least one purification or isolation step. According to certain embodiments, an isolated antibody may be substantially free of other cellular material and/or chemicals.

[00054] The anti-ANGPTL8 antibodies described herein may comprise one or more amino acid substitutions, insertions and/or deletions in the framework and/or CDR regions of the heavy and light chain variable domains. Such mutations can be readily ascertained by comparing the amino acid sequences disclosed herein to sequences available from, for example, public antibody sequence databases. Once obtained, antibodies and antigen-binding fragments that contain one or more mutations can be easily tested for one or more desired property such as, improved binding specificity, increased binding affinity, improved or enhanced antagonistic or agonistic biological properties (as the case may be), reduced immunogenicity, etc. Antibodies and antigen-binding fragments obtained in this general manner are encompassed within the present invention.

[00055] The anti-ANGPTL8 antibodies described herein also comprise variants of any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein having one or more conservative substitutions. For example, they may have HCVR, LCVR, and/or CDR amino acid sequences with, *e.g.*, 10 or fewer, 8 or fewer, 6 or fewer, 4 or fewer, etc. conservative amino acid substitutions relative to any of the HCVR, LCVR, and/or CDR amino acid sequences set forth in Table 1 herein.

[00056] A “blocking antibody” or a "neutralizing antibody", as used herein (or an "antibody that neutralizes ANGPTL8 activity"), is intended to refer to an antibody whose binding to and/or interaction with ANGPTL8 results in inhibition of at least one biological

activity of ANGPTL8. For example, an antibody described herein may inhibit the lipoprotein lipase inhibitory activity of ANGPTL8, or it may lower plasma triglycerides through a mechanism other than through inhibition of the LPL inhibitory activity of ANGPTL8. This inhibition of the biological activity of ANGPTL8 can be assessed by measuring one or more indicators of ANGPTL8 biological activity by one or more of several standard *in vitro* or *in vivo* assays known in the art. An alternate activity is the triglyceride lowering activity associated with an antibody.

[00057] The term "capture mAb" refers to the antibody that is used to coat the wells of the assay plate. It is specific for binding the analyte, or the target antigen (human ANGPTL8 in this system), and immobilizes it from the biological sample.

[00058] The term "detection mAb" refers to the antibody that is used to detect levels of the analyte, human ANGPTL8, in the ELISA system described herein. It is conjugated to biotin molecules, in order to utilize the biotin-avidin interaction, when streptavidin is added in the ELISA. This allows for the detection of the target analyte, human ANGPTL8.

[00059] "Enzyme-linked immunosorbent assay (ELISA)" is a general plate-based technology used to detect and quantify analytes in various biological samples. In this technique, the analyte is immobilized to a surface (in this case, to an antibody that is attached to the well surface of an assay plate). This analyte is then detected via another antibody specific for it, a detection antibody, which is conjugated to tag molecules.

[00060] To measure the "efficacy" or "outcome" of a certain therapy, various clinical measurements are utilized. The "efficacy" or "outcome" of the therapy takes into account several clinical parameters, including a measurement of symptoms (with emphasis on alleviating the symptoms associated with the particular disorder or condition) and the need for concomitant medications. Herein, the efficacy is evaluated in terms of the level of ANGPTL8 in a sample from a subject.

[00061] The term "surface plasmon resonance", or "SPR", as used herein, refers to an optical phenomenon that allows for the analysis of real-time biomolecular interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using a BIACORE™ system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, N.J.), or a MASS-1 system (Sierra Sensors, Hamburg, Germany and Greenville, RI).

[00062] The term "K_D ", as used herein, is intended to refer to the equilibrium dissociation constant of a particular antibody-antigen interaction.

[00063] The term "epitope" refers to an antigenic determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope.

A single antigen may have more than one epitope. Thus, different antibodies may bind to different areas on an antigen and may have different biological effects. Epitopes may be either conformational or linear. A conformational epitope is produced by spatially juxtaposed amino acids from different segments of the linear polypeptide chain. A linear epitope is one produced by adjacent amino acid residues in a polypeptide chain. In certain circumstance, an epitope may include moieties of saccharides, phosphoryl groups, or sulfonyl groups on the antigen.

[00064] The term "substantial identity" or "substantially identical," when referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 95%, and more preferably at least about 96%, 97%, 98% or 99% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, such as FASTA, BLAST or Gap, as discussed below. A nucleic acid molecule having substantial identity to a reference nucleic acid molecule may, in certain instances, encode a polypeptide having the same or substantially similar amino acid sequence as the polypeptide encoded by the reference nucleic acid molecule.

[00065] As applied to polypeptides, the term "substantial similarity" or "substantially similar" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 95% sequence identity, even more preferably at least 98% or 99% sequence identity. Preferably, residue positions that are not identical differ by conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well known to those of skill in the art. *See, e.g.*, Pearson (1994) Methods Mol. Biol. 24: 307-331, herein incorporated by reference. Examples of groups of amino acids that have side chains with similar chemical properties include (1) aliphatic side chains: glycine, alanine, valine, leucine and isoleucine; (2) aliphatic-hydroxyl side chains: serine and threonine; (3) amide-containing side chains: asparagine and glutamine; (4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; (5) basic side chains: lysine,

arginine, and histidine; (6) acidic side chains: aspartate and glutamate, and (7) sulfur-containing side chains are cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine. Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet *et al.* (1992) Science 256: 1443-1445, herein incorporated by reference. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[00066] Sequence similarity for polypeptides is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG software contains programs such as GAP and BESTFIT, which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. See, *e.g.,* GCG Version 6.1. Polypeptide sequences also can be compared using FASTA with default or recommended parameters; a program in GCG Version 6.1. FASTA (*e.g.,* FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson (2000) *supra*). Another preferred algorithm when comparing a sequence of the invention to a database containing a large number of sequences from different organisms is the computer program BLAST, especially BLASTP or TBLASTN, using default parameters. See, *e.g.,* Altschul *et al.* (1990) J. Mol. Biol. 215: 403 410 and (1997) Nucleic Acids Res. 25:3389 402, each of which is herein incorporated by reference.

[00067] By the phrase "therapeutically effective amount" is meant an amount that produces the desired effect for which it is administered. The exact amount will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (see, for example, Lloyd (1999) The Art, Science and Technology of Pharmaceutical Compounding).

[00068] The term "treating" or "treatment" or "therapy", as used herein, refers to alleviating symptoms, eliminating the causation of symptoms either on a temporary or permanent basis, or preventing or slowing the appearance of symptoms. The term "treating" or "treatment" or "therapy", as used herein, also refers to an approach for

obtaining beneficial or desired clinical results. In one embodiment of the invention, a beneficial or desired clinical result includes, but is not limited to, an improvement in blood triglyceride levels, or an improvement in any one or more conditions, diseases, or symptoms associated with, or resulting from, elevated levels of triglycerides, including, but not limited to hypertriglyceridemia, etc. Standard therapy may include fluid administration, or administration of any other pharmaceutical agents useful for lowering blood triglycerides, or lipids, or for weight reduction.

[00069] ANGPTL8-associated conditions or disorders (conditions or disorders associated with elevated expression and/or activity of ANGPTL8) that are monitored using methods according to the invention include, without limitation, conditions or diseases associated with, or characterized in part by high blood triglyceride levels, or at least one symptom or complication associated with the condition or disease; hypertriglyceridemia, or at least one symptom associated with hypertriglyceridemia, or risk for acquiring hypertriglyceridemia, for example, in a patient who has a genetic predisposition for developing hypertriglyceridemia, *e.g.* familial hypertriglyceridemia or familial dysbetalipoproteinemia; any disease or condition improved, ameliorated, inhibited or prevented, (or at least one symptom associated with the disease reduced in severity or frequency of occurrence, compared to that without anti-hANGPTL8 antibody treatment (*e.g.*, ANGPTL8-mediated diseases or disorders), by removing, inhibiting, reducing, or otherwise interfering with, ANGPTL8 activity; diseases or disorders involving lipid metabolism, such as hyperlipidemia, hyperlipoproteinemia and dyslipidemia, including atherogenic dyslipidemia, diabetic dyslipidemia, hypertriglyceridemia, including severe hypertriglyceridemia with TG > 1000 mg/dL and associated acute pancreatitis, hypercholesterolemia, chylomicronemia, mixed dyslipidemia (obesity, metabolic syndrome, diabetes, etc.), lipodystrophy, lipoatrophy, and the like, which are caused by, for example, decreased LPL activity and/or LPL deficiency, altered ApoC2, ApoE deficiency, increased ApoB, increased production and/or decreased elimination of very low-density lipoprotein (VLDL), certain drug treatment (*e.g.*, glucocorticoid treatment-induced dyslipidemia), any genetic predisposition, diet, life style, and the like; diseases or disorders associated with or resulting from triglyceridemia, hypertriglyceridemia, hyperlipidemia, hyper-lipoproteinemia, and/or dyslipidemia, including, but not limited to, cardiovascular diseases or disorders, such as atherosclerosis, aneurysm, hypertension, angina, stroke, cerebrovascular diseases, congestive heart failure, coronary artery diseases, myocardial infarction, peripheral vascular diseases, and the like; acute pancreatitis;

nonalcoholic steatohepatitis (NASH); blood sugar disorders, such as diabetes; obesity, and the like; metabolic syndrome associated dyslipidemia, obesity; other conditions that predispose a patient to high levels of triglycerides, for example, certain medications such as beta blockers, birth control pills, diuretics, steroids, or the use of tamoxifen; conditions, or complications associated with high levels of triglycerides, such as atherosclerosis, stroke, heart attack, and other cardiac conditions; and/or certain other conditions that may lead to high levels of triglycerides, including obesity, poorly controlled diabetes, hypothyroidism, kidney disease, or alcohol consumption.

[00070] As used herein, the term “subject" means any human or non-human animal. The terms “subject” and “patient” are used interchangeably herein. As used herein, the term "a subject in need thereof" means any human or non-human animal who: (a) is prone to hypertriglyceridemia and/or other disorders or conditions associated with elevated triglyceride and lipid levels; (b) has previously exhibited symptoms of hypertriglyceridemia and/or other disorders or conditions associated with elevated triglyceride and lipid levels; (c) has a known history of hypertriglyceridemia and/or other disorders or conditions associated with elevated triglyceride and lipid levels; and/or (d) exhibits a sign or symptom of, or is diagnosed as having hypertriglyceridemia and/or other disorders or conditions associated with elevated triglyceride and lipid levels.

[00071] The term “sample” or “biological sample” or “subject sample” or "patient sample" may include any tissue sample, including both solid tissue (or extracts thereof), biological fluids, or blood samples. The blood sample may be whole blood, plasma, or serum. The tissue sample or extract thereof, or biological fluid may be any tissue sample or bodily fluid that contains immunoglobulin-expressing cells. In certain embodiments, the sample may require further processing before being added to the inventive ELISA system.

Anti-ANGPTL8 antibodies

[00072] In one embodiment, the antibody employed in the method of the invention is a monoclonal antibody or antigen-binding fragment thereof that specifically binds to ANGPTL8. In another embodiment, the antibody or antigen-binding fragment thereof exhibits one or more of the following characteristics:

a) is a fully human monoclonal antibody;

b) neutralizes, inhibits, blocks, abrogates, reduces, or interferes with at least one activity associated with ANGPTL8;

c) comprises a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 266, 274, 282, 290, 298, 306, 314 and 330;

d) comprises a light chain variable region (LCVR) having an amino acid sequence selected from the group consisting of SEQ ID NO: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, and 322; or

e) cross-competes with a reference antibody, wherein the reference antibody comprises a heavy chain variable region (HCVR) and a light chain variable region (LCVR) amino acid sequence selected from the group consisting of any of the HCVR and LCVR amino acid sequences of Table 1.

[00073] Fully human antibodies specific for ANGPTL8 have been described that may be used in a clinical setting to treat diseases, or conditions characterized by elevated levels of triglycerides, including hypertriglyceridemia (U.S. Patent No. 9,018,356, incorporated herein in its entirety). Methods for generating monoclonal antibodies, including fully human monoclonal antibodies are known in the art.

[00074] VELOCIMMUNE® technology (see, for example, US 6,596,541, Regeneron Pharmaceuticals, VELOCIMMUNE®) or any other known method for generating monoclonal antibodies can be used to initially isolate high affinity chimeric antibodies to an allergen having a human variable region and a mouse constant region, as described in U.S. Patent No. 9,018,356. As further described therein, the high affinity chimeric antibodies, which are isolated having a human variable region and a mouse constant region, are characterized and selected for desirable characteristics, including affinity, selectivity, epitope, etc. The mouse constant regions are then replaced with a desired human constant region to generate the fully human antibody of the invention, for example wild-type or modified IgG1 or IgG4. While the constant region selected may vary according to specific use, high affinity antigen binding and target specificity characteristics reside in the variable region.

[00075] In general, the antibodies of the instant invention possess very high affinities, typically possessing K_D of from about 10^{-12} through about 10^{-9} M, when measured by binding to antigen either immobilized on solid phase or in solution phase.

[00076] Exemplary anti-ANGPTL8 antibodies are listed in Tables 1 and 2 herein. Table 1 sets forth the amino acid sequence identifiers of the heavy chain variable regions (HCVRs), light chain variable regions (LCVRs), heavy chain complementarity

determining regions (HCDR1, HCDR2 and HCDR3), and light chain complementarity determining regions (LCDR1, LCDR2 and LCDR3) of exemplary anti-ANGPTL8 antibodies. Table 2 sets forth the nucleic acid sequence identifiers of the HCVRs, LCVRs, HCDR1, HCDR2 HCDR3, LCDR1, LCDR2 and LCDR3 of the exemplary anti-ANGPTL8 antibodies.

[00077] The inventive method employs, as capture and/or detection antibodies, antibodies, or antigen-binding fragments thereof, comprising an HCVR comprising an amino acid sequence selected from any of the HCVR amino acid sequences listed in Table 1, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[00078] The inventive method also employs, as capture and/or detection antibodies, antibodies, or antigen-binding fragments thereof, comprising an LCVR comprising an amino acid sequence selected from any of the LCVR amino acid sequences listed in Table 1, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity thereto.

[00079] The inventive method also employs, as capture and/or detection antibodies, antibodies, or antigen-binding fragments thereof, comprising an HCVR and an LCVR amino acid sequence pair (HCVR/LCVR) comprising any of the HCVR amino acid sequences listed in Table 1 paired with any of the LCVR amino acid sequences listed in Table 1.

[00080] In one embodiment, the method employs, as capture and/or detection antibodies, an isolated antibody or antigen-binding fragment thereof that binds specifically to and/or inhibits at least one activity associated with ANGPTL8, wherein the antibody or antigen-binding fragment comprises a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154, 162/170, 178/186, 194/202, 210/218, 226/234, 242/250, 258/250, 266/250, 274/250, 282/250, 290/250, 306/250, 314/322, and 330/322.

[00081] In another embodiment, the method employs, as capture and/or detection antibodies, an isolated antibody or antigen-binding fragment thereof that binds specifically to and/or inhibits at least one activity associated with ANGPTL8, wherein the antibody or antigen-binding fragment comprises a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 34/42, 162/170, 226/234, and 290/250.

[00082] In one embodiment, the inventive method employs, as capture and/or detection antibodies, an isolated antibody or antigen-binding fragment thereof that binds to and/or

inhibits at least one activity associated with ANGPTL8, wherein the antibody or antigen-binding fragment comprises: (a) three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2 and HCDR3) contained within any one of the heavy chain variable region (HCVR) sequences as set forth in Table 1; and (b) three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within any one of the light chain variable region (LCVR) sequences as set forth in Table 1.

[00083] In one embodiment, the method employs, as capture and/or detection antibodies, an isolated antibody or antigen-binding fragment thereof that binds specifically to and/or inhibits at least one activity associated with ANGPTL8, wherein the antibody or antigen-binding fragment comprises:

(a) a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 20, 36, 52, 68, 84, 100, 116, 132, 148, 164, 180, 196, 212, 228, 244, 260, 268, 276, 284, 292, 300, 308, 316 and 332;

(b) a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 6, 22, 38, 54, 70, 86, 102, 118, 134, 150, 166, 182, 198, 214, 230, 246, 262, 270, 278, 286, 294, 302, 310, 318, and 334;

(c) a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 24, 40, 56, 72, 88, 104, 120, 136, 152, 168, 184, 200, 216, 232, 248, 264, 272, 280, 288, 296, 304, 312, 320 and 336;

(d) a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 12, 28, 44, 60, 76, 92, 108, 124, 140, 156, 172, 188, 204, 220, 236, 252 and 324;

(e) a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 30, 46, 62, 78, 94, 110, 126, 142, 158, 174, 190, 206, 222, 238, 254, and 326; and

(f) a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 32, 48, 64, 80, 96, 112, 128, 144, 160, 176, 192, 208, 224, 240, 256 and 328.

[00084] The inventive method also employs, as capture and/or detection antibodies, antibodies, or antigen-binding fragments thereof, comprising a heavy chain CDR1 (HCDR1) comprising an amino acid sequence selected from any of the HCDR1 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[00085] The inventive method also employs, as capture and/or detection antibodies, antibodies or antigen-binding fragments thereof, comprising a heavy chain CDR2 (HCDR2) comprising an amino acid sequence selected from any of the HCDR2 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[00086] The inventive method also employs, as capture and/or detection antibodies, antibodies, or antigen-binding fragments thereof, comprising a heavy chain CDR3 (HCDR3) comprising an amino acid sequence selected from any of the HCDR3 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[00087] The inventive method also employs, as capture and/or detection antibodies, antibodies, or antigen-binding fragments thereof, comprising a light chain CDR1 (LCDR1) comprising an amino acid sequence selected from any of the LCDR1 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[00088] The inventive method also employs, as capture and/or detection antibodies, antibodies, or antigen-binding fragments thereof, comprising a light chain CDR2 (LCDR2) comprising an amino acid sequence selected from any of the LCDR2 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[00089] The inventive method also employs, as capture and/or detection antibodies, antibodies, or antigen-binding fragments thereof, comprising a light chain CDR3 (LCDR3) comprising an amino acid sequence selected from any of the LCDR3 amino acid sequences listed in Table 1 or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[00090] The inventive method also employs, as capture and/or detection antibodies, antibodies, or antigen-binding fragments thereof, comprising an HCDR3 and an LCDR3 amino acid sequence pair (HCDR3/LCDR3) comprising any of the HCDR3 amino acid sequences listed in Table 1 paired with any of the LCDR3 amino acid sequences listed in Table 1. According to certain embodiments, the inventive method also employs, as capture and/or detection antibodies, antibodies, or antigen-binding fragments thereof, comprising an HCDR3/LCDR3 amino acid sequence pair contained within any of the exemplary anti-ANGPTL8 antibodies listed in Table 1.

[00091] The inventive method also employs, as capture and/or detection antibodies, antibodies, or antigen-binding fragments thereof, comprising a set of six CDRs (*i.e.*, HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3) contained within any of the exemplary anti-ANGPTL8 antibodies listed in Table 1. In certain embodiments, the HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 amino acid sequence set is selected from the group consisting of SEQ ID NOs: 36-38-40-44-46-48 (*e.g.*, H4H15318P); 164-166-168-172-174-176 (*e.g.*, H4H15341P); 228-230-232-236-238-240 (*e.g.*, H4H15347P); 292-294-296-252-254-256 (*e.g.*, H4H15361P2).

[00092] The inventive method also employs, as capture and/or detection antibodies, antibodies, or antigen-binding fragments thereof, comprising a set of six CDRs (*i.e.*, HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3) contained within an HCVR/LCVR amino acid sequence pair as defined by any of the exemplary anti-ANGPTL8 antibodies listed in Table 1. For example, the inventive method also employs, as capture and/or detection antibodies, antibodies, or antigen-binding fragments thereof, comprising the HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 amino acid sequences set contained within an HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 34/42 *(e.g.*, H4H15318P), 162/170 (*e.g.*, H4H15341P), 226/234 (*e.g.*, H4H15347P), and 290/250 (*e.g.*, H4H15361P2). Methods and techniques for identifying CDRs within HCVR and LCVR amino acid sequences are well known in the art and can be used to identify CDRs within the specified HCVR and/or LCVR amino acid sequences disclosed herein. Exemplary conventions that can be used to identify the boundaries of CDRs include, *e.g.*, the Kabat definition, the Chothia definition, and the AbM definition. In general terms, the Kabat definition is based on sequence variability, the Chothia definition is based on the location of the structural loop regions, and the AbM definition is a compromise between the Kabat and Chothia approaches. *See*, *e.g.*, Kabat, "Sequences of Proteins of Immunological Interest," National Institutes of Health, Bethesda, Md. (1991); Al-Lazikani *et al.*, *J. Mol. Biol. 273*:927-948 (1997); and Martin *et al.*, *Proc. Natl. Acad. Sci. USA 86*:9268-9272 (1989). Public databases are also available for identifying CDR sequences within an antibody.

[00093] The inventive method also employs, as capture and/or detection antibodies, antibodies and antigen-binding fragments thereof that compete for specific binding to ANGPTL8 with a reference antibody or antigen-binding fragment thereof comprising the CDRs of a HCVR and the CDRs of a LCVR, wherein the HCVR and LCVR each has an amino acid sequence selected from the HCVR and LCVR sequences listed in Table 1.

[00094] In one embodiment, the inventive method employs, as capture and/or detection antibodies, an isolated monoclonal antibody or antigen-binding fragment thereof that competes for binding to ANGPTL8 with a reference antibody comprising an HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154, 162/170, 178/186, 194/202, 210/218, 226/234, 242/250, 258/250, 266/250, 274/250, 282/250, 290/250, 306/250, 314/322, and 330/322.

[00095] The inventive method also employs, as capture and/or detection antibodies, antibodies and antigen-binding fragments thereof that bind the same epitope on ANGPTL8 as a reference antibody or antigen-binding fragment thereof comprising the CDRs of a HCVR and the CDRs of a LCVR, wherein the HCVR and LCVR each has an amino acid sequence selected from the HCVR and LCVR sequences listed in Table 1.

[00096] In one embodiment, the inventive method employs, as capture and/or detection antibodies, an isolated monoclonal antibody or antigen-binding fragment thereof that binds to the same epitope on ANGPTL8 as a reference antibody comprising an HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154, 162/170, 178/186, 194/202, 210/218, 226/234, 242/250, 258/250, 266/250, 274/250, 282/250, 290/250, 306/250, 314/322, and 330/322.

[00097] In one embodiment, the inventive method employs, as capture and/or detection antibodies, an isolated antibody that binds specifically to and/or inhibits at least one activity associated with ANGPTL8, and that is a recombinantly produced human monoclonal antibody.

[00098] In one embodiment, the inventive method employs, as capture and/or detection antibodies, an isolated antibody that binds specifically to and/or inhibits at least one activity associated with ANGPTL8, and that is a recombinantly produced human monoclonal antibody having a HCVR and/or an LCVR sequence selected from the amino acid sequences found in Table 1.

[00099] In one embodiment, the inventive method employs, as capture and/or detection antibodies, an isolated antibody that binds specifically to and/or inhibits at least one activity associated with ANGPTL8, and that is a recombinantly produced human monoclonal antibody having a HCVR /LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106,

114/122, 130/138, 146/154, 162/170, 178/186, 194/202, 210/218, 226/234, 242/250, 258/250, 266/250, 274/250, 282/250, 290/250, 306/250, 314/322, and 330/322.

[000100] The inventive method also employs, as capture and/or detection antibodies, an isolated antibody that binds specifically to and/or inhibits at least one activity associated with ANGPTL8, and that is a recombinantly produced human monoclonal antibody having a HCVR and/or a LCVR encoded by a nucleic acid sequence selected from the nucleic acid sequences found in Table 2.

[000101] The inventive method also employs, as capture and/or detection antibodies, anti-ANGPTL8 antibodies that bind to the same epitope as any of the specific exemplary antibodies described herein (*e.g.* antibodies comprising any of the amino acid sequences as set forth in Table 1 herein). Likewise, the inventive method employs, as capture and/or detection antibodies, anti-ANGPTL8 antibodies that compete for binding to ANGPTL8 with any of the specific exemplary antibodies described herein (*e.g.* antibodies comprising any of the amino acid sequences as set forth in Table 1 herein).

[000102] One can easily determine whether an antibody binds to the same epitope as, or competes for binding with, a reference anti-ANGPTL8 antibody by using routine methods known in the art and exemplified herein. For example, to determine if a test antibody binds to the same epitope as a reference anti-ANGPTL8 antibody of the invention, the reference antibody is allowed to bind to an ANGPTL8 protein. Next, the ability of a test antibody to bind to the ANGPTL8 molecule is assessed. If the test antibody is able to bind to ANGPTL8 following saturation binding with the reference anti-ANGPTL8 antibody, it can be concluded that the test antibody binds to a different epitope than the reference anti-ANGPTL8 antibody. On the other hand, if the test antibody is not able to bind to the ANGPTL8 molecule following saturation binding with the reference anti-ANGPTL8 antibody, then the test antibody may bind to the same epitope as the epitope bound by the reference anti-ANGPTL8 antibody of the invention. Additional routine experimentation (*e.g.*, peptide mutation and binding analyses) can then be carried out to confirm whether the observed lack of binding of the test antibody is in fact due to binding to the same epitope as the reference antibody or if steric blocking (or another phenomenon) is responsible for the lack of observed binding. Experiments of this sort can be performed using ELISA, RIA, Biacore, flow cytometry or any other quantitative or qualitative antibody-binding assay available in the art. In accordance with certain embodiments of the present invention, two antibodies bind to the same (or overlapping) epitope if, *e.g.*, a 1-, 5-, 10-, 20- or 100-fold excess of one antibody inhibits binding of the

other by at least 50% but preferably 75%, 90% or even 99% as measured in a competitive binding assay (see, e.g., Junghans et al., Cancer Res. 1990:50:1495-1502). Alternatively, two antibodies are deemed to bind to the same epitope if essentially all amino acid mutations in the antigen that reduce or eliminate binding of one antibody reduce or eliminate binding of the other. Two antibodies are deemed to have "overlapping epitopes" if only a subset of the amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

[000103] To determine if an antibody competes for binding (or cross-competes for binding) with a reference anti-ANGPTL8 antibody, the above-described binding methodology is performed in two orientations: In a first orientation, the reference antibody is allowed to bind to a ANGPTL8 protein under saturating conditions followed by assessment of binding of the test antibody to the ANGPTL8 molecule. In a second orientation, the test antibody is allowed to bind to an ANGPTL8 molecule under saturating conditions followed by assessment of binding of the reference antibody to the ANGPTL8 molecule. If, in both orientations, only the first (saturating) antibody is capable of binding to the ANGPTL8 molecule, then it is concluded that the test antibody and the reference antibody compete for binding to ANGPTL8. As will be appreciated by a person of ordinary skill in the art, an antibody that competes for binding with a reference antibody may not necessarily bind to the same epitope as the reference antibody, but may sterically block binding of the reference antibody by binding an overlapping or adjacent epitope.

Uses of the inventive ELISA assay system

[000104] In one embodiment, by allowing the clinician monitoring or treating a patient to assess ANGPTL8 levels in the patient, the methods described herein may be used to prevent the onset of a disease or disorder characterized in part by elevated blood triglyceride levels, or to prevent the likelihood of developing such disease or disorder, or to mitigate the severity of the disease or disorder, or at least one symptom associated with the disease or disorder, or to prevent long term complications associated with a disease characterized by high triglyceride levels.

[000105] In one embodiment, the method of the invention may be used in assessing the need for or efficacy of a medicament for treating any disease or disorder characterized in part by elevated levels of triglycerides.

[000106] The method of the invention may be used to monitor progress in short-term therapy in an acute setting, or in long-term use as chronic therapy.

Sandwich ELISA

Principle of Assay

[000107] In a basic chromogenic sandwich ELISA, the wells of an assay plate are coated with a capture antibody that is specific for the target antigen of interest. The capture antibody is immobilized on the well surface. Samples are then added, where the target antigen binds to the antibody coating the well. Next, another antibody that is specific for the target antigen is added, and also binds to the immobilized target antigen. This detection antibody is bound to a tag, usually biotin. Streptavidin conjugated to horseradish peroxidase (HRP) is then applied. Having high affinity for biotin, streptavidin binds to biotin in the complex. When 3,3′,5,5′-Tetramethylbenzidine (TMB), the substrate for HRP, is added, an enzymatic reaction occurs. The products of this reaction are quantified by reading the absorbance with a spectrophotometer. (Figure 1).

Exemplary Materials and Equipment

[000108] In certain embodiments, reagents employed include: as capture mAb: anti-human C19orf80 (ANGPTL8) (H4H15347P), as capture mAb: anti-human C19orf80 (ANGPTL8) (H4H15361P2); as detection mAb: biotinylated anti-human C19orf80 (ANGPTL8) (biotin-H4H15318P); as protein standard: untagged human ANGPTL8 protein (outsourced); as protein standard: human ANGPTL8 dimer protein with mouse Fc tag; as protein standard: human ANGPTL8 monomer protein with myc-myc-his tag.

[000109] Additional reagents employed in certain embodiments include: Phosphate-buffered saline (PBS), 1X, pH 7.2; Fraction V bovine serum albumin (BSA); Blocking buffer: 1% Fraction V BSA in PBS; Tween-20 nonionic detergent; Assay diluent: 1% Fraction V BSA/0.1% Tween-20 in PBS; Wash buffer: 0.1% Tween-20 in PBS; 3,3′,5,5′-Tetramethylbenzidine liquid substrate (TMB); 2 Normal sulfuric acid; Horseradish peroxidase-conjugated streptavidin (streptavidin-HRP).

[000110] Instruments and Labware employed in certain embodiments include: 96-well, clear, high-binding surface assay plates; Biotek EL406 plate washer dispenser with accompanying Liquid Handling Control software; Molecular Devices SpectraMax i3 microplate reader system with accompanying SoftMax Pro application (version 6.3.1).

Assay Procedure

[000111] Proteins and mAbs stored at -80°C were thawed and kept on ice, as they were needed. The wells of the ELISA plate were coated with capture mAb, at a concentration of 4µg/mL in PBS, and a volume of 100µL/well. The plate was covered with a plate sealer, and stored at 4°C overnight (approximately 18 hours). The wells of the

plate were then washed with 350µL/well wash buffer (0.1% Tween-20 in PBS), for a total of 3 washes, using an automated plate washer. Excess wash remnants were removed by inverting and tapping the plate against clean paper towels. Blocking buffer (1% Fraction V BSA in PBS) was added at 300µL/well. The plate was covered with a plate sealer, and placed on a plate shaker at room temperature, shaking at 300rpm for a minimum of 1 hour.

[000112] During this period of blocking, the standard curves and samples were prepared for duplicate in the ELISA. The standard curve dilutions were typically prepared in U-bottom dilution plates, where the most concentrated standard was prepared at 200ng/mL in PBS, and then serially-diluted 2-fold, to generate a 12-point curve, where the final point is the blank (PBS only) at 0ng/mL protein. Enough volume of each concentration was prepared to assay in duplicate wells, with 100µL/well. Serum or plasma samples require troubleshooting in order to determine the proper dilution in PBS so as to obtain absorbance values that lie within the linear range of the standard curve. Typically, several dilutions were made in PBS in U-bottom dilution plates.

[000113] After the blocking period, the wells of the ELISA plate were washed again, and excess wash remnants were removed, as previously described. The standards and the diluted samples were added to the plate in duplicate, with 100µL/well. The plate was covered with a plate sealer and placed on a plate shaker at room temperature, shaking at 500rpm for 2 hours. The wells of the ELISA plate were washed again, and excess wash remnants were removed, as previously described. The detection mAb was added at a concentration of 400ng/mL in assay diluent (1% Fraction V BSA/0.1% Tween-20 in PBS), with 150µL/well. The plate was covered with a plate sealer and placed on a plate shaker at room temperature, shaking at 500rpm for 1 hour. The wells of the ELISA plate were washed again, and excess wash remnants were removed, as previously described. Horseradish peroxidase-conjugated streptavidin was added at a concentration of 100ng/mL in assay diluent, with 150µL/well. The plate was covered with a plate sealer and placed on a plate shaker at room temperature, shaking at 500rpm for 30 minutes. The wells of the ELISA plate were washed again, as previously described, but 4 times, and excess wash remnants were removed. 3,3′,5,5′-Tetramethylbenzidine liquid substrate (TMB) was added at 100µL/well. The plate was covered with a plate sealer, and protected from light, covered with aluminum foil. The plate was placed on a plate shaker at room temperature, shaking for approximately 15 minutes. As the liquid in the wells of the assay plate turned from clear to blue in color during this incubation period, the color was carefully monitored so as to avoid undersaturation and oversaturation of substrate. Sulfuric acid (2N) was

added to each well at 100µL/well in order to stop the reaction. The bottom of the plate was quickly wiped with ethanol in order to remove any possible remnant contamination that may affect the reading of the plate by spectrophotometry. The plate was read immediately, at most within 15 minutes of adding the stop solution, using a spectrophotometric plate reader, set to 450nm, with a wavelength correction set to 540nm.

Data Analysis

[000114] The data obtained in SoftMax Pro file format were exported to Microsoft Excel, where wavelength correction was calculated by subtracting the absorbance reading at 540nm from the absorbance reading at 450nm. These true optical density values were then transferred to GraphPad Prism, where wells were masked on a need basis. Standard curves were generated, using nonlinear, 3-parameter Hills equation curve fit. Optical density values of samples that fell within the linear range of the appropriate standard curve were transformed in order to account for dilution factor of the loaded sample.

Detection Strategies for ELISA System

[000115] The final stage in the ELISA system is the detection/measurement step, after an enzyme substrate is introduced (unless a radioactive or fluorescent tag was used). The substrate is converted to a detectable product by the enzyme. The intensity of signal produced when the substrate is added is directly proportional to the amount of hANGPTL8 captured in the plate and bound by the detection antibodies. Thus, enzyme-conjugated antibodies (for example, with horseradish peroxidase, HRP) offer flexibility in the instant ELISA detection/measurement method because of the myriad substrates available for chromogenic, chemifluorescent and chemiluminescent imaging.

[000116] Chromogenic ELISA substrates allow direct visualization and are detected with standard absorbance plate readers common to many laboratories. HRP catalyzes the conversion of chromogenic substrates (e.g., TMB, OPD, DAB, ABTS) into colored products; it can be conjugated to a labeled molecule. HRP produces a colored derivative of the labeled molecule when incubated with a proper substrate, allowing it to be detected and quantified. HRP is often used in conjugates to determine the presence of a molecular target. It is commonly used in ELISA systems due to its monomeric nature and the ease with which it produces colored products.

[000117] Chemifluorescent ELISA substrates are for use with a fluorescent plate reader.

[000118] Chemiluminescent ELISA substrates are for use with a luminometer or other plate reader that can measure total luminescence.

EXAMPLES

[000119] Before the present methods are described, it is to be understood that this invention is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

[000120] 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 this invention belongs. As used herein, the term "about," when used in reference to a particular recited numerical value, means that the value may vary from the recited value by no more than 1%. For example, as used herein, the expression "about 100" includes 99 and 101 and all values in between (*e.g.*, 99.1, 99.2, 99.3, 99.4, etc.).

[000121] Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference in their entirety.

Example 1. Selection of purified candidate mAbs to use as pairs in ELISA-based system for determination of human ANGPTL8 levels

[000122] Anti-ANGPTL8 antibodies were obtained by immunizing a VELOCIMMUNE® mouse (*i.e.*, an engineered mouse comprising DNA encoding human immunoglobulin heavy and kappa light chain variable regions) with an immunogen comprising a recombinant human ANGPTL8 expressed with a C-terminal mouse IgG2a tag (See SEQ ID NO: 340). The antibody immune response was monitored by an ANGPTL8-specific immunoassay. When a desired immune response was achieved, several fully human anti-ANGPTL8 antibodies were generated from antigen-positive B cells as described in US 2007/0280945A1, incorporated by reference herein in its entirety.

[000123] Table 1 sets forth the amino acid sequence identifiers of the heavy and light chain variable regions and CDRs of selected anti-ANGPTL8 antibodies utilized in the

assay described herein. The corresponding nucleic acid sequence identifiers are set forth in Table 2.

Table 1: Amino Acid Sequence Identifiers

	SEQ ID NOs:							
Antibody Designation	**HCVR**	**HCDR1**	**HCDR2**	**HCDR3**	**LCVR**	**LCDR1**	**LCDR2**	**LCDR3**
H4H15314P2	2	4	6	8	10	12	14	16
H4H15316P	18	20	22	24	26	28	30	32
H4H15318P	34	36	38	40	42	44	46	48
H4H15319P	50	52	54	56	58	60	62	64
H4H15321P	66	68	70	72	74	76	78	80
H4H15323P	82	84	86	88	90	92	94	96
H4H15330P	98	100	102	104	106	108	110	112
H4H15331P	114	116	118	120	122	124	126	128
H4H15334P	130	132	134	136	138	140	142	144
H4H15335P	146	148	150	152	154	156	158	160
H4H15341P	162	164	166	168	170	172	174	176
H4H15343P	178	180	182	184	186	188	190	192
H4H15345P	194	196	198	200	202	204	206	208
H4H15346P	210	212	214	216	218	220	222	224
H4H15347P	226	228	230	232	234	236	238	240
H4H15350P2	242	244	246	248	250	252	254	256
H4H15353P2	258	260	262	264	250	252	254	256
H4H15354P2	266	268	270	272	250	252	254	256
H4H15355P2	274	276	278	280	250	252	254	256
H4H15357P2	282	284	286	288	250	252	254	256
H4H15361P2	290	292	294	296	250	252	254	256
H4H15362P2	298	300	302	304	250	252	254	256
H4H15363P2	306	308	310	312	250	252	254	256
H4H15367P2	314	316	318	320	322	324	326	328
H4H15369P2	330	332	334	336	322	324	326	328

Table 2: Nucleic Acid Sequence Identifiers

SEQ ID NOs:								
Antibody Designation	**HCVR**	**HCDR1**	**HCDR2**	**HCDR3**	**LCVR**	**LCDR1**	**LCDR2**	**LCDR3**
H4H15314P2	1	3	5	7	9	11	13	15
H4H15316P	17	19	21	23	25	27	29	31
H4H15318P	33	35	37	39	41	43	45	47
H4H15319P	49	51	53	55	57	59	61	63
H4H15321P	65	67	69	71	73	75	77	79
H4H15323P	81	83	85	87	89	91	93	95
H4H15330P	97	99	101	103	105	107	109	111
H4H15331P	113	115	117	119	121	123	125	127
H4H15334P	129	131	133	135	137	139	141	143
H4H15335P	145	147	149	151	153	155	157	159
H4H15341P	161	163	165	167	169	171	173	175
H4H15343P	177	179	181	183	185	187	189	191
H4H15345P	193	195	197	199	201	203	205	207
H4H15346P	209	211	213	215	217	219	221	223
H4H15347P	225	227	229	231	233	235	237	239
H4H15350P2	241	243	245	247	249	251	253	255
H4H15353P2	257	259	261	263	249	251	253	255
H4H15354P2	265	267	269	271	249	251	253	255
H4H15355P2	273	275	277	279	249	251	253	255
H4H15357P2	281	283	285	287	249	251	253	255
H4H15361P2	289	291	293	295	249	251	253	255
H4H15362P2	297	299	301	303	249	251	253	255
H4H15363P2	305	307	309	311	249	251	253	255
H4H15367P2	313	315	317	319	321	323	325	327
H4H15369P2	329	331	333	335	321	323	325	327

[000124] Antibodies are typically referred to herein according to the following nomenclature: Fc prefix (*e.g.* "H1H," "H1M," "H2M", "H4H", etc.), followed by a numerical identifier (*e.g.* "15321," "15341," "15350," etc.), followed by a "P" or "N" suffix, as shown in Tables 1 and 2. Thus, according to this nomenclature, an antibody may be referred to herein as, *e.g.*, "H4H15321P", etc. The H4H prefix on the antibody

designations used herein indicate the particular Fc region isotype of the antibody. For example, an "H4H" antibody has a human IgG4 Fc, an "H1M" antibody has a mouse IgG1 Fc, and an "H2M" antibody has a mouse IgG2 Fc, (all variable regions are fully human as denoted by the first 'H' in the antibody designation). As will be appreciated by a person of ordinary skill in the art, an antibody having a particular Fc isotype can be converted to an antibody with a different Fc isotype (*e.g.*, an antibody with a mouse IgG1 Fc can be converted to an antibody with a human IgG4, etc.), but in any event, the variable domains (including the CDRs) – which are indicated by the numerical identifiers shown in Tables 1 and 2 – will remain the same, and the binding properties are expected to be identical or substantially similar regardless of the nature of the Fc domain.

[000125] In order for an ELISA method of protein detection and quantification to be reliable, there is a need for quality capture and detection mAbs that can recognize the target analyte in biological samples. This initial step was executed in order to select potential capture mAbs and detection mAbs that are able to target and bind to the human ANGPTL8 analyte in the ELISA system.

[000126] The selection process involved performing a series of immunoprecipitations (IPs), in which each mAb was incubated with cell conditioned media (CM) containing the human ANGPTL8 protein. A resin that identifies human IgG4 was then applied to the mAb/CM mixtures. The resin beads targeted the mAbs, binding the mAb-human ANGPTL8 complex. A series of Western blots probing for human ANGPTL8 were run on the various fractions of the IPs, and anti-human ANGPTL8 mAbs were selected based on high human ANGPTL8 expression in the IP fractions, with low human ANGPTL8 expression in the unbound fractions.

[000127] mAbs found to strongly and specifically bind human ANGPTL8 in the immunoprecipitated fractions, with minimal human ANGPTL8 protein detected in the unbound fractions, included: H4H15318P, H4H15347P, H4H15361P2, and H4H15341P (Figure 2). Therefore, these four mAbs were selected to be tested in the ELISA, with each as the capture mAb and each as the biotinylated detection mAb.

Example 2: Evaluation of various combinations of candidate capture mAbs and detection mAbs in ELISA; determination of proper purified human ANGPTL8 protein to use as ELISA standard; testing of native condition versus denatured condition of human ANGPTL8 standard protein in human ANGPTL8 detection

[000128] This series of ELISAs had a three-fold purpose: (1) to select which pair of candidate capture and detection mAbs performed best in the ELISA; (2) to test both a

monomer form and a dimer form of the human ANGPTL8 protein as a standard protein; (3) to test whether native condition or acid-denatured condition of the human ANGPTL8 protein enables stronger detection of ANGPTL8.

[000129] Each of the four selected candidate mAbs was tested as a capture mAb and as a detection mAb, forming possible pairs. Each possible mAb pair was tested using both the monomer and the dimer forms of human ANGPTL8 as the standard protein, to generate a standard curve, using a 10-point, 2-fold serial dilution series, ranging from 50ng/mL-0ng/mL. Each possible mAb pair, with each form of human ANGPTL8 standard was tested under both native condition and acid-denatured condition.

[000130] Both H4H15347P and H4H15361P2 performed best as the capture mAbs, while biotin-H4H15318P performed best as a detection mAb, with the selected capture mAbs. The monomer form of human ANGPTL8 protein produced better standard curves, with wider linear ranges, and with more points in the linear range. Human ANGPTL8 is detectable under native conditions, and undetectable under acid-denatured conditions, using the purified mAbs and proteins listed herein (Figure 3).

Example 3: Comparison of human ANGPTL8 detection in *ex vivo* serum samples, using selected purified candidate mAb pairs

[000131] It is crucial that the ELISA platform, using the selected capture and detection mAbs, is able to detect human ANGPTL8 protein in various types of biological samples that contain the protein. At the same time, it is also important that the ELISA described herein shows specificity for the target analyte, by not detecting it in samples from a different species. This study evaluated the specificity of the selected human ANGPTL8 capture and detection mAbs in the ELISA, using various *ex vivo* serum samples from untreated and treated mice, as well as from human donors.

[000132] The ELISAs were performed as described, diluting serum samples 100-fold, interpolating the serum human ANGPTL8 concentration by comparing the optical densities of the diluted samples to those of the monomer human ANGPTL8 protein (human ANGPTL8 dimer protein with mouse Fc tag) standard curve.

[000133] The selected Regeneron capture and detection mAbs show specificity for human ANGPTL8 and no cross-reactivity to mouse ANGPTL8 (Figure 4).

Example 4: Testing of various human ANGPTL8 proteins in a standard curve

[000134] H4H15347P was selected as capture, and biotin-H4H15318P as detection, mAbs for the further ELISA development. In order to quantify human ANGPTL8 levels in biological samples, it is critical for the ELISA to utilize a human ANGPTL8 protein as a

standard to produce reliable optical densities from which to interpolate the concentrations of human ANGPTL8 in test samples. Ideally, there should be a sigmoidal, dose-response curve, with a wide linear range, with several quantified points within that range, and low background signal. The instant experiment compared several forms of human ANGPTL8 protein as a standard in the ELISA.

[000135] The following human ANGPTL8 protein forms were serially-diluted 2-fold in PBS, in a 12-point curve, from 50ng/mL-0ng/mL: human ANGPTL8 dimer with mouse Fc tag (human ANGPTL8 monomer protein with myc-myc-his tag), human ANGPTL8 monomer with mouse myc-myc-his tag (human ANGPTL8 dimer protein with mouse Fc tag), untagged human ANGPTL8 protein in buffer containing detergent (outsourced production, Genscript), and untagged human ANGPTL8 protein in buffer without detergent (outsourced production, Genscript).

[000136] The two human ANGPTL8 untagged proteins from Genscript (in different buffers) produced the best dose-response standard curves (comparable to each other), with the lowest background signal, the widest linear range, and the most number of points along the curve (Figure 5).

Example 5: Validation of standard curve using serum spike-in

[000137] It is important that the ELISA platform can detect human ANGPTL8 in biological samples, and that the optical densities obtained are true read-outs of the target protein concentration, unaffected by the many cofactors that are endogenously present in serum and plasma.

[000138] The instant experiment serially-diluted the untagged human ANGPTL8 protein standard as previously described in PBS, and compared this standard curve to those obtained by diluting the standard protein in the same manner, but in 1% mouse *Angptl8*$^{-/-}$ serum matrix and in 1% human serum matrix.

[000139] The optical densities obtained in the standard curve are true measurements of human ANGPTL8 protein, and not influenced by the presence of endogenous serum cofactors (Figure 6).

Example 6: Validation of human ANGPTL8 ELISA specificity

[000140] For this ELISA system to be a reliable method for human ANGPTL8 quantification, specificity for the human ANGPTL8 protein is key. While a previous ELISA experiment confirmed that the mAbs of this ELISA are specific for the human form of ANGPTL8, the instant experiment verifies that the mAbs are specific for human ANGPTL8, as opposed to similar proteins, human ANGPTL3 and human ANGPTL4,

which are members of the same family of proteins. Furthermore, it has been established that ANGPTL3 and ANGPTL8 proteins interact with each other for biological effect. Therefore, it needs to be determined whether the ELISA system described herein is able to detect human ANGPTL8 in the presence of human ANGPTL3, and that human ANGPTL3 presence in no way affects the measurement of human ANGPTL8 protein levels.

[000141] Standard curves of the following human proteins were generated using 2-fold serial dilutions, 12 points, from 200ng/mL-0ng/mL in PBS: ANGPTL8, ANGPTL3, ANGPTL4, and ANGPTL8 with spiked-in ANGPTL3. These curves were compared to one another.

[000142] The ELISA described herein is specific for the human ANGPTL8 protein, and does not detect human ANGPTL3 or human ANGPTL4. The presence of human ANGPTL3 with human ANGPTL8 does not affect the system's ability to detect human ANGPTL8 (Figure 7).

Example 7. Measurement of human ANGPTL8 concentration in human serum and plasma samples

[000143] Often, biological samples are too concentrated with proteins to run undiluted, or "neat," in a plate-based ELISA system; therefore, it is usually necessary to dilute the samples, so that the target protein can be properly detected, and its concentration can be measured. Troubleshooting with various dilutions of sample is typical in ELISA development, with the goal being the determination of a dilution factor that results in the optical densities of the samples falling within the linear range of the standard curve. Since the goal of the ELISA described herein is to reliably detect and quantify the concentration of human ANGPTL8 protein in human samples (serum and plasma), it is important to validate this system using serum and plasma samples collected from human donors. It is also necessary to optimize the ELISA described herein by determining a proper dilution factor of human serum and plasma samples so that, when applied as samples, the optical densities fall within the linear range of the human ANGPTL8 standard curve. This way, it can be certain that the interpolated values of the samples (taking into account the linear range of the standard curve, and the dilution factor of the samples) are reliable measurements of circulating human ANGPTL8 protein concentration.

[000144] Serum (collected without anti-coagulant) and plasma (collected with lithium heparin as anti-coagulant) samples were purchased from commercial vendor, Bioreclamation IVT, who obtained them from male and female human donors. Each of these samples were run in the ELISA as described, using H4H15347P as capture mAb,

applying the following dilutions of each sample: 1:5, 1:10, and 1:50 in PBS. The optical densities of each sample, at each dilution, were examined, and compared to the standard curve, in order to determine which dilution factor results in optical densities lying within the linear range of the human ANGPTL8 protein standard curve.

[000145] Human ANGPTL8 was able to be detected in human male and female, serum (N=10/gender) and plasma (N=10/gender) samples. Using H4H15347P as the capture mAb in the ELISA, a dilution of 1:10 serum or plasma:PBS proved to be the optimal dilution. At the 1:10 dilution, the sample optical densities fell within the linear range of the standard curve, and the human ANGPTL8 protein concentrations were determined by interpolating the values from the standard curves and transforming the values according to the dilution factor of 10. Using H4H15347P as the capture mAb in the ELISA, the 1:5 dilution factor resulted in similar interpolated human ANGPTL8 protein concentrations in the samples, while the 1:50 dilution factor proved to be too dilute to obtain any data. Males and females showed similar mean human ANGPTL8 concentrations to each other, for both serum and plasma.

What is claimed is:

1. A method for detecting and/or quantifying human Angiopoietin-like protein 8 (hANGPTL8) in a sample, the method comprising:

   Obtaining a sample from a subject;

   Adding the sample to the wells of an Enzyme Linked Immunosorbent Assay (ELISA) plate coated with a capture antibody, wherein the capture antibody is a first anti-hANGPTL8 antibody;

   Adding a detection antibody to the wells of the plate, wherein the detection antibody is a second anti-hANGPTL8 antibody bound to a tag;

   Adding an agent that binds the tag, wherein the agent is conjugated to an enzyme;

   Adding a substrate for the enzyme; and

   Measuring the amount of product of the enzyme-substrate reaction.

2. The method of claim 1, wherein the sample is plasma or serum.
3. The method of claim 2, wherein the serum or plasma is diluted 1:10 in PBS.
4. The method of any one of claims 1-3, wherein the subject is human.
5. The method of any one of claims 1-4, wherein the capture antibody is H4H15347P or H4H15361P2.
6. The method of any one of claims 1-5, wherein the detection antibody is H4H15318P.
7. The method of any one of claims 1-6, wherein the capture antibody is H4H15347P, and the detection antibody is H4H15318P.
8. The method of any one of claims 1-7, wherein the tag is biotin.
9. The method of any one of claims 1-8, wherein the agent is streptavidin.
10. The method of any one of claims 1-9, wherein the enzyme is horseradish peroxidase (HRP).
11. The method of any one of claims 1-10, wherein the substrate is a chromogenic substrate.
12. The method of claim 11, wherein the chromogenic substrate is 3,3',5,5'-tetramethylbenzidine (TMB).
13. The method of any one of claims 1-12, wherein the method does not detect (and/or quantify) non-human ANGPTL8.

14. The method of any one of claims 1-13, wherein the method does not detect (and/or quantify) hANGPTL3 nor hANGPTL4.
15. A method for assessing the efficacy and/or outcome of treating an ANGPTL8-associated disorder in a subject, the method comprising:

    Obtaining a sample from a subject;

    Adding the sample to the wells of an Enzyme Linked Immunosorbent Assay (ELISA) plate coated with a capture antibody, wherein the capture antibody is a first anti-hANGPTL8 antibody;

    Adding a detection antibody to the wells of the plate, wherein the detection antibody is a second anti-hANGPTL8 antibody bound to a tag;

    Adding an agent that binds the tag, wherein the agent is conjugated to an enzyme;

    Adding a substrate for the enzyme; and

    Measuring the amount of product of the enzyme-substrate reaction.
16. The method of claim 15, wherein a decrease in the amount of product of the enzyme-substrate reaction indicates a proportionate decrease in the level of ANGPTL8 in the sample, which indicates efficacy and/or a positive outcome of the treatment.
17. A method for determining whether a subject is at risk for developing an ANGPTL8-associated disorder, the method comprising:

    Obtaining a sample from a subject;

    Adding the sample to the wells of an Enzyme Linked Immunosorbent Assay (ELISA) plate coated with a capture antibody, wherein the capture antibody is a first anti-hANGPTL8 antibody;

    Adding a detection antibody to the wells of the plate, wherein the detection antibody is a second anti-hANGPTL8 antibody bound to a tag;

    Adding an agent that binds the tag, wherein the agent is conjugated to an enzyme;

    Adding a substrate for the enzyme; and

    Measuring the amount of product of the enzyme-substrate reaction.
18. The method of claim 17, wherein an augmented amount of product of the enzyme-substrate reaction indicates an augmented level of ANGPTL8 in the sample, which indicates risk for developing an ANGPTL8-associated disorder.

19. A method for treating an ANGPTL8-associated disorder in a subject, the method comprising treating the subject, and, before, during, and/or after the treatment, respectively:

Obtaining a sample from a subject;

Adding the sample to the wells of an Enzyme Linked Immunosorbent Assay (ELISA) plate coated with a capture antibody, wherein the capture antibody is a first anti-hANGPTL8 antibody;

Adding a detection antibody to the wells of the plate, wherein the detection antibody is a second anti-hANGPTL8 antibody bound to a tag;

Adding an agent that binds the tag, wherein the agent is conjugated to an enzyme;

Adding a substrate for the enzyme; and

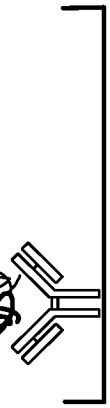
Measuring the amount of product of the enzyme-substrate reaction;

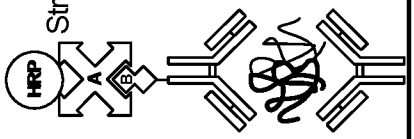
the method further comprising comparing the amounts measured before, during, and/or after treatment with one another, and continuing or discontinuing treatment based on the comparison.

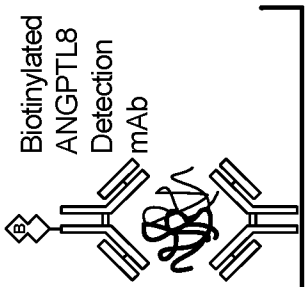
20. A kit for determining if a patient is responsive to treatment of an ANGPTL8-associated disorder/condition, the kit comprising: an Enzyme Linked Immunosorbent Assay (ELISA) plate coated with a capture antibody, wherein the capture antibody is a first anti-hANGPTL8 antibody; a detection antibody, wherein the detection antibody is a second anti-hANGPTL8 antibody bound to a tag, an agent conjugated to an enzyme, a substrate for the enzyme, instructions for obtaining a sample from a subject, and instructions for measuring the amount of ANGPTL8 in the sample.

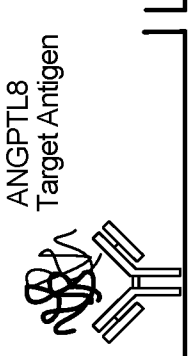
21. A chromogenic sandwich ELISA assay for detecting and/or determining the level of human ANGTPL8 in a sample, wherein the assay plate wells are coated with a capture antibody (a first anti-hANGPTL8 antibody) before adding the sample to the wells; wherein a detection antibody (a second anti-hANGPTL8 antibody bound to a tag) is then added to the wells, followed by an agent conjugated to an enzyme, and then a substrate for the enzyme, allowing for subsequent measurement of the amount of product of the enzyme-substrate reaction.

FIG. 1


TMB
Chromagen
TMB
HRP
A
B


Streptavidin-HRP
Complex
HRP
A
B


Biotinylated
ANGPTL8
Detection
mAb
B


ANGPTL8
Target Antigen


ANGPTL8
Capture mAb

FIG. 2

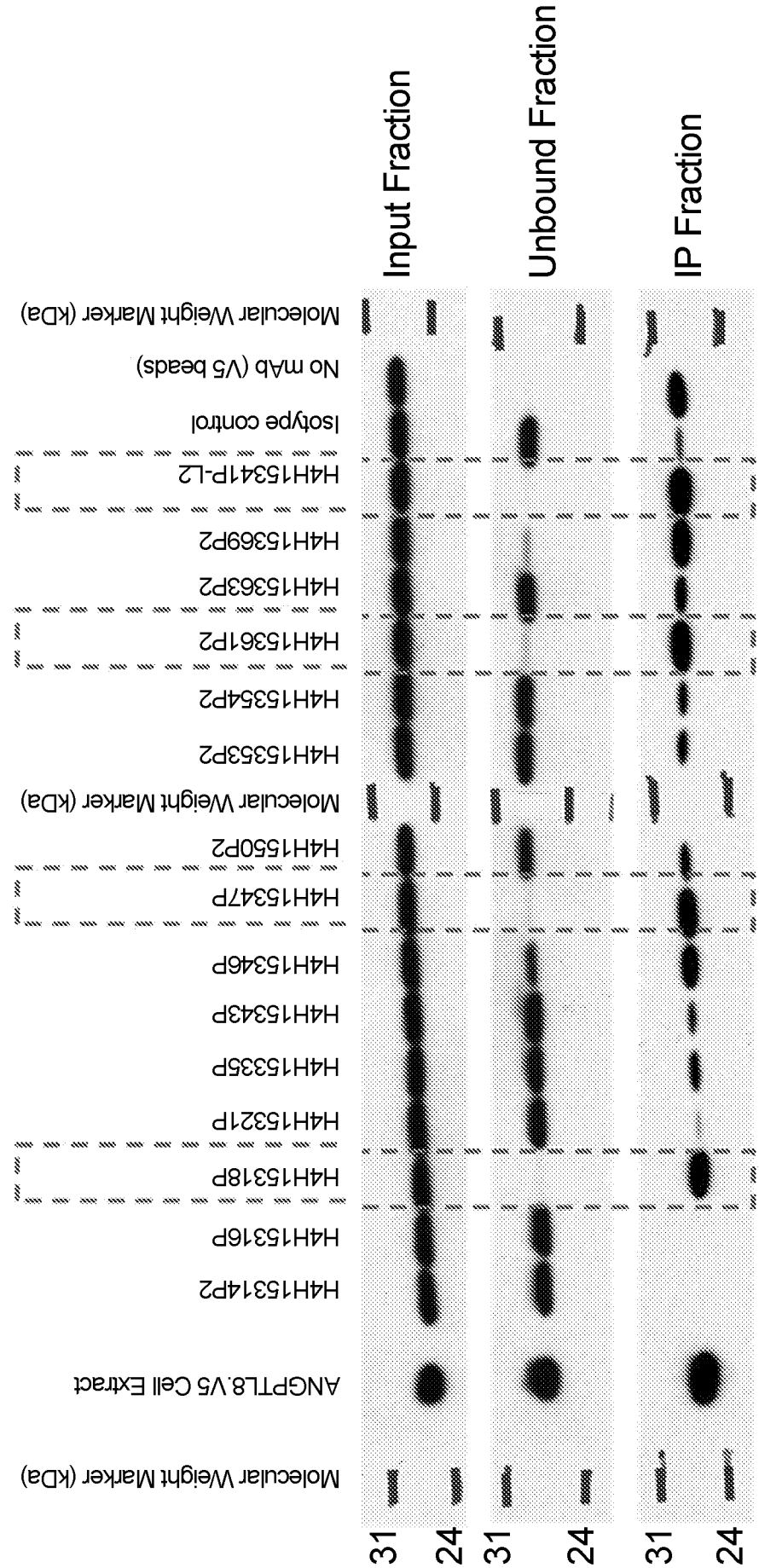

Input Fraction
Unbound Fraction
IP Fraction
31
24
Molecular Weight Marker (kDa)
ANGPTL8.V5 Cell Extract
H4H15314P2
H4H15316P
H4H15318P
H4H15321P
H4H15335P
H4H15343P
H4H15346P
H4H15347P
H4H15350P2
Molecular Weight Marker (kDa)
H4H15353P2
H4H15354P2
H4H15361P2
H4H15363P2
H4H15369P2
H4H15341P-L2
Isotype control
No mAb (V5 beads)
Molecular Weight Marker (kDa)

FIG. 3A

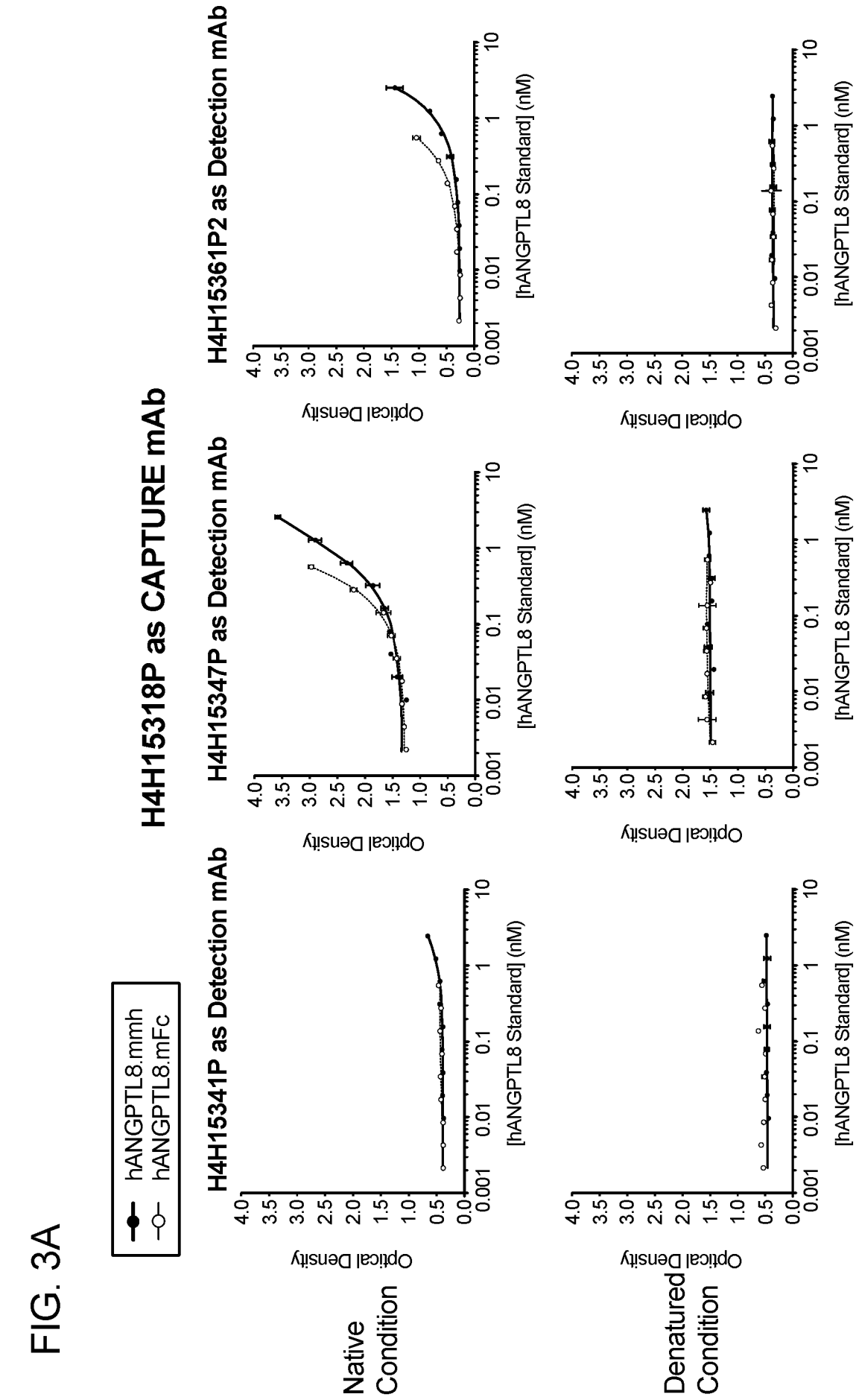

hANGPTL8.mmh
hANGPTL8.mFc
H4H15318P as CAPTURE mAb
H4H15341P as Detection mAb
H4H15347P as Detection mAb
H4H15361P2 as Detection mAb
Native Condition
Denatured Condition
Optical Density
[hANGPTL8 Standard] (nM)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
0.001
0.01
0.1
1
10

FIG. 3B

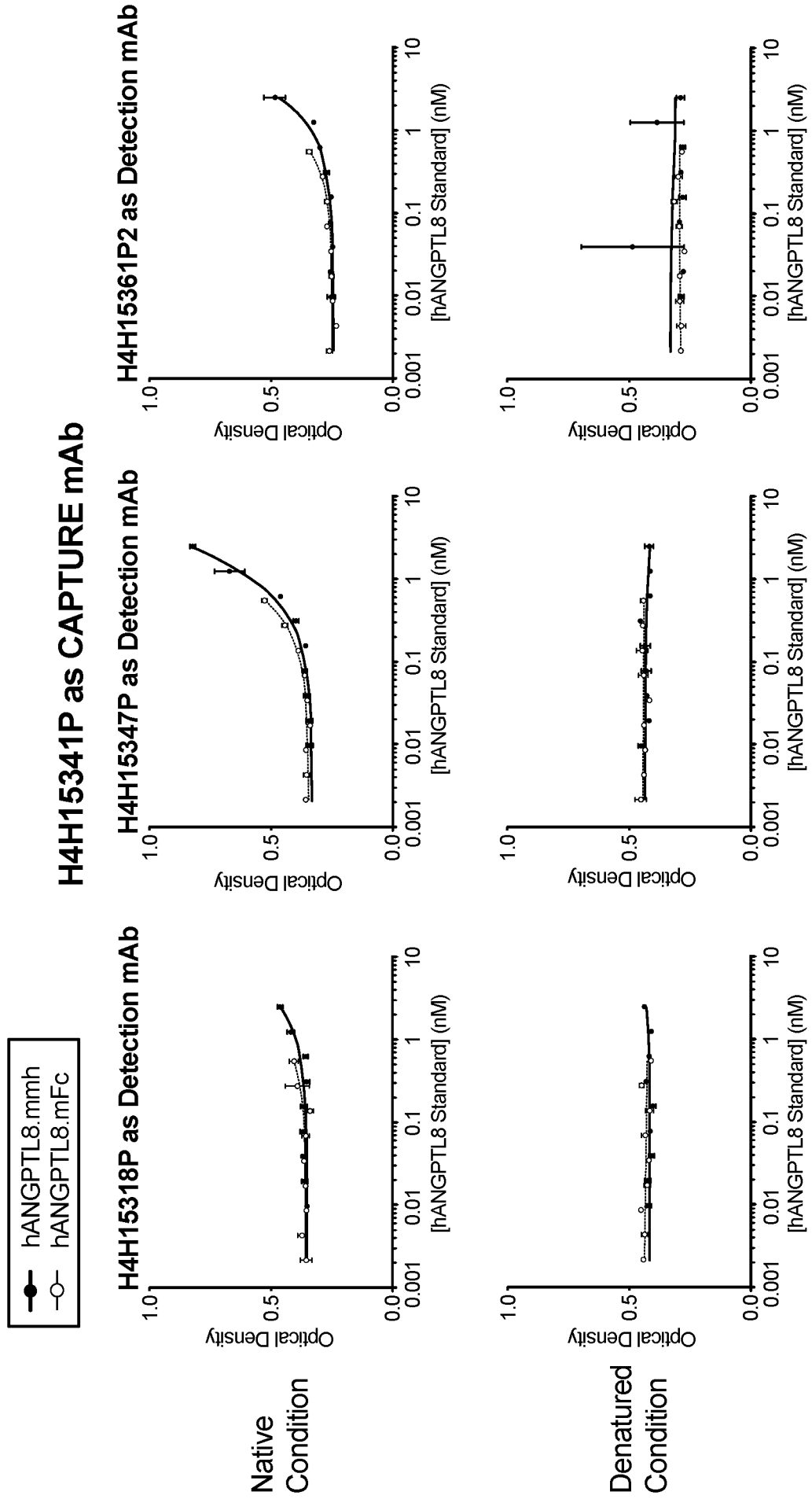

hANGPTL8.mmh
hANGPTL8.mFc
H4H15341P as CAPTURE mAb
H4H15318P as Detection mAb
H4H15347P as Detection mAb
H4H15361P2 as Detection mAb
Native Condition
Denatured Condition
Optical Density
[hANGPTL8 Standard] (nM)
0.0
0.5
1.0
0.001
0.01
0.1
1
10

FIG. 3C

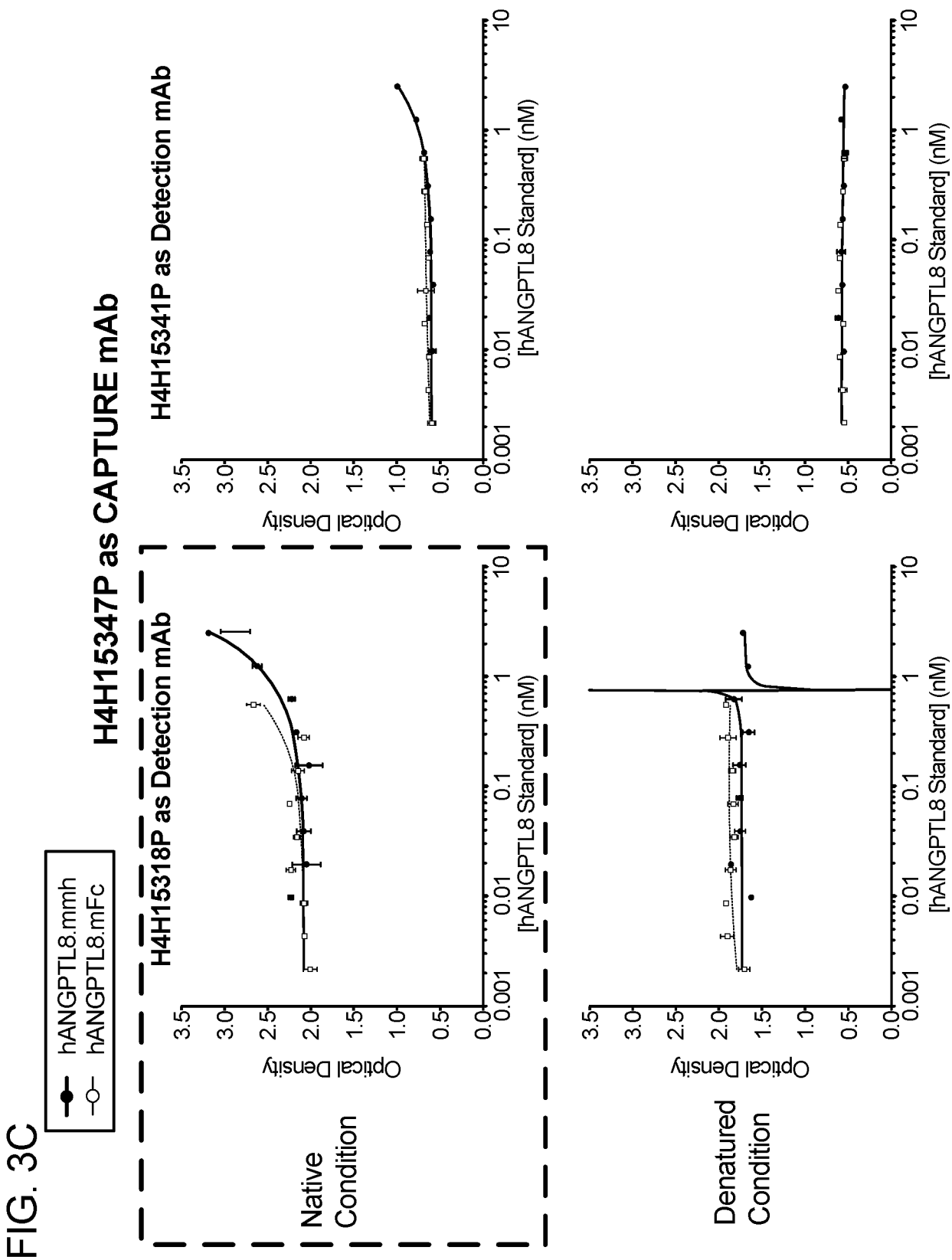

hANGPTL8.mmh
hANGPTL8.mFc
H4H15347P as CAPTURE mAb
H4H15318P as Detection mAb
H4H15341P as Detection mAb
Native Condition
Denatured Condition
Optical Density
[hANGPTL8 Standard] (nM)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
0.001
0.01
0.1
1
10

FIG. 3D

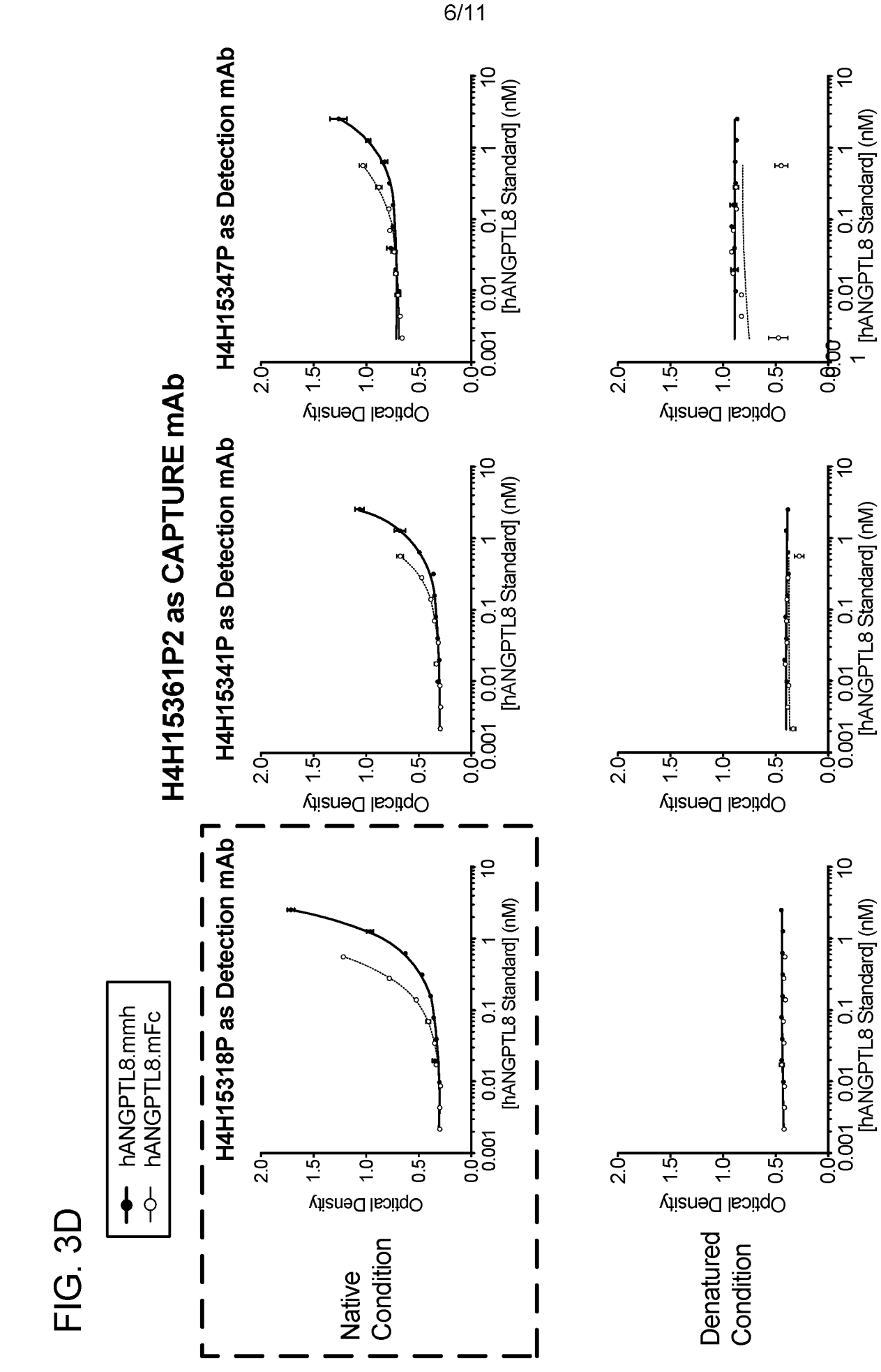

hANGPTL8.mmh
hANGPTL8.mFc
H4H15361P2 as CAPTURE mAb
H4H15318P as Detection mAb
H4H15341P as Detection mAb
H4H15347P as Detection mAb
Native Condition
Denatured Condition
Optical Density
[hANGPTL8 Standard] (nM)

FIG. 4

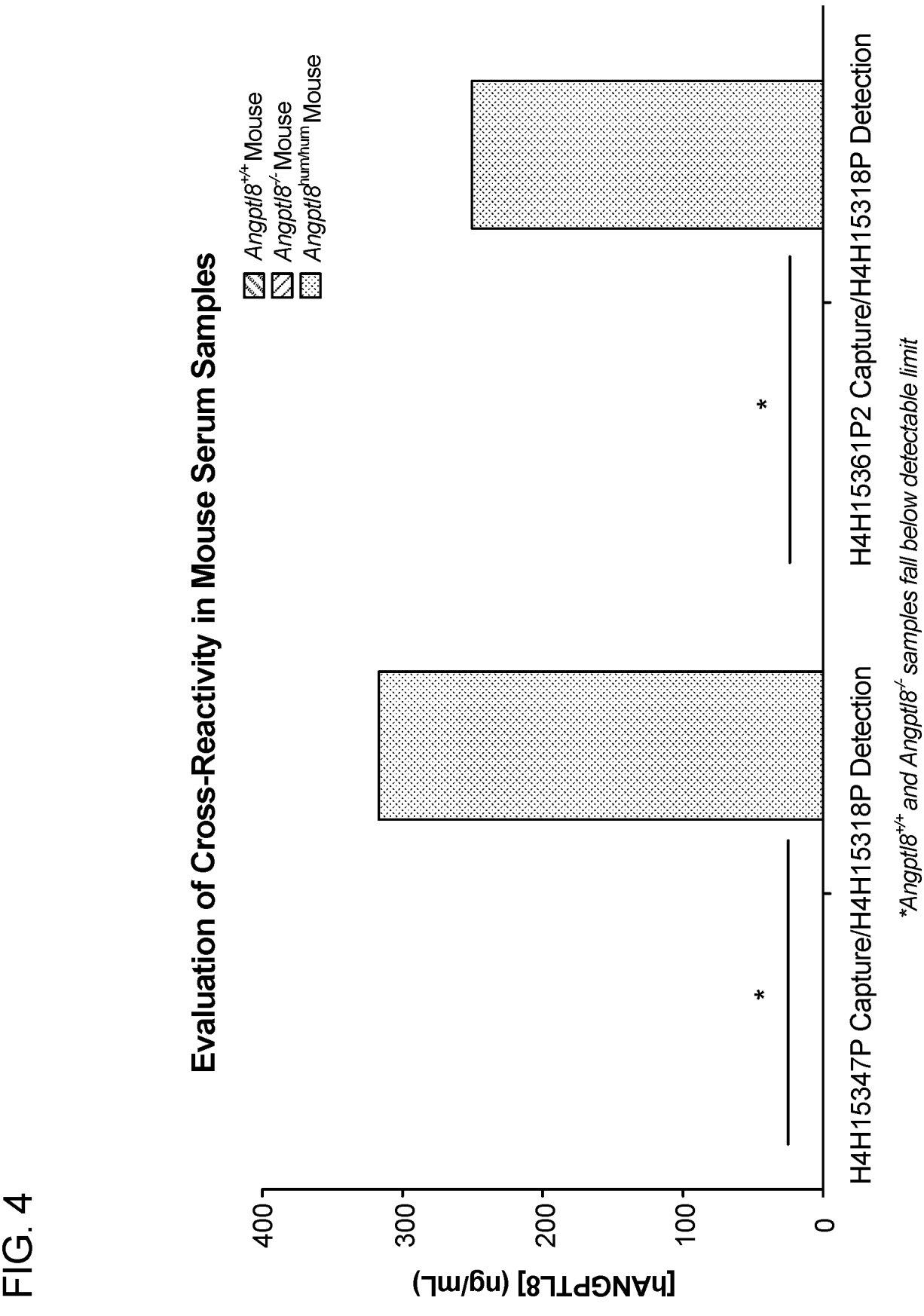
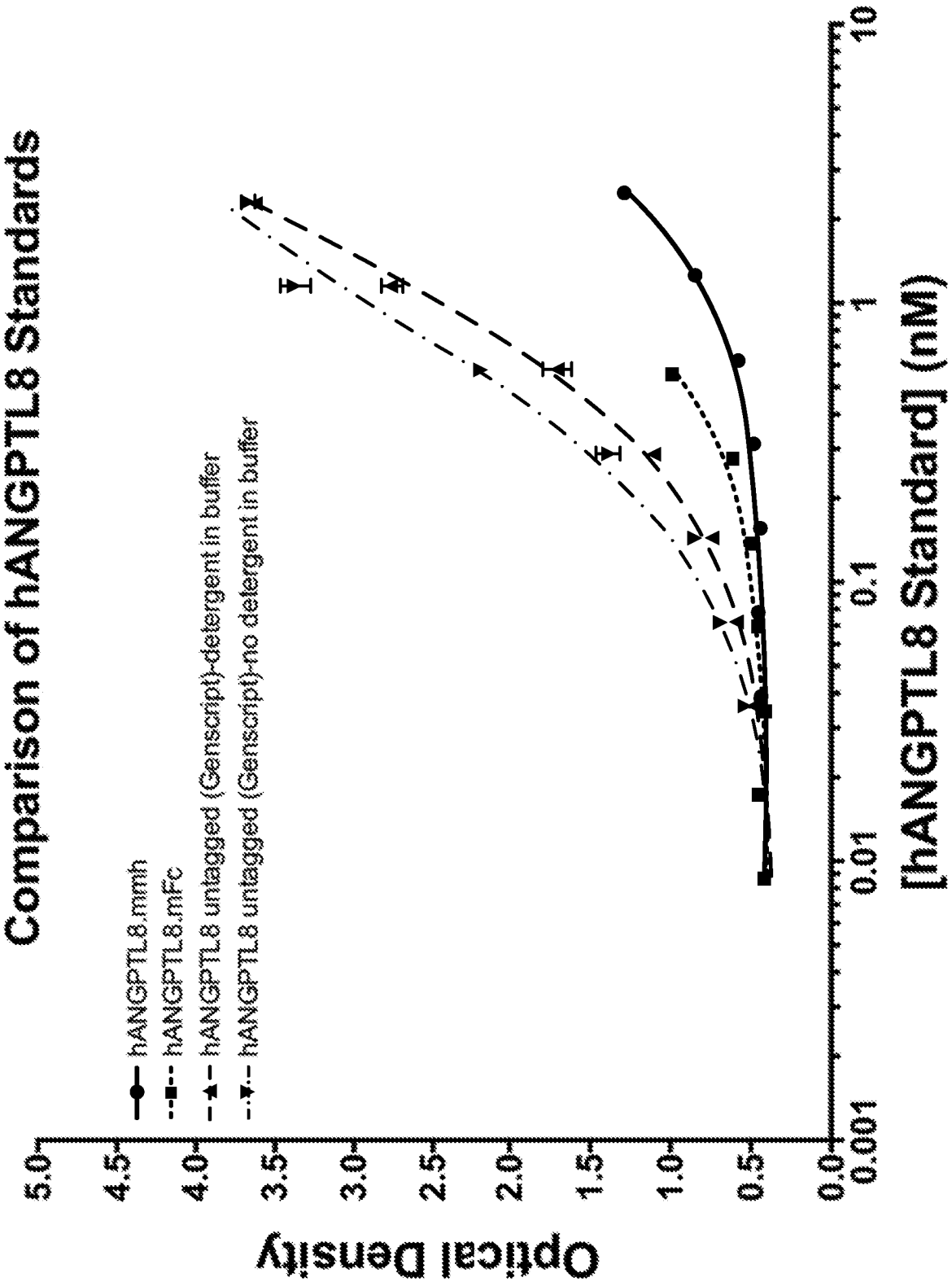

Evaluation of Cross-Reactivity in Mouse Serum Samples
Angptl8+/+ Mouse
Angptl8-/- Mouse
Angptl8hum/hum Mouse
[hANGPTL8] (ng/mL)
400
300
200
100
0
*
*
H4H15347P Capture/H4H15318P Detection
H4H15361P2 Capture/H4H15318P Detection
*Angptl8+/+ and Angptl8-/- samples fall below detectable limit

FIG. 5


Comparison of hANGPTL8 Standards
hANGPTL8.mmh
hANGPTL8.mFc
hANGPTL8 untagged (Genscript)-detergent in buffer
hANGPTL8 untagged (Genscript)-no detergent in buffer
Optical Density
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.001
0.01
0.1
1
10
[hANGPTL8 Standard] (nM)

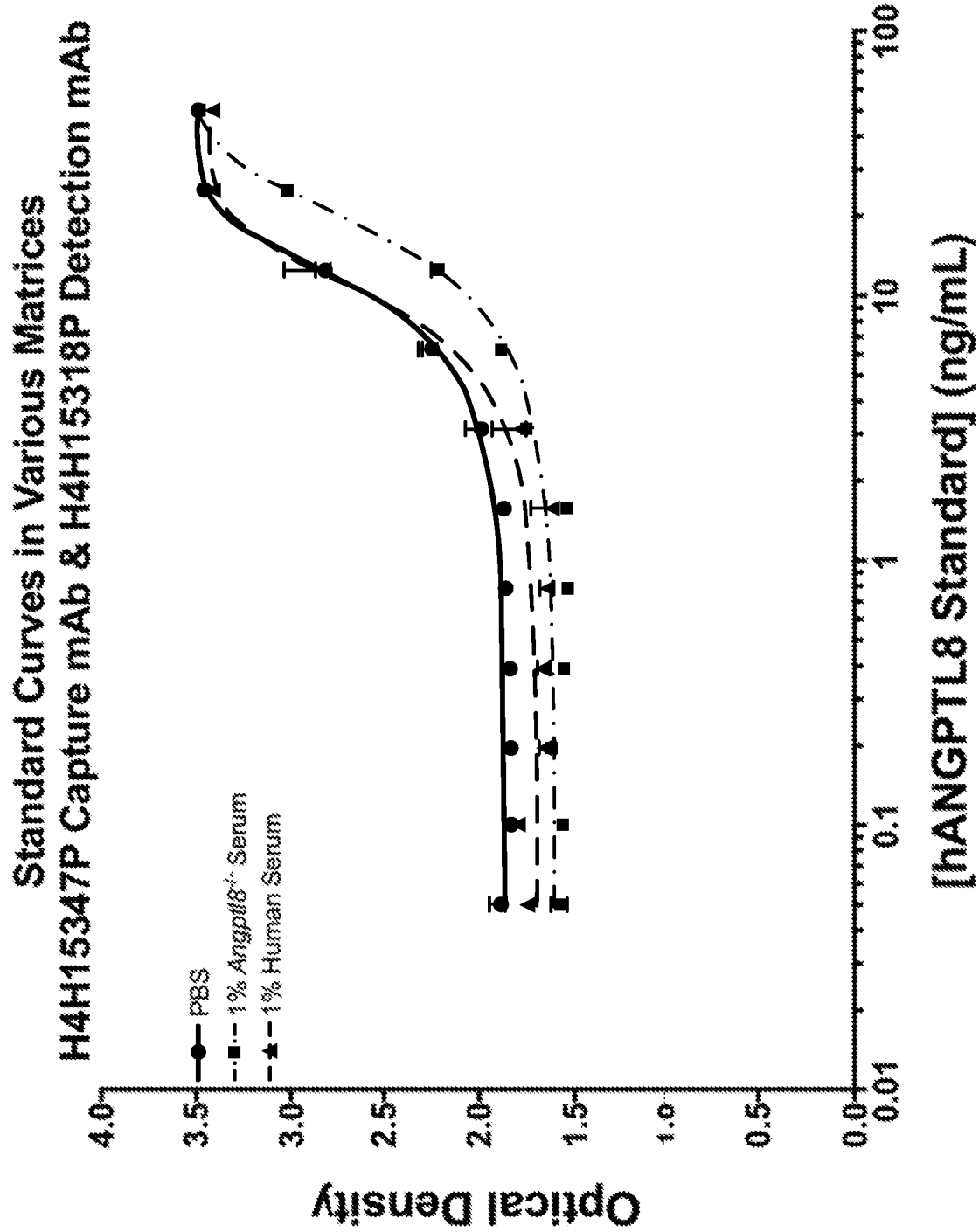

Standard Curves in Various Matrices
H4H15347P Capture mAb & H4H15318P Detection mAb
PBS
1% Angptl8-/- Serum
1% Human Serum
Optical Density
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.01
0.1
1
10
100
[hANGPTL8 Standard] (ng/mL)

FIG. 6

FIG. 7

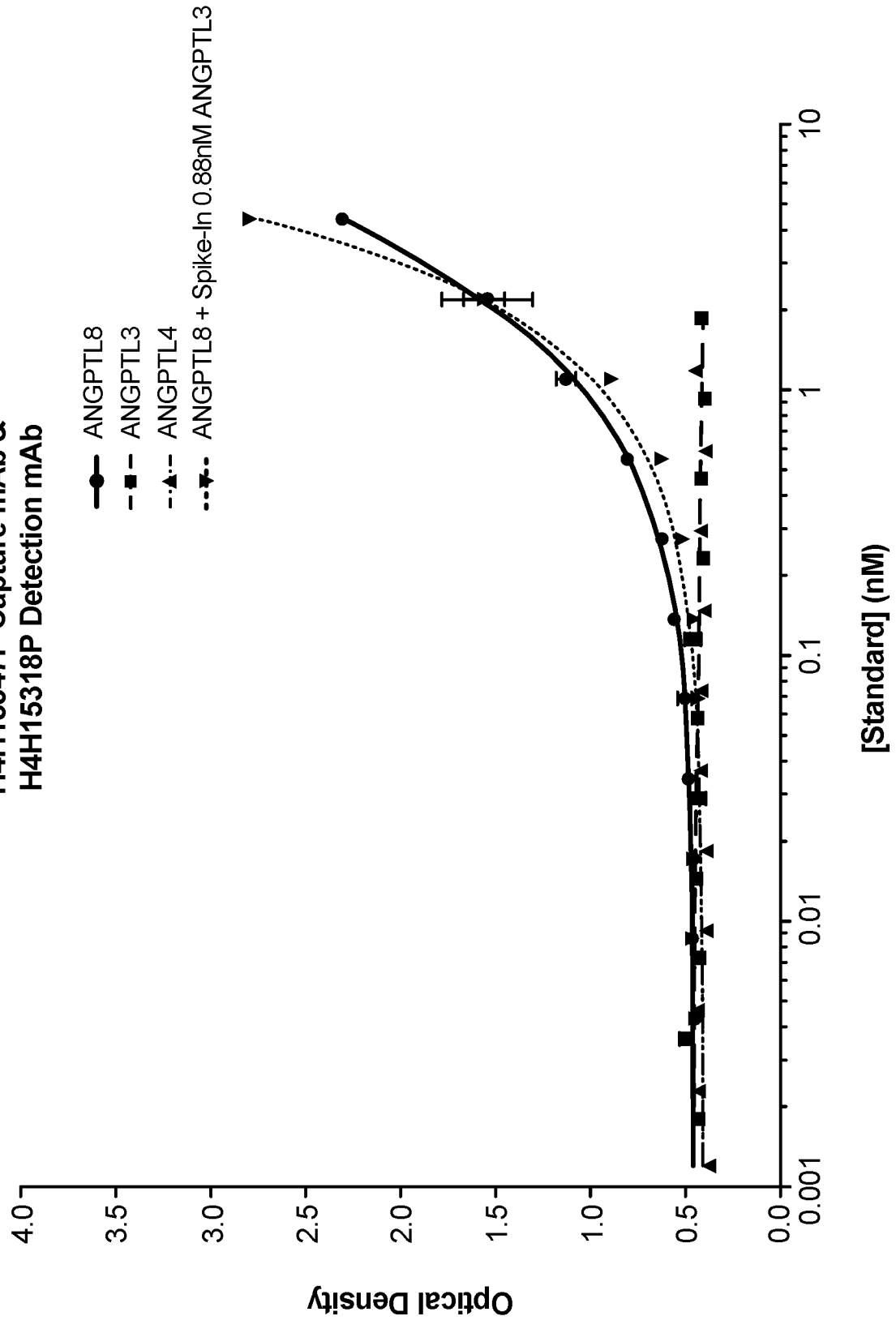

Various ANGPTL Protein Standard Curves
H4H15347P Capture mAb &
H4H15318P Detection mAb
ANGPTL8
ANGPTL3
ANGPTL4
ANGPTL8 + Spike-In 0.88nM ANGPTL3
Optical Density
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.001
0.01
0.1
1
10
[Standard] (nM)

FIG. 8A

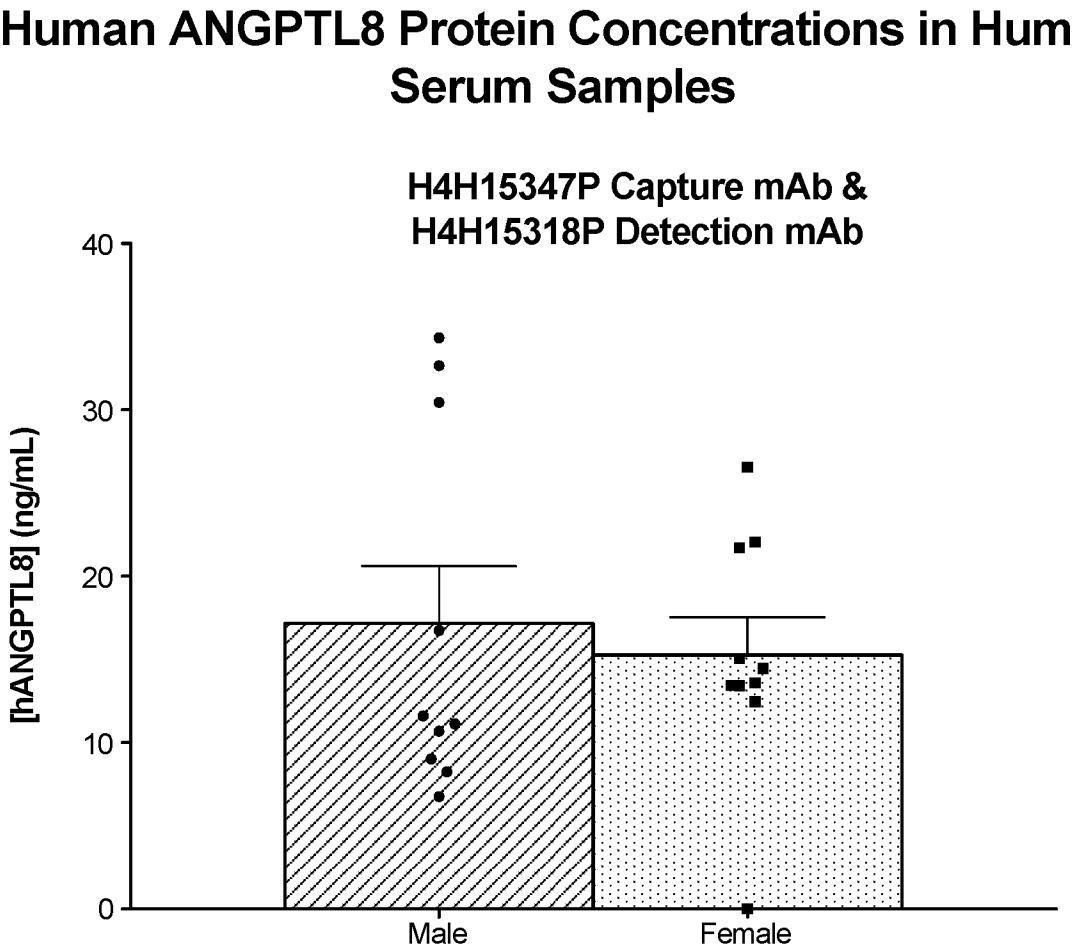
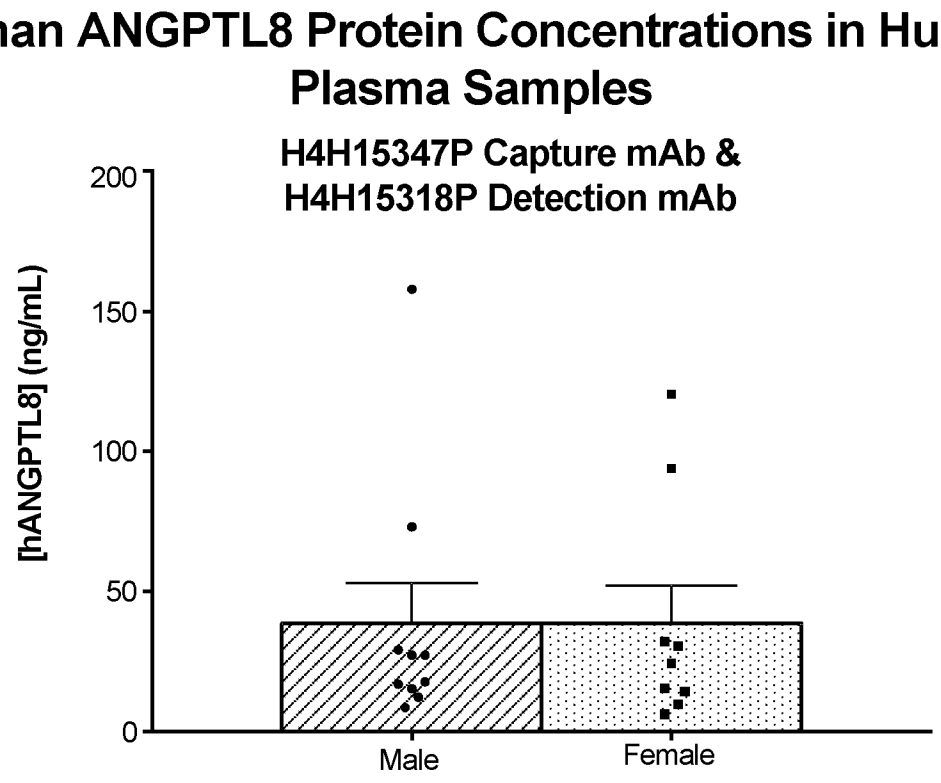

Human ANGPTL8 Protein Concentrations in Human Serum Samples
H4H15347P Capture mAb &
H4H15318P Detection mAb
[hANGPTL8] (ng/mL)
40
30
20
10
0
Male
Female

FIG. 8B


Human ANGPTL8 Protein Concentrations in Human Plasma Samples
H4H15347P Capture mAb &
H4H15318P Detection mAb
[hANGPTL8] (ng/mL)
200
150
100
50
0
Male
Female

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2018/066266

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/74
ADD.

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)
G01N

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2017/027316 A1 (REGENERON PHARMA [US]) 16 February 2017 (2017-02-16) ([0189-0191])([0150], [0211]); abstract; table 7 -----	1-21
A	ZHIYAO FU ET AL: "A lipasin/Angptl8 monoclonal antibody lowers mouse serum triglycerides involving increased postprandial activity of the cardiac lipoprotein lipase", SCIENTIFIC REPORTS, vol. 5, no. 1, 21 December 2015 (2015-12-21), XP055557023, DOI: 10.1038/srep18502 abstract -----	1-21

☐ 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

"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
14 February 2019	25/02/2019

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Bigot-Maucher, Cora

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2018/066266

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2017027316 A1	16-02-2017	AR 105600 A1	18-10-2017
		AU 2016307430 A1	22-02-2018
		CA 2993976 A1	16-02-2017
		CN 107922478 A	17-04-2018
		EA 201890453 A1	31-07-2018
		EP 3331908 A1	13-06-2018
		JP 2018525383 A	06-09-2018
		KR 20180038020 A	13-04-2018
		TW 201713690 A	16-04-2017
		US 2017037124 A1	09-02-2017
		WO 2017027316 A1	16-02-2017