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[Continued on next page]

(54) Title: IMMUNOASSAY STANDARDS AND MEASUREMENT OF CLINICAL BIOMARKERS USING INTRA-ASSAY CALIBRATION STANDARDS

(57) Abstract: The present invention provides novel compositions and methods for creating quantitative standards to calibrate analytes. These compositions and methods enable the creation of standards and calibrators for analyzing analytes and measuring clinical biomarkers. Also provided are kits comprising the novel compositions for use in assays, for example sandwich immunoassays.

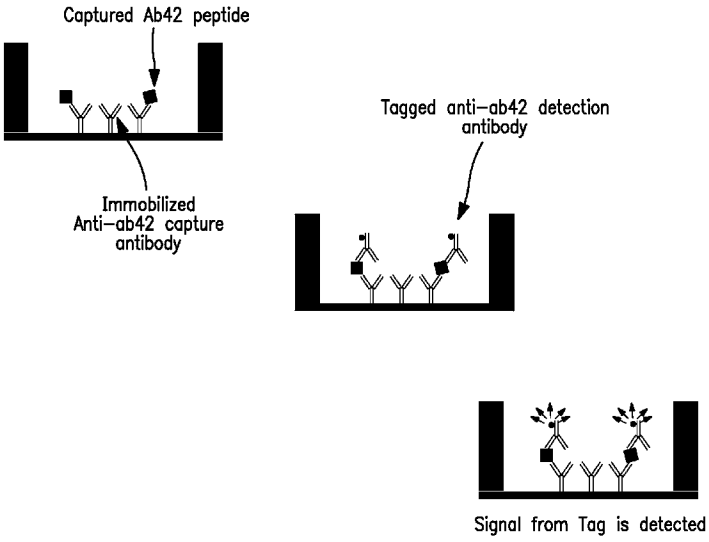

FIG. 1

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IMMUNOASSAY STANDARDS AND MEASUREMENT OF CLINICAL BIOMARKERS USING INTRA-ASSAY CALIBRATION STANDARDS

FIELD OF THE INVENTION

[0001] This invention relates to novel compositions that can be used as reference standards and calibrators in order to measure clinical biomarkers in an immunoassay. This invention also relates to methods of using the compositions and kits comprising the compositions.

BACKGROUND OF THE INVENTION

[0002] Amyloid Beta (Aβ) peptides are generated from the cleavage of Amyloid Precursor Protein (APP) via beta secretase and gamma secretase enzymatic complexes (Wolfe, *Biochemistry*, 45:7931-7939 (2006)). Beta secretase generates the N-terminal ends of these amyloid peptides and gamma secretase generates the C-terminal ends (Wolfe, *Biochemistry*, 45:7931-7939 (2006)). Several species of peptides are subsequently generated, typically ranging from 38 to 42 amino acids in length depending on where the gamma secretase cleaves the APP. Aβ peptides have an extracellular domain (amino acids 1-28) and a transmembrane region (amino acids 29-42) that is embedded in the lipid bilayer. Amyloid peptides that are 42 amino acids long ($A\beta_{42}$) are believed to be the putative neurotoxic species, either alone or as aggregates. These aggregates are suspected to contribute to the neurodegeneration of the brain resulting in Alzheimer's disease and dementia. The hypothesis that $A\beta_{42}$ contributes to clinical dementias is called the amyloid cascade hypothesis as described by Hardy et al. (*Science*, 256:184-185 (1992)).

[0003] One of the characteristics of the Aβ peptides is the ability to self-assemble into oligomers at physiological concentrations (Burdick et al., *Journal of Biological Chemistry*, 267:546-554 (1992); Cerf et al., *Biochemical Journal*, 421:415-423 (2009).). The $A\beta_{42}$ species is more prone to forming oligomers as compared to the $A\beta_{40}$ and $A\beta_{38}$ species. The mechanism of oligomer formation has been shown to originate from a small, five amino acid region located at amino acids 16 to 20 (KLVFF) which mediates the binding of Aβ peptides in an anti-parallel manner. This small region has thus been termed the "aggregation domain" (Tjernberg et al., *Journal of Biological Chemistry*,

271:8545-8548 (1996)). Aβ peptide aggregates assemble rapidly (*i.e.*, within minutes) under certain conditions especially at lower pH ranges, with slightly slower kinetics at neutral or higher pH (Burdick et al., *Journal of Biological Chemistry*, 267:546-554 (1992)). The aggregates are poorly soluble in aqueous solutions, especially in the presence of salts. The C-terminal ends of the Aβ peptides fold back over the core of the dimer via salt bridges thereby increasing their hydrophobicity and promoting further polymerization of the peptides into filaments or fibrils. The additional two C-terminal residues in the $A\beta_{42}$ species provides increased hydrophobicity in comparison to the other Aβ species (Kim et al., *Journal of Biological Chemistry*, 280:35069-35076 (2005)).

[0004] Clinical data suggests that the degree of dementia and cognitive decline has a higher correlation with $A\beta_{42}$ concentration than either the $A\beta_{40}$ or $A\beta_{38}$ species. This observation, in conjunction with the rapid aggregation properties of $A\beta_{42}$, has led to the hypothesis that inhibition of $A\beta_{42}$ aggregation may have clinical benefits. There have been numerous studies showing different mechanisms that can be used to inhibit the formation of $A\beta_{42}$ aggregates. Tjernberg et al. (*Journal of Biological Chemistry*, 271: 8545-8548 (1996)) showed that peptides comprising the aggregation domain bind well to Aβ peptides and inhibit the formation of aggregates. Several other molecules that bind to the aggregation domain have also been shown to inhibit amyloid peptide aggregation (Martharu et al., *Journal of Neurological Sciences*, 280:49-58 (2009); Kim et al., *Biochemical and Biophysical Research Communications*, 303:576-279 (2003)). Substitution of amino acids in the aggregation core domain or the deletion of the entire aggregation domain also prevents Aβ peptide aggregation and fibril formation (Tjernberg et al., *Journal of Biological Chemistry*, 274:12619-12625 (1999)). In addition, various drugs have been designed to inhibit gamma secretase activity in order to lower the amount of $A\beta_{42}$ and related peptide species. The usefulness of these approaches in the clinic is currently under investigation.

[0005] In order to assess the effectiveness of a molecule to inhibit the generation of $A\beta_{42}$ or to prevent its aggregation, it is necessary to measure the amount of $A\beta_{42}$ accurately. There are several techniques that are used to detect and quantitate $A\beta_{42}$ in biological samples including both immunoassays (Olsson et al., *Clinical Chemistry*, 51:336-345 (2005); Verwey et al., *Journal of Immunological Methods*, 348:57-66 (2009); Sjogren et al., *Journal of Neural Transmission*, 107:563-679 (2000)) and mass

spectrometry (MS) based methods (Cantone et al., *Journal of Neuroscience Methods*, 180:255-260 (2009); *Journal of Mass Spectrometry*, 40:142-145 (2005)). The MS based methods, including those of MALDI-TOF and SELDI-TOF along with liquid chromatography prepared mass spectrometry, are able to detect many of the amyloid beta species in a biological sample, but do not presently provide sufficient quantitative values that are needed for measuring $A\beta_{42}$ in clinical samples.

[0006] Immunoassay methods are based on a double sandwich immunoassay that comprises one antibody that is specific to the N-terminus and a second antibody that is highly specific for the $A\beta_{42}$ C-terminus (*i.e.*, does not recognize other Aβ peptide species). There are two basic versions of the immunoassays. The first version captures Aβ peptides in biological samples via a solid surface immobilized N-terminal region specific antibody. The $A\beta_{42}$ specific antibody carrying a tag is added to the immunoassay in order to complete the antibody sandwich. The second version captures Aβ peptides in biological samples via an $A\beta_{42}$ C-terminal region specific antibody immobilized on a solid surface. The N-terminal region specific antibody carrying a tag is added to the immunoassay. In either version, the tag incorporated via the second antibody enables the detection of the complete complex. These assays are made quantitative by the use of $A\beta_{42}$ reference standards, which are added in lieu of biological samples. The resulting signal measured from the reference standards are used to generate a standard curve which is subsequently used to quantify the amount of $A\beta_{42}$ in the biological samples.

[0007] Until now, the use of $A\beta_{42}$ reference standards in immunoassays has relied on synthetic, full length $A\beta_{42}$ peptides which are typically generated with minimal difficulty. However, these peptides have strong hydrophobic properties and, therefore, are not soluble in aqueous solutions. In addition, the storage and use of $A\beta_{42}$ as reference standards presents many issues. As discussed, $A\beta_{42}$ forms aggregates rapidly and this formation occurs more readily at room temperatures and neutral pH. Long term storage at low temperatures (below -20 °C) and low pH helps to minimize aggregation during storage but it does not prevent it. Reconstitution of $A\beta_{42}$ in buffers that are amenable to immunoassays can also prove difficult. These solutions are almost always aqueous, buffered at a neutral pH, contain salts, and used at room temperature; all the conditions that accelerate $A\beta_{42}$ aggregation. $A\beta_{42}$ peptides that have aggregated are not useful as reference standards in immunoassays because of the insoluble precipitates and non-

uniformity in both size and availability to be recognized by either detection or capture antibodies.

[0008] Thus, the present invention fulfills a need in the art by providing methods useful for generating an $A\beta_{42}$ peptide or protein construct and compositions thereof that can be used as a reference standard or calibrator in an immunoassay or other format to measure the abundance of $A\beta_{42}$ peptide accurately in a fluid or tissue extract sample. Specifically, the compositions and methods of the present invention are aimed at creating non-aggregating peptide reference standards for $A\beta_{42}$ for use in immunoassay formats. The compositions and methods described herein have a broad applicability to many other peptides that are difficult to measure and quantitate.

BRIEF SUMMARY OF THE INVENTION

[0009] In one aspect, the invention provides a composition comprising an N-terminal immunoreactive region, a C-terminal immunoreactive region and a linker region. In another aspect, the invention provides a composition comprising an N-terminal immunoreactive region, a C-terminal immunoreactive region and a linker region, wherein the composition is used as a reference standard in an immunoassay. In one embodiment, the immunoassay is selected from the group consisting of a sandwich immunoassay, a single antibody assay, a double sandwich immunoassay and a competition assay. In another embodiment, the composition is selected from the group consisting of a protein, a peptide, a fragment and a modified protein. In one embodiment, the N-terminal immunoreactive region binds $A\beta_{42}$, $A\beta_{40}$, $A\beta_{38}$, tau or insulin growth factor receptor 1. In another embodiment, the C-terminal immunoreactive region binds $A\beta_{42}$, $A\beta_{40}$, $A\beta_{38}$, tau or insulin growth factor receptor 1. In another embodiment, the linker region is a non-immunoreactive domain. In another embodiment, the linker region comprises a linker selected from the group consisting of polyethylene glycol, a glutamine residue, an alanine residue, a lysine residue, a lipid, a globular protein, a nucleic acid (including but not limited to DNA, RNA and PNA) and an alkyl chain.

[0010] In another aspect, the invention provides an isolated peptide molecule having an amino acid sequence of SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8, 9. 10, 11, 12, 13, 14, 15, 17, 19, 21, 22, 23, 24, 25, 26 or 27.

[0011] In another aspect, the invention provides a method of measuring the quantity of an analyte in a biological sample, the method comprising: attaching a reference standard to at least two beads thereby forming a first bead set and a second bead set, wherein the reference standard comprises an epitope recognized by a first detection antibody and wherein each bead set comprises a different concentration of the reference standard; attaching a capture antibody specific to the analyte to a third bead set; mixing all of the bead sets together to form a suspension array; applying the biological sample to the suspension array whereby the analyte binds to the capture antibody on the third bead set; adding a first detection antibody to the suspension array, wherein the first detection antibody binds the reference standard and analyte bound to the capture antibody; measuring a first signal from the first detection antibody bound to the reference standard in the first bead set; measuring a second signal from the first detection antibody bound to the reference standard in the second bead set; generating a standard curve based upon the first and second signals; and quantitating the amount of the analyte in the third bead set by measuring a third signal from the first detection antibody and comparing the third signal to the first and second signal measurements on the standard curve.

[0012] In one embodiment, the reference standard comprises a composition comprising an N-terminal immunoreactive region, a C-terminal immunoreactive region and a linker region. In another embodiment, the reference standard comprises a peptide or modified peptide having an amino acid sequence of SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8, 9. 10, 11, 12, 13, 14, 15, 17, 19, 21, 22, 23, 24, 25, 26 or 27.

[0013] In another embodiment, the biological sample is selected from the group consisting of blood, serum, plasma, peripheral blood mononuclear cells, peripheral blood lymphocytes, tissue, cerebrospinal fluid and cells. In another embodiment, the analyte is selected from the group consisting of $A\beta_{42}$, $A\beta_{40}$, $A\beta_{38}$, tau or insulin growth factor receptor 1.

[0014] In another embodiment, the method is performed in a multi-well plate, nitrocellulose filter, glass fiber or on a glass slide. In another embodiment, the first signal and second signal is a signal selected from the group consisting of phycoerythrin, alexa 532, streptavidin-phycoerythrin and streptavidin-Alexa 532. In another embodiment, the reference standard is covalently attached to the bead. In another embodiment, the capture

antibody is covalently attached to the bead. In another embodiment, the covalent attachment is a carbodimide bond.

[0015] In another aspect, the present invention provides a kit for conducting an immunoassay to detect $A\beta_{42}$ peptide, the kit comprising a composition of the present invention.

BRIEF DESCRIPTION OF THE TABLES

[0016] Table 1 shows the desirable physical properties of the Aβ peptides and the tests used to measure those properties.

[0017] Table 2 shows $A\beta_{42}$ peptide and modified peptide sequences and descriptions.

[0018] Table 3 shows tau peptide and modified peptide sequences and descriptions.

[0019] Table 4 shows the measured concentration of $A\beta_{42}$ peptide in three human cerebrospinal (CSF) samples.

[0020] Table 5 shows a list of the novel Aβ peptides that were characterized.

[0021] Table 6 shows a summary of dynamic light scattering (DLS) data.

BRIEF DESCRIPTION OF THE FIGURES

[0022] Figure 1 shows a schematic of a two-sided or sandwich soluble standard.

[0023] Figure 2 shows a calibration curve from an exemplary $A\beta_{42}$ sandwich immunoassay.

[0024] Figure 3 shows an exemplary $A\beta_{42}$ sandwich assay using modified $A\beta_{42}$ peptide standards.

[0025] Figure 3A shows a calibration curve from modified standard 2 (SEQ ID NO:2) and 4 (SEQ ID NO:4).

[0026] Figure 3B shows a calibration curve from modified standard 12 (SEQ ID NO:12) and 13 (SEQ ID NO:13).

[0027] Figure 3C shows a calibration curve from modified standard 14 (SEQ ID NO:14) and 6 (SEQ ID NO:6).

[0028] Figure 4 shows a schematic of the $A\beta_{42}$ intra-assay bead approach.

[0029] Figure 5A shows the measured Median Fluorescence Intensity (MFI) of 6 different Luminex bead sets covalently coupled with different concentrations of Aβ 1-40 peptide (SEQ ID NO:15).

[0030] Figure 5B shows 4-PL generated calibration curves from either the $A\beta_{40}$ intra-assay standards (circles) or from native $A\beta_{42}$ peptides as a soluble standard (triangles), similar to the curve shown in Figure 2.

[0031] Figure 5C shows the measured $A\beta_{42}$ peptides in human CSF samples using either a calibration curve generated from soluble $A\beta_{42}$ peptides or from the intra-assay $A\beta_{40}$ standards.

[0032] Figure 6 shows the levels of phosphorylated insulin growth factor receptor 1 (IGF-R1) in human peripheral blood mononuclear cell lysates. A 4 parameter calibration curve was generated from the MFI values on the different bead sets (Figure 6A) and used to determine the relative levels of phosphorylated IGF-R1 in PBMC lysates (Figure 6B).

[0033] Figure 7 shows DLS data.

[0034] Figure 8 shows circular dichroism analysis.

[0035] Figure 9 shows peptide stability data. The full length $A\beta_{42}$ and seven modified peptides were subjected to stability studies at different temperatures for up to 40 days (Figures 9A-9F).

[0036] Figure 10 shows a standard curve comparison between full length $A\beta_{42}$ and seven modified peptides.

[0037] Figure 11 shows the standard curve analysis of full length versus modified peptides.

[0038] Figure 12 shows a CSF sample analysis using full length versus modified peptides.

[0039] Figure 13 shows the structures of the polyethylene glycol spacers incorporated into the modified $A\beta_{(1-42)}$ peptides.

DETAILED DESCRIPTION OF THE INVENTION

[0040] The present invention may be understood more readily by reference to the following detailed description of the preferred embodiments of the invention and the Examples included herein.

[0041] This invention relates to novel compositions and methods that can be used as reference standards and calibrators in order to measure clinical biomarkers in an immunoassay. This invention also relates to methods of using the compositions and kits comprising the compositions. Specifically, the compositions and methods of the present

invention are aimed at creating non-aggregating peptide reference standards for $A\beta_{42}$ or tau for use in immunoassay formats.

[0042] This invention also relates to kits comprising the compositions of the invention.

Definitions

[0043] As used herein, the term "Aβ" refers to amyloid beta.

[0044] As used herein, the term "$A\beta_{42}$" refers to Amyloid Beta 1-42. "$A\beta_{42}$" refers to a 42 amino acid length peptide that has an amino acid sequence as noted in Table 2, SEQ ID NO:1.

[0045] As used herein, the term "$A\beta_{38}$" refers to Amyloid Beta 1-38. "$A\beta_{38}$" refers to a 38 amino acid length peptide that has the sequence DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGG (SEQ ID NO:17).

[0046] As used herein, the term "$A\beta_{40}$" refers to Amyloid Beta 1-40. "$A\beta_{40}$" refers to a 40 amino acid length peptide that has the sequence as noted in Table 2, SEQ ID NO:15.

[0047] As used herein, the term "tau" refers to the native tau protein corresponding to the amino acid sequence as noted in Table 3, SEQ ID NO:20.

[0048] As used herein, the term "antibody" is used in the broadest sense, and specifically covers monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (*i.e.*, bispecific antibodies), and antibody fragments (*i.e.*, Fab, F(ab').sub.2 and Fv) so long as they exhibit binding activity or affinity for a selected antigen. "Antibody" can also refer to an antibody or antibody fragments hanging or fused to carrier proteins/organisms such as phage or other display carriers that have the same properties as isolated antibodies.

[0049] As used herein, the term "isolated", as used herein with reference to the subject proteins and protein complexes, refers to a preparation of protein or protein complex that is essentially free from contaminating proteins that normally would be present with the protein or complex (*i.e.*, in the cellular milieu in which the protein or complex is found endogenously). Thus, an isolated protein complex is isolated from cellular components that normally would "contaminate" or interfere with the study of the complex in isolation, for instance while screening for modulators thereof. It is to be

understood, however, that such an "isolated" complex may incorporate other proteins the modulation of which, by the subject protein or protein complex, is being investigated.

[0050] As used herein, the term "isolated" as also used herein with respect to nucleic acids, such as DNA or RNA, refers to molecules in a form which does not occur in nature. Moreover, an "isolated nucleic acid" is meant to include nucleic acid fragments which are not naturally occurring as fragments and would not be found in the natural state.

[0051] As used herein, the term "nucleic acid" refers to polynucleotide such as deoxyribonucleic acid (DNA), and, where appropriate, ribonucleic acid (RNA). The term should also be understood to include, as equivalents, analogs of either RNA or DNA made from nucleotide analogs, and, as applicable to the embodiment being described, single-stranded (such as sense or antisense) and double-stranded polynucleotide. "Nucleic acid" can also refer to a peptide nucleic acid "PNA" or an artificially synthesized DNA or RNA.

[0052] As used herein, the terms "peptides", "proteins" and "polypeptides" are used interchangeably herein. The term "purified protein" refers to a preparation of a protein or proteins that are preferably isolated from, or otherwise substantially free of, other proteins normally associated with the protein(s) in a cell or cell lysate. As used herein, the term "modified peptide" refers a peptide that has been modified relative to the native sequence of that peptide. For example, a modification may include the removal of a deleterious domain or the addition of a linker within the native peptide sequence.

[0053] As used herein, the term "binding" refers to a direct association between two molecules, due to, for example, covalent, electrostatic, hydrophobic, ionic and/or hydrogen-bond interactions under physiological conditions. Likewise, "complex formation," between two or more polypeptides, refers to a direct association between polypeptides, due to, for example, covalent, electrostatic, hydrophobic, ionic and/or hydrogen-bond interactions under physiological conditions.

[0054] As used herein, the term "domain" refers to a region of a protein that comprises a particular structure and/or performs a particular function (*i.e.*, aggregation domain, "phosphorylation domain"). The term "aggregation domain" as used herein refers to a five amino acid region located at amino acids 16 to 20 (KLVFF (SEQ ID NO:18)) which mediates the binding of Aβ peptides in an anti-parallel manner.

[0055] As used herein, the term "immunoreactive domain" refers to a region of a protein that comprises a particular amino acid sequence that can be recognized by an antibody. This region includes amino acid sequences that contain modifications such as glycosylation, methylation, phosphorylation or any other post-translational modification known to one of ordinary skill in the art. Examples of amino acids that could be phosphorylated are tyrosine, serine, or threonine amino acids. An "immunoreactive domain" that includes amino acids that are phosphorylated would also be characterized as a phosphorylation domain. An "immunoreactive" domain would also include two or more regions of a protein that are in close proximity to one another in the proteins native folded state, which together comprise an antibody binding site.

[0056] As used herein, the term "immunoassay" as used herein refers to a biochemical test that utilizes one or more antibodies to measure the presence or concentration of an analyte in a biological matrix. This assay can produce a measurable signal in response to a specific binding of an antibody to an immunoreactive domain of a specific protein or peptide.

Reference Standards

[0057] In one aspect, this invention relates to compositions that can be used as reference standards and calibrators in order to measure clinical biomarkers. In one embodiment, the reference standard comprises a peptide. The peptide may be a modified peptide. The modified peptide may comprise a linker, a deletion or substitution in a non-immunoreactive domain. In another embodiment, the non-immunoreactive domain is an aggregation domain or phosphorylation domain.

Alterations in the Aggregation or Non-immunoreactive Domain

[0058] In one aspect, the present invention provides modified peptides which can be used as reference standards. There are known domains or amino-acid sequences which lead to the self-aggregation and non-specific interactions of sticky peptides like $A\beta_{42}$ with itself and other molecules. As such, it is possible to construct standards or calibrators which lack these deleterious domains. The present invention provides several ways in which to modify the peptides in order to remove the deleterious domains. In one embodiment, the amino acids comprising the deleterious domains are deleted from the

amino acid sequence and the N-terminal immunoreactive domain is connected to the C-terminal immunoreactive domain, lacking the central 17-20 amino acid sequence as well as various lengths of adjacent C-terminal peptide in the case of $A\beta_{42}$. Examples of $A\beta_{42}$ peptides with the central domains deleted are shown in Table 2, SEQ ID NOs:2, 3, and 4. In another embodiment, the central aggregation domain plus the adjacent amino acids are replaced with a linker or spacer consisting of many different types of matter.

[0059] In another embodiment, amino acids that do not aggregate are contemplated as the linker. In one embodiment, the amino acids that do not aggregate are in the form of a hydrophilic spacer or linker of the amino acid sequence or form EERP, shown with both the C-terminal 37-42 sequences of $A\beta_{42}$ (SEQ ID NO:5) and the C-terminal 32-42 portions of $A\beta_{42}$ (SEQ ID NO:6). In another embodiment, the $A\beta_{42}$ peptide includes a longer hydrophilic linker, for example the amino acid sequence DREPNR (SEQ ID NO:16), with both the C-terminal 37-42 (SEQ ID NO:7) and C-terminal 32-42 (SEQ ID NO:8) portions of $A\beta_{42}$.

[0060] In yet another embodiment, a series of charged residues are used in the form of the linker between the N-terminal and C-terminal immunoreactive domains. In another embodiment, a linker is created consisting of an integer number *m* Lysine residues (SEQ ID NO:9) or integer number *n* Glutamic acid residues (SEQ ID NO:10). In another embodiment, a string of neutral residues is used as a linker. In another embodiment, a construct consisting of an integer number *p* alanine residues (SEQ ID NO:11) is contemplated.

[0061] In another embodiment of the present invention, various forms of polyethylene glycol (PEG) are used as a linker. In a preferred embodiment, PEG-6atom and PEG-20atom are used with various C-terminal portions (SEQ ID NOs:12-14). In another embodiment, any polymer with chemistry able to couple to amino acid residues is used as a linker or spacer. This polymer includes those of a linear form as well as those of known branched topology, like dendrimers and branched co-polymers, to simulate the immunoreactivity of oligomers of $A\beta_{42}$ and other similar sticky or self aggregating molecules.

[0062] Phosphorylated regions of Tau may also be used to generate modified peptides that may be used as reference standards. The abnormally hyperphosphorylated tau is associated with neurofibrillary tangles. There are multiple phosphorylation sites of Tau;

each of them has different effect on its biological function. Measurement of phosphorylated Tau at Ser202/Thr181/Thr212/Thr231 or Ser262 may help to understand which one correlates with cognitive decline in MCI subject. Thus, in another embodiment, a modified tau peptide is contemplated as the reference standard. Examples of modified tau peptides are shown in Table 3.

[0063] In another embodiment, the linker comprises any one of the following molecules: lipids, globular proteins, nucleic acids (including but not limited to DNA, RNA and PNA), alkyl chains, or any other linkage that adds to the stability of the two immune epitopes of interest in the immunoassay. In another embodiment, the bond between the peptide backbone and linker comprises a covalent bond, avidin-biotin complex or any other stable bond. In another embodiment, the construct does not lead to self aggregation or non specific absorption to laboratory plastics, in particular polypropylene, polystyrene, polycarbon and other laboratory plastic resins of which pipette tips, tubes, plates and other vessels which hold fluids in which the analyte of interest can be measured.

[0064] In another embodiment, the novel $A\beta_{42}$ or tau immunoassay standards or calibrators require the presence of the N-terminal epitope that is recognized by the N-terminal specific antibody. There are many $A\beta_{42}$ N-terminal binding antibodies that are known in the art. For example, 6E10 is known to recognize the $A\beta_{3-8}$ epitope whereas 3D6 is known to recognize the N-terminal epitope of Aβ. An overlapping epitope may be designed to allow a selection of several N-terminal specific antibodies to be used, depending on the immunoassay requirements and detection system.

[0065] In another embodiment, the novel $A\beta_{42}$ immunoassay standards or calibrators require the presence of the C-terminal epitope that is recognized by the C-terminal $A\beta_{42}$ specific antibody. Examples of well characterized C-terminal $A\beta_{42}$ neo-epitope antibodies include G2-11 (from Heidelberg University), 21F12 (from Athena Diagnostics), 4D7A3 (from Innogenetics), and 12F4 (from Covance, formerly Signet). An overlapping c-terminal epitope may be designed to allow a selection of several C-terminal $A\beta_{42}$ specific antibodies to be used, depending on the immunoassay requirements and detection system.

[0066] In another embodiment, any N-terminal binding, C-terminal binding or phosphorylated tau binding antibody known to one of ordinary skill in the art may be used herein.

[0067] The invention also relates to methods for generating a peptide or protein construct and compositions thereof that can be used as a reference standard or calibrator in an immunoassay to measure the abundance of a peptide accurately in a fluid or tissue extract sample. An immunoassay often requires biologically specific capture reagents, such as antibodies, to capture the analytes or biomarkers of interest. Antibodies can be produced by methods well known in the art, *i.e.*, by immunizing animals with the biomarkers as antigens. Biomarkers can be isolated from samples based on their binding characteristics. Alternatively, if the amino acid sequence of a polypeptide biomarker is known, the polypeptide can be synthesized and used to generate antibodies by methods well known in the art. Examples of biomarkers include Aβ peptides and tau.

[0068] This invention contemplates traditional immunoassays including, for example, sandwich immunoassays including ELISA or fluorescence-based immunoassays, as well as other enzyme immunoassays. In the SELDI-based immunoassay, a biospecific capture reagent for the biomarker is attached to the surface of an mass spectrometry (MS) probe, such as a pre-activated PROTEINCHIP® array. The biomarker is then specifically captured on the biochip through this reagent, and the captured biomarker is detected by mass spectrometry.

[0069] Thus, in one aspect, the invention relates to methods of measuring the clinical markers with the reference standards of the invention. In one embodiment, the reference standard is measured by immunoassay. In another embodiment, the immunoassay is a sandwich immunoassay. In another embodiment, the immunoassay is a single antibody immunoassay, often run in a competitive or "competition" mode for immunoreactive binding sites. In yet another embodiment, the immunoassay is a double sandwich immunoassay or enzyme linked immunosorbant assay (ELISA).

[0070] In a preferred embodiment, the method of measuring the clinical markers is by using an immunoassay comprising the steps of attaching a reference standard to at least two beads thereby forming a first bead set and a second bead set, wherein the reference standard comprises an epitope recognized by a first detection antibody and wherein each bead set comprises a different concentration of the reference standard; attaching a capture

antibody specific to the analyte to a third bead set; mixing all of the bead sets together to form a suspension array; applying the biological sample to the suspension array whereby the analyte binds to the capture antibody on the third bead set; adding a first detection antibody to the suspension array, wherein the first detection antibody binds the reference standard and analyte bound to the capture antibody; measuring a first signal from the first detection antibody bound to the reference standard in the first bead set; measuring a second signal from the first detection antibody bound to the reference standard in the second bead set; generating a standard curve based upon the first and second signals; and quantitating the amount of the analyte in the third bead set by measuring a third signal from the first detection antibody and comparing the third signal to the first and second signal measurements on the standard curve. It is understood without limitation to the present invention that a bead set could be replaced by any other solid phase in which multiplexed information can be measured independently in a particular detection technology or instrument.

[0071] In one embodiment, the reference standard comprises a composition described herein. In another embodiment, the biological sample is selected from the group consisting of blood, serum, plasma, peripheral blood mononuclear cells, peripheral blood lymphocytes tissue, cerebrospinal fluid and cells. In yet another embodiment, the analyte is $A\beta_{42}$, $A\beta_{40}$, $A\beta_{38}$, tau or insulin growth factor receptor 1.

[0072] The analyte and/or reference standard may be bound to a variety of surface. A surface could be any solid phase surface to which an antibody or reference standard can be immobilized by covalent linkage, passive absorbance, biotin-strepavidin or any other linkage known to one of ordinary skill in the art. For example, the surface may be a bead, plate, slides, fiber, surface plamon resonance sensors or any solid surface.

[0073] In another embodiment, the method is performed in a multi-well plate, nitrocellulose filter or on a glass slide. In another embodiment, the first and second signals are detected by fluorescence. For example, the first signal and second signal may be a signal selected from the group consisting of phycoerythrin, alexa 532, streptavidin-phycoerythrin and streptavidin-Alexa 532. In another embodiment, the signal is detected by enzymatic activity (*i.e.*, horseradish peroxidase or alkaline phosphatase), chemiluminescence, radioactivity, infra-red emission, fluorescence resonance energy transfer (FRET) or any other method known to one of ordinary skill in the art.

[0074] In another aspect, the invention comprises a kit for conducting an immunoassay to detect an $A\beta_{42}$ or tau peptide, the kit comprising a reference standard of the invention.

Performance Comparison of Novel Immunoassay Standard or Calibrator to Native $A\beta_{42}$

[0075] The performance of novel immunoassay standards or calibrators should have comparable performance to the native $A\beta_{42}$ in an immunoassay. Native full length $A\beta_{42}$ peptides may be synthesized using standard solid phase techniques or they may be purchased commercially from a number of vendors as a catalog item (Anaspec Inc., American Peptide Company, or Invitrogen Inc.). Standard methods can be used to verify the abundance of the full length construction from truncated species using mass spectrometry techniques such as amino acid analysis that are well known in the field (Kanu et al., *Journal of Mass Spectrometry*, 43:1-22 (2008); Bernstein et al., *Journal of American Chemical Society*, 127:2075-2084 (2005); Li et al., *Encyclopedia of Analytical Chemistry*, Meyers, R.A., ed., John Wiley & Sons Ltd. (2009)).

[0076] By way of example, Table 1 lists the desirable physical properties of $A\beta_{42}$ peptides and the various methods used to test these properties. These methods can be employed to determine if the properties of the reference standard are comparable to those of the native $A\beta_{42}$ peptide.

TABLE 1

Property	Test	Target Range	Reference
Solubility	SDS-PAGE	Greater than 90% monomeric form	*Analy. Biochem.*, 316:223-231 (2003)
	Dynamic light scattering	Less than 5% aggregated peptides	*Meth. Enzymology*, 309:429-459 (1999); *J. Biol. Chem.*, 274:25945-25952 (1999)

Property	Test	Target Range	Reference
Non-specific adsorption	Spike recovery into CSF or buffer $A\beta_{42}$ immunoassay	Recovery between 80 and 120%	Aβ Immunoassay Olsson et al., *Clinical Chemistry*, 51:336-345 (2005)
Aggregation	SDS-PAGE Western blotting	Greater than 90% monomeric form	*PNAS*, 100:330-335 (2003); *Analy. Biochem.*, 316:223-231 (2003)
	Thioflavin T assay	Greater than 90% monomeric form	*Meth. Enzymology*, 309:274-284 (1999)
Fibril formation	Microscopy (transmission electron microscopy, optical, Microscopy (optical, atomic force)	Minimal levels of observable oligomers or fibrils	*Protein Pep. Letters*, 13:261-270 (2006); *J. Am. Chem. Soc.*, 125:15359-15365 (2003)
Stability at 25 °C, -20 °C, and -80 °C	Less than 20% CV loss of signal compared to *freshly* prepared $A\beta_{42}$ Via $A\beta_{42}$ immunoassay	2 hrs 25 °C 3 months -20 °C, 6 months -80 °C	Olsson et al., *Clinical Chemistry*, 51:336-345 (2005); Verwey et al., *Journal of Immunological Methods*, 348:57-66 (2009)

Use of Modified Reference Standards or Calibrators in Antibody Based Immunoassays

[0077] In another aspect of the present invention, the modified reference standard is used in a single antibody based assay. In one embodiment, immunoassays containing a single antibody can be used to measure $A\beta_{42}$ or tau in a biological sample by a competition immunoassay. A single antibody specific to $A\beta_{42}$ or tau is immobilized to a solid surface such as the well of a microtiter plate, a bead, or other immunoassay relevant

surface. The antibody may be covalently linked via many different methods such as EDC mediated linkage of carboxyl and amine groups, or via passive absorbance or through a Protein A or Protein G interface. An $A\beta_{42}$ or tau competitor is then generated from an $A\beta_{42}$ or tau standard or calibrator containing the full length $A\beta_{42}$ or tau peptide or a modified version that retains the epitope of the capture antibody. The $A\beta_{42}$ or tau competitor is used to generate competition between the native $A\beta_{42}$ or tau in the biological sample and the binding site (paratope) on the immobilized antibody. A paratope is a term used to describe the binding region on the antibody that recognizes the epitope or immunoreactive domain on the analyte. The $A\beta_{42}$ or tau competitor is tagged for detection purposes. In one embodiment, the tag is an enzyme such as horseradish peroxidase or alkaline phosphatase. In another embodiment, the tag is a fluorescein such as phycoerythrin. In yet another embodiment, the tag is another tag such as biotin or ruthenium. In yet another embodiment, the tag is a nucleic acid such as DNA, RNA or PNA, where by detection of the antibody is quantitated using sensitive technology to detect the nucleic acid flag, such as Polymerase Chain Reaction (PCR). A single concentration of the $A\beta_{42}$ or tau competitor is used in the assay and would be determined based on the ability to compete with the natural levels of $A\beta_{42}$ or tau found in biological samples.

[0078] In another embodiment, the assay is made quantitative by establishing a calibration curve. In another embodiment, quantitation is performed by making a set of $A\beta_{42}$ or tau standards or calibrators that are either the full length $A\beta_{42}$ or tau peptide or a modified version that retains the epitope of the capture antibody. These untagged standards or calibrators are prepared in either buffer or a biological matrix that does not contain $A\beta_{42}$ or tau. In one embodiment, the calibration curve is established by mixing one of concentrations of the untagged standards or calibrators with the tagged $A\beta_{42}$ or tau competitor to the immobilized antibody. The resulting signal value from each tested concentration of untagged standard or calibrator is used to generate a standard curve; plotting the concentration of the untagged $A\beta_{42}$ or tau standards or calibrators versus the resulting signal values. Once a standard quantitative curve is established, an assay is used to determine the levels of native $A\beta_{42}$ or tau in biological samples by mixing the tagged $A\beta_{42}$ or tau competitor at the same fixed concentration with the biological sample. The

resulting signal value is plotted on the standard curve to determine the level of $A\beta_{42}$ or tau in the biological sample.

[0079] In another aspect of the present invention, the modified reference standard is used in a sandwich based immunoassay.

Use of Modified Standards or Calibrators in Intra-assay Reference Standard Based Immunoassays

[0080] In another aspect of the present invention, $A\beta_{42}$ or tau peptides are incorporated into an intra-assay calibration system. In this approach, a multiplex immunoassay format such as the Luminex bead based system or the Meso-Scale Discovery ECL plate based system could be used. Peptides containing amino acid residues that encompass the antibody binding epitope of the detection antibody are generated. In one embodiment, these peptides include modifications that enable them to be covalently coupled to a solid phase or modifications that increase their solubility and use in aqueous immunoassays. These peptides are immobilized at different concentrations to the relevant solid phase as defined by multiplexed immunoassay systems, in order to create a set of well defined standards from which to create a standard curve.

[0081] The measurement of soluble biomarkers in clinical samples is often done using double antibody sandwich assays. These assays require two antibodies that are specific to the biomarker and a technology in which to detect the captured biomarker using the second "reporter" or "detection" antibody. Protein reference standards are required in order to make the assay quantitative. These standards are often in the form of recombinant proteins; however, they may also be obtained from biological samples. Traditional assay formats for these assays include ELISA techniques that provide quantitation suitable for the analysis of clinical samples. However, they are often limited to one biomarker assay per well. Newer technologies have been developed that allow multiple biomarkers to be analyzed in a single well or reaction vessel. Some of the multiplexed technologies utilize antibodies spotted onto a solid surface such as glass slides or specialized microtiter plates. Another approach is via suspension arrays where the antibodies are bound to latex beads which are mixed together in solution to form the array.

[0082] In one embodiment, suspension array technology is used. In another embodiment, the suspension array technology is the Luminex *x*MAP technology. Luminex *x*MAP technology uses latex beads that contain a ratio of two fluorescent dyes. Different bead 'sets' are created by altering the ratio of these two dyes. The beads are mixed together to form a suspension array. The bead mixture is analyzed by an instrument that identifies each bead by the fluorescence ratio as it passes in front of a laser. These bead sets have different modifications on their surface that are used for the covalent attachment of molecules such as proteins, peptides, antibodies, etc. This allows assay to be performed on the surface of these beads. Assays are quantitated through the incorporation of a third fluorescent label such as phycoerythrin to a reporter antibody directed at the analytes of interest. A second laser in the instrument measures the fluorescence of this reporter label as the beads move through the instrument.

EXAMPLES

EXAMPLE 1 - SANDWICH BASED ASSAY THAT CAN BE USED TO MEASURE $A\beta_{42}$

[0083] A schematic of a two-sided or sandwich soluble standard is shown in Figure 1. Briefly, capture antibodies specific to the C-terminus of $A\beta_{42}$ were immobilized to a solid surface. A biological sample was added thereby allowing $A\beta_{42}$ to be captured by the immobilized antibody. A second tagged detection antibody was added that was specific to the N-terminus of the $A\beta_{42}$. The measured signal generated by the tagged detection antibody was used for quantitation.

[0084] An example of a sandwich assay that can be used to measure $A\beta_{42}$ is shown in Figure 2. Briefly, a biotin labeled anti-C-terminal $A\beta_{42}$ antibody (565) was immobilized to a 96 well Meso-Scale Discovery streptavidin coated plate (MesoScale Discovery Inc., Gaithersburg, Maryland ((MSD)). Reference standard $A\beta_{42}$ peptides (full length with native sequence) were added to different wells. $A\beta_{42}$ peptides were captured by the immobilized capture antibody. A second ruthenium tagged (Ru) antibody to the N-terminus of $A\beta_{42}$ (26D6) was added, completing the sandwich. The complex was detected using an MSD sector 6000 instrument using ECL. The raw fluorescence units (RFU) measured by the instrument were fit to a 4-parameter logistic model to create a

standard curve. In another example, several modified $A\beta_{42}$ peptides are used as a reference standard (Table 2, SEQ ID NOs:2, 4, 6, 12, 13, and 14). The standard curves generated by these modified calibrators are shown in Figure 3.

TABLE 2: $A\beta_{42}$ Peptide and Modified Peptide Sequences and Descriptions

Peptide/Modified Peptide Sequence	Description of Peptide/Modified Peptide Sequence
DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAII GLMVGGVVIA (SEQ ID NO:1)	native $A\beta_{42}$ sequence
DAEFRHDSGYEVHMVGGVVIA (SEQ ID NO:2)	native $A\beta_{42}$ amino acids (1-14)(35-42) with no spacer in between
DAEFRHDSGYEVHHQKGGVVIA (SEQ ID NO:3)	native $A\beta_{42}$ amino acids (1-16)(37-42) with no spacer in between
DAEFRHDSGYEVHHQKIGLMVGGVVIA (SEQ ID NO:4)	native $A\beta_{42}$ amino acids (1-16)(32-42) with no spacer in between
DAEFRHDSGYEVHHQKEERPGGVVIA (SEQ ID NO:5)	native $A\beta_{42}$ amino acids (1-16)-EERP-native $A\beta_{42}$ amino acids (37-42)
DAEFRHDSGYEVHHQKEERPIGLMVGGVVIA (SEQ ID NO:6)	native $A\beta_{42}$ amino acids (1-16)-EERP-native $A\beta_{42}$ amino acids (32-42)
DAEFRHDSGYEVHHQKDREERPGGVVIA (SEQ ID NO:7)	native $A\beta_{42}$ amino acids (1-16)-DREERP hydrophilic linker-native $A\beta_{42}$ amino acids (37-42)
DAEFRHDSGYEVHHQKDREPNRIGLMVGGVV IA (SEQ ID NO:8)	native $A\beta_{42}$ amino acids (1-16)-DREPNR hydrophilic linker - native $A\beta_{42}$ amino acids (32-42)
(1-16)-(Lys)*m*-(37-42) (SEQ ID NO:9)	native $A\beta_{42}$ amino acids (1-16)- up to 20 Lysine residues- native $A\beta_{42}$ amino acids (37-42)

Peptide/Modified Peptide Sequence	Description of Peptide/Modified Peptide Sequence
(1-16)-(Glu)*n*-(37-42) (SEQ ID NO:10)	native $A\beta_{42}$ amino acids (1-16)- up to 20 Glutamic acid residues- native $A\beta_{42}$ amino acids (37-42)
(1-16)-(Ala)*p*-(37-42) (SEQ ID NO:11)	native $A\beta_{42}$ amino acids (1-16)- up to 20 Alanine residues- native $A\beta_{42}$ amino acids (37-42)
DAEFRHDSGYEVHHQK-PEG(20-ATOMS)3-GGVVIA (SEQ ID NO:12)	native $A\beta_{42}$ amino acids (1-16)-(PEG_20)3 linker- native $A\beta_{42}$ amino acids (37-42)
DAEFRHDSGYEVHHQK-PEG(9-ATOMS)6-MVGGVVIA (SEQ ID NO:13)	native $A\beta_{42}$ amino acids (1-16)-(PEG_9)6 linker- native $A\beta_{42}$ amino acids (35-42)
DAEFRHDSGYEVHHQK-PEG(9-ATOMS)5-IGLMVGGVVIA (SEQ ID NO:14)	native $A\beta_{42}$ amino acids (1-16)-PEG_9)5 linker- native $A\beta_{42}$ amino acids (32-42)
(X-Y) -Linker-(Z-42)	Generic: X,Y,Z, Linker to be specified
DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV (SEQ ID NO:15)	$A\beta_{40}$ native sequence

[0085] An immunoassay can also be used to measure tau. Examples of modified tau peptide sequences that can be used as reference standards of the instant invention are depicted below in Table 3.

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TABLE 3: Tau Peptide Sequences and Constructs

Peptide/Modified Peptide Sequence	Description of Peptide/Modified Peptide Sequence
MAEPRQEFEVMEDHAGTYGLGDRKDQGGYTMHQDQ EGDTDAGLKESPLQTPTEDGSEEPGSETSDAKSTPTAE DVTAPLVDEGAPGKQAAAQPHTEIPEGTTAEEAGIGDT PSLEDEAAGHVTQARMVSKSKDGTGSDDKKAKGADG KTKIATPRGAAPPGQKGQANATRIPAKTPPAPKTPPSSG EPPKSGDRSGYSSPGSPGTPGSRSRTPSLPTPPTREPKKV AVVRTPPKSPSSAKSRLQTAPVPMPDLKNVKSKIGSTE NLKHQPGGGKVQIINKKLDLSNVQSKCGSKDNIKHVP GGGSVQIVYKPVDLSKVTSKCGSLGNIHHKPGGGQVE VKSEKLDFKDRVQSKIGSLDNITHVPGGGNKKIETHKL TFRENAKAKTDHGAEIVYKSPVVSGDTSPRHLSNVSST GSIDMVDSPQLATLADEVSASLAKQGL (SEQ ID NO:20)	native tau sequence
EVMEDHAGTYGL-generic linker-GAAPPGQKGQAN (SEQ ID NO:21)	native tau amino acids (9-20)-generic linker- native tau amino acids (156 -167)
EVMEDHAGTYGL-generic linker-SGDRSGYSSP (SEQ ID NO:22)	native tau amino acids (9-20)-generic linker- native tau amino acids- (191-200)
GAAPPGQKGQAN-generic linker- IPAKTPPAPKT$_{(PO4)}$PPSSGEPPK (SEQ ID NO:23)	native tau amino acids (156 - 167)-generic linker- native tau amino acids (171-190); amino acid T^{180} is phosphorylated

Peptide/Modified Peptide Sequence	Description of Peptide/Modified Peptide Sequence
GAAPPGQKGQAN-generic linker-REPKKVAVVRT$_{(PO4)}$PPKSPSSAK (SEQ ID NO:24)	native tau amino acids (156-167)-generic linker-native tau amino acids (221-240); amino acid T^{231} is phosphorylated
GAAPPGQKGQAN-generic linker-SGDRSGYSSP (SEQ ID NO:25)	native tau amino acids (156-167)-generic linker-native tau amino acids (191-200)
IPAKTPPAPKT$_{(PO4)}$PPSSGEPPK-generic linker-SGDRSGYSSP (SEQ ID NO:26)	native tau amino acids (171-190)-generic linker-native tau amino acids (191-200); amino acid T^{180} is phosphorylated
SGDRSGYSSP-generic linker-REPKKVAVVRT$_{(PO4)}$PPKSPSSAK (SEQ ID NO:27)	native tau amino acids (191-200)-generic linker-native tau amino acids (221-240); amino acid T^{231} is phosphorylated

EXAMPLE 2 - SANDWICH $A\beta_{42}$ ASSAY MEASURING $A\beta_{42}$ IN BIOLOGICAL SAMPLES

[0086] An example of a sandwich $A\beta_{42}$ assay measuring $A\beta_{42}$ in biological samples is shown in Table 3. Biotin labeled anti-C-terminal $A\beta_{42}$ antibody (565) was immobilized to a 96 well MSD streptavidin coated plate. Reference standard $A\beta_{42}$ peptides (full length with native sequence) or a modified $A\beta_{42}$ reference standard (SEQ ID NO:2) were added to different wells. Human CSF samples at different dilutions were placed in different wells. The plate was incubated 2 hours at room temperature to allow $A\beta_{42}$ peptides to be captured by the immobilized capture antibody. A second ruthenium tagged (Ru) antibody

to the N-terminus of $A\beta_{42}$ (26D6) was added, completing the sandwich. The complex was detected using an MSD sector 6000 instrument using ECL. The raw fluorescence units (RFU) measured by the instrument were fit to a 4-parameter logistic model to create a standard curve. The measured concentrations of $A\beta_{42}$ in human CSF samples are shown in Table 3.

TABLE 4: Measured Concentration of $A\beta_{42}$ Peptide in Three Human Cerebrospinal (CSF) Samples

		$A\beta_{42}$ Concentration (pg/ml)	
CSF sample	CSF dilution	Full length Aβ 1-42 Peptide	Modified Aβ xx-42 peptide
CSF-1	1:2 dil	Below limit of detection	Below limit of detection
CSF-1	1:4 dil	Below limit of detection	Below limit of detection
CSF-2	1:2 dil	26.2	27.8
CSF-2	1:4 dil	54.0	56.8
CSF-3	1:2 dil	88.4	80.4
CSF-3	1:4 dil	134.4	126.4

EXAMPLE 3 - Aβ PEPTIDE BASED INTRA-ASSAY LUMINEX ASSAY

[0087] Figure 4 shows a schematic of the $A\beta_{42}$ intra-assay bead approach. Bead sets coupled with different concentrations of $A\beta_{42}$ standard peptides (or other native or modified Aβ peptides) are combined with a bead set coupled with an anti-$A\beta_{42}$-C-terminal specific capture antibody to form a suspension array. The array is incubated with a biological sample, where the $A\beta_{42}$ peptide in the biological sample is captured by the bead coupled with anti-$A\beta_{42}$ capture antibody. A tagged detection antibody specific to the N-terminus of the $A\beta_{42}$ peptide is added to the suspension array, thereby binding to the captured $A\beta_{42}$ peptide and also to the beads that have $A\beta_{42}$ peptides coupled to their surface. The MFI values obtained from the beads with $A\beta_{42}$ peptides coupled to their surface is used to generate an intra-assay calibration curve. The amount of $A\beta_{42}$ in the biological sample is determined from the amount of captured $A\beta_{42}$ on the bead coupled with the anti-$A\beta_{42}$ capture using the intra-assay standard curve.

Antibodies and Reference Standards

[0088] The native full length $A\beta_{42}$ and full length $A\beta_{40}$ peptides (SEQ ID NOs:1 and 15, respectively) were obtained from American Peptide Company. The 116B565.1 mouse anti-human $A\beta_{42}$ C-terminus antibody and the 26D6-B2-B3 mouse anti-human $A\beta_{42}$ N-terminus antibody were obtained via protein-G purification of culture supernatants produced by the relevant BMS owned hybridoma cell lines.

[0089] Phycoerythrin-streptavidin conjugate was obtained from Jackson Immunoresearch (West Grove, PA). Tween-20, 1-ethyl-3-[3 dimethylaminopropyl] carbodiimide hydrochloride (EDC), sodium azide, IgG free Bovine Serum Albumin (BSA), and sodium phosphate were purchased from Sigma-Aldrich Corporation (St. Louis, MO). Phosphate buffered saline (PBS) was obtained from Mediatech Incorporated (Herndon, VA). Carboxylated Luminex beads were purchased from Bio-Rad Incorporated (Hercules, CA). Phycoerythrin label goat anti-mouse IgG antibody was obtained from Jackson Immunoresearch (West Grove, PA).

Covalent Coupling of Anti-$A\beta_{42}$ Capture Antibody to Luminex Beads

[0090] The 116B565.1 mouse anti-human $A\beta_{42}$ C-terminus antibody was covalently coupled to the surface of carboxylated beads using a two-step carbodimide procedure. The beads were washed by centrifuging 1.25 x 10^7 beads for 5 min at 14000 x *g* 4 °C in an Eppendorf 5415D centrifuge (Westbury, NY). The supernatant was carefully removed and another 1.25 x 10^7 beads were dispensed and centrifuge for 5 min at 14000 x *g* 4 °C. The supernatant was again carefully removed and resuspended in 800 µl of 0.1M sodium phosphate buffer pH 4.8 (activation buffer). The beads were then vortexed for 15 seconds and sonicated for 15 seconds using a SPER SCIENTIFIC® LTD Ultrasonic Cleaner (Scottsdale, AZ). The beads were washed 2 additional times with activation buffer, and resuspended in 200 µl of freshly prepared 5 mg/ml EDC prepared in activation buffer. The beads were incubated in a rotator for 20 min RT protected from light. At the end of the EDC step, the beads were washed and resuspended in 1000 µl of 250 µg/ml capture antibody prepared in PBS and incubated for 1hrs RT in a rotator protected from light. The beads were washed and incubated with 1 ml of blocking buffer (PBS, 1% (w/v) BSA, 0.02% (w/v) Tween-20) in a rotator for 1 hr RT protected from light. Finally, the beads

were counted with a hemacytometer, resuspended in blocking buffer at 2 x 10^6 beads/ml, and stored protected from light at 4 °C until ready for use.

Surface Testing of Covalent Coupling Efficiency of $A\beta_{42}$ Capture Antibodies to Luminex Beads

[0091] The presence of capture antibody on the bead surface was confirmed using surface testing. 50 µl of assay buffer (PBS, 1% (w/v) BSA, 0.05% (w/v) Tween 20, 0.05% (w/v) azide) containing 2500 beads were added to Millipore filter bottom plate wells (Bedford, MA). The beads were washed by placing the plate over a Millipore vacuum manifold (Bedford, MA) to remove the liquid and then resuspended in 100 µl/well of PBST wash buffer. Finally, the wash buffer was removed from the wells via vacuum and the beads were incubated with 100 µl/well of PE-Goat anti-mouse IgG diluted 1/100 in assay buffer. The plate was incubated on a 96-well plate shaker (Lab Line Instruments, Melrose Park, IL) at 300 rpm for 30 min RT protected from light. The beads were subsequently washed 3 times via vacuum filtration using 100 µl/well of wash buffer and resuspended in 100 µl/well of assay buffer. The MFI of at least 50 beads/well was measured using a Luminex^{100} instrument obtained from Bio-Rad Laboratories (Hercules, CA) running Bioplex manager 4.1.1 software. An MFI of at least 20,000 was used to confirm the presence of usable quantities of antibody on the bead surface.

Covalent Coupling of Intra-assay $A\beta_{40}$ Peptides to Luminex Beads

[0092] $A\beta_{40}$ native full length peptides (SEQ ID NO:15) were prepared by reconstituting the lyophilized peptide in 2.5 ml PBS to give a final concentration of 10mg/ml. $A\beta_{40}$ peptides were selected for this assay in lieu of $A\beta_{42}$ peptides because they still expressed the N-terminal $A\beta_{42}$ epitopes needed for the binding of 26D6 antibodies and the $A\beta_{40}$ peptides were more stable in aqueous buffers needed for conjugation. $A\beta_{40}$ peptides were diluted to different concentrations (shown in Figure 5A) using diluent buffer (PBS, 1% (w/v) BSA, 0.02% (w/v) Tween-20). Each preparation of $A\beta_{40}$ peptide was covalently coupled to the surface of a selected bead set (different bead set for each concentration) using a two-step carbodimide procedure. Each bead set was washed by centrifuging 1.25 x 10^7 beads for 5 min at 14000 x *g* 4 °C in an Eppendorf 5415D centrifuge (Westbury, NY). The supernatant was carefully removed and 800 µl of 0.1M

sodium phosphate buffer pH 4.8 (activation buffer) was added. The beads were then vortexed for 15 seconds and sonicated for 15 seconds using a SPER SCIENTIFIC® LTD Ultrasonic Cleaner (Scottsdale, AZ). The beads were washed an additional time with activation buffer, and resuspended in 200 µl of freshly prepared 5 mg/ml EDC prepared in activation buffer. The beads were incubated in a rotator for 20 min RT protected from light. At the end of the EDC step, each bead set was washed and resuspended with the predetermined concentrations of $A\beta_{40}$ peptides and incubated for 1hr RT in a rotator protected from light. The bead sets were washed and incubated with 1 ml of blocking buffer (PBS, 1% (w/v) BSA, 0.02% (w/v) Tween-20) in a rotator for 1 hr RT protected from light. Finally, the concentration of each bead set preparation was assessed by counting the beads with a hemacytometer. Each bead set was then resuspended in blocking buffer at 2 x 10^6 beads/ml, and stored protected from light at 4 °C until ready for use.

Testing of $A\beta_{40}$ Peptide Coupled Luminex Beads

[0093] The presence of $A\beta_{40}$ peptides which contain the N-terminal epitopes for the $A\beta_{42}$ N-terminal specific antibodies on the bead surface was confirmed using surface testing. 50 µl of assay buffer (PBS, 1% (w/v) BSA, 0.05% (w/v) Tween 20, 0.05% (w/v) azide) containing 2500 beads were added to Millipore filter bottom plate wells (Bedford, MA). The beads were washed by placing the plate over a Millipore vacuum manifold (Bedford, MA) to remove the liquid and the beads were resuspended in 100 µl/well of PBST wash buffer. Finally, the wash buffer was removed from the wells via vacuum and the beads were incubated with 100 µl/well of biotin labeled 26D6-B2-B3 antibody diluted in assay buffer. The plate was incubated on a 96-well plate shaker (Lab Line Instruments, Melrose Park, IL) at 300 rpm for 30 min RT protected from light. Following the incubation step, the beads were washed 4 times, resuspended in 50 µl/well of 1µg/ml of Phycoerthrin-streptavidin conjugate, and incubated on a plate shaker for 20 min at RT protected from light. The beads were subsequently washed 3 times via vacuum filtration using 100 µl/well of wash buffer and resuspended in 100 µl/well of assay buffer. The MFI of at least 50 beads/well was measured using a Luminex^{100} instrument obtained from Bio-Rad Laboratories (Hercules, CA) running Bioplex manager 4.1.1 software. The MFI measured on each of the bead sets is shown in Figure 5A.

$A\beta_{42}$ Intra-Assay Analysis of Biological Samples

[0094] Samples analysis using the intra-assay Luminex based assay was performed by first mixing bead sets that were coupled with anti-C-terminal specific $A\beta_{42}$ 565 capture antibodies and bead sets that were coupled with different concentrations of $A\beta_{40}$ peptides (shown in Figure 5A). 50 µl/well of the combined mixture of all bead sets at 50,000 beads/ml suspension prepared in assay buffer was added to each well of a pre-wet filter bottom 96-well plate. The beads were washed with 100 µl/well of assay buffer via vacuum filtration. The capture beads were resuspended in 50 µl of diluted human CSF samples, quality control samples (QC), different concentrations of full length native $A\beta_{42}$ peptides as reference standards, or different concentrations of modified $A\beta_{42}$ peptide standards in duplicate wells and incubated on a plate shaker for 1 hr at RT protected from light. 1.0 µg/ml anti-$A\beta_{42}$ 26D6 reporter antibody labeled with biotin was added and then incubated on a plate shaker for 0.5 hrs at RT protected from light. Following the incubation step, the beads were washed 4 times, resuspended in 50 µl/well of 1µg/ml of Phycoerthrin-streptavidin conjugate, and incubated on a plate shaker for 20 min at RT protected from light. Finally, the beads were washed 4 times and resuspended in 100 µl/well of assay buffer. The MFI of at least 50 beads per well was measured using a Bioplex Luminex instrument running Bioplex manager 5.1 software (Bio-Rad Laboratories, Hercules, CA). Standard curves were generated from the soluble $A\beta_{42}$ peptides or from the signals generated from the bead sets coated with different concentrations of $A\beta_{40}$ peptides (intra-standard) using a weighted 4-parameter logistic curve fit (Figure 5B). The concentration of $A\beta_{42}$ peptides in the CSF or QC samples were calculated from the relevant standard curve, and are shown in Figure 5C.

EXAMPLE 4 - PEPTIDE BASED INTRA-ASSAY FOR IGFR1 WITH LUMINEX BEADS

[0095] Below is another example of a peptide intra-assay based assay for the detection of phosphorylated tyrosine residues, 1162 and 1163, on human IGF-R1 receptors. Phosphorylated regions of Tau may also be used in the same manner.

Antibodies and Reference Standards

[0096] A custom made IGF1R [$PYPY^{1162/1163}$] peptide was provided by Cambridge Research Biochemicals Ltd (UK). Mouse anti-IGF1R capture antibody was obtained from Calbiochem (San Diego, CA) and the rabbit anti-phospho-tyrosine (1162/1163) - IGF1R reporter antibody was purchased from Millipore (Billerica, MA). Phycoerythrin label goat anti-rabbit antibody was obtained from Jackson Immunoresearch (West Grove, PA). Tween-20, 1-ethyl-3-[3 dimethylaminopropyl] carbodiimide hydrochloride (EDC), sodium azide, IgG free Bovine Serum Albumin (BSA), and sodium phosphate were purchased from Sigma-Aldrich Corporation (St. Louis, MO). Phosphate buffered saline (PBS) was obtained from Mediatech Incorporated (Herndon, VA). Carboxylated Luminex beads were purchased from Bio-Rad Incorporated (Hercules, CA). Normal healthy PBMC samples were obtained from In house donors (BMS, NJ). The PBMC samples were treated with either PBS or 100ng/ml of purified human IGF1 (Genetex Inc, TX) for 10 min at 37 °C to induce phosphorylation of IGFR present on the cells. The cells were washed, lysed with a modified RIPA buffer, and stored at -80 °C.

Covalent Coupling of Anti-IGF-R1 Capture Antibody to Luminex Beads

[0097] The mouse anti-human IGF1R capture antibody was covalently coupled to the surface of carboxylated beads using a two-step carbodimide procedure. The beads were washed by centrifuging 1.25 x 10^7 beads for 5 min at 14000 x *g* 4 °C in an Eppendorf 5415D centrifuge (Westbury, NY). The supernatant was carefully removed and another 1.25 x 10^7 beads were dispensed and centrifuged for 5 min at 14000 x *g* 4 °C. The supernatant was again carefully removed and re-suspended in 800 µl of 0.1M sodium phosphate buffer pH 4.8 (activation buffer). The beads were then vortexed for 15 seconds and sonicated for 15 seconds using a SPER SCIENTIFIC® LTD Ultrasonic Cleaner (Scottsdale, AZ). The beads were washed 2 additional times with activation buffer, and resuspended in 200 µl of freshly prepared 5 mg/ml EDC prepared in activation buffer. The beads were incubated in a rotator for 20 min RT protected from light. At the end of the EDC step, the beads were washed and resuspended in 1000 µl of 250 µg/ml capture antibody prepared in PBS and incubated for 1hr RT in a rotator protected from light. The beads were washed and incubated with 1 ml of blocking buffer (PBS, 1% (w/v) BSA, 0.02% (w/v) Tween-20) in a rotator for 1 hr RT protected from light. Finally, the beads

were counted with a hemacytometer, resuspended in blocking buffer at 2 x 10^6 beads/ml, and stored protected from light at 4 °C until ready for use.

Testing of Covalent Coupled IGF-R1 Capture Antibodies to Luminex Beads

[0098] The presence of capture antibody on the bead surface was confirmed using surface testing. 50 µl of assay buffer (PBS, 1% (w/v) BSA, 0.05% (w/v) Tween 20, 0.05% (w/v) azide) containing 2500 beads were added to Millipore filter bottom plate wells (Bedford, MA). The beads were washed by placing the plate over a Millipore vacuum manifold (Bedford, MA) to remove the liquid and the beads were resuspended in 100 µl/well of PBST wash buffer. Finally, the wash buffer was removed from the wells via vacuum and the beads were incubated with 100 µl/well of PE-GAM diluted 1/100 in assay buffer. The plate was incubated on a 96-well plate shaker (Lab Line Instruments, Melrose Park, IL) at 300 rpm for 30 min RT protected from light. The beads were subsequently washed 3 times via vacuum filtration using 100 µl/well of wash buffer and resuspended in 100 µl/well of assay buffer. The MFI of at least 50 beads/well was measured using a Luminex^{100} instrument obtained from Bio-Rad Laboratories (Hercules, CA) running Bioplex manager 4.1.1 software. An MFI of at least 20,000 was used to confirm the presence of usable quantities of antibody on the bead surface.

Covalent Coupling of Intra-assay IGF-R1 Peptide Standards to Luminex Beads

[0099] IGF1R [PYPY1162/1163] reference standard peptides were prepared by reconstituting the lyophilized peptide in 2.5 ml PBS to give a final concentration of 10mg/ml. Initial 10 fold dilution with subsequent 10-fold were made using diluent buffer (PBS, 1% (w/v) BSA, 0.02% (w/v) Tween-20). The phospho-IGF1R peptide was covalently coupled to the surface of four different sets of carboxylated beads at four different concentrations using a two-step carbodimide procedure. The bead sets were washed by centrifuging 1.25 x 10^7 beads for 5 min at 14000 x *g* 4 °C in an Eppendorf 5415D centrifuge (Westbury, NY). The supernatant was carefully removed and 800 µl of 0.1M sodium phosphate buffer pH 4.8 (activation buffer) was added. The beads were then vortexed for 15 seconds and sonicated for 15 seconds using a SPER SCIENTIFIC® LTD Ultrasonic Cleaner (Scottsdale, AZ). The beads were washed an additional time with activation buffer, and resuspended 200 µl of freshly prepared 5 mg/ml EDC prepared

in activation buffer. The beads were incubated in a rotator for 20 min RT protected from light. At the end of the EDC step, each beads were washed and resuspended with individual concentration of 1000, 100, 10 and 1 ng/ml of phospho-IGF1R peptide made from a 10-fold serial dilutions of 10mg/ml prepared in PBS. The beads were then incubated for 1hr RT in a rotator protected from light. The beads were washed and incubated with 1 ml of blocking buffer (PBS, 1% (w/v) BSA, 0.02% (w/v) Tween-20) in a rotator for 1 hr RT protected from light. Finally, the beads were counted with a hemacytometer, resuspended in blocking buffer at 2 x 10^6 beads/ml, and stored protected from light at 4 °C until ready for use.

Testing of IGF-R1 Peptide Coupled Luminex Beads

[00100] The presence of phospho-IGF1R peptide on the bead surface was confirmed using surface testing. 50 µl of assay buffer (PBS, 1% (w/v) BSA, 0.05% (w/v) Tween 20, 0.05% (w/v) azide) containing 2500 beads were added to Millipore filter bottom plate wells (Bedford, MA). The beads were washed by placing the plate over a Millipore vacuum manifold (Bedford, MA) to remove the liquid and the beads were resuspended in 100 µl/well of PBST wash buffer. Finally, the wash buffer was removed from the wells via vacuum and the beads were incubated with 100 µl/well of rabbit anti-phospho-IGF-R1 antibody diluted in assay buffer. The plate was incubated on a 96-well plate shaker (Lab Line Instruments, Melrose Park, IL) at 300 rpm for 30 min RT protected from light. Following the incubation step, the beads were washed 4 times, resuspended in 50 µl/well of 1µg/ml of phycoerthrin labeled goat anti-rabbit IgG conjugate, and incubated on a plate shaker for 20 min at RT protected from light. The beads were subsequently washed 3 times via vacuum filtration using 100 µl/well of wash buffer and resuspended in 100 µl/well of assay buffer. The MFI of at least 50 beads/well was measured using a Luminex^{100} instrument obtained from Bio-Rad Laboratories (Hercules, CA) running Bioplex manager 4.1.1 software. phospho-IGF1R peptide,

IGF-R1 Intra-assay Analysis of Biological Samples

[00101] Sample analysis using the Luminex based assay was performed by adding 50 µl of a 50,000 beads/ml of capture antibody suspension prepared in assay buffer to each well of a pre-wet filter bottom 96-well plate. The beads were washed with 100 µl/well of

assay buffer via vacuum filtration. The capture beads were resuspended in 50 µl of diluted samples, or quality control samples (QC) in duplicate wells and incubated on a plate shaker for 1 hr at RT protected from light. The beads were washed 4 times, resuspended in 50 µl of a 50,000 beads/ml of the four different bead sets of peptide mentioned in section 2.2.3. The beads were filtered, resuspended in 50 µl/well of 1.0 µg/ml anti-phospho IGF1R reporter antibody, and incubated on a plate shaker for 0.5 hrs at RT protected from light. Following the incubation step, the beads were washed 4 times, resuspended in 50 µl/well of 1µg/ml of PE-Goat anti rabbit IgG, and incubated on a plate shaker for 20 min at RT protected from light. Finally, the beads were washed 4 times and resuspended in 100 µl/well of assay buffer. The MFI of at least 50 beads per well was measured using a Bioplex Luminex instrument running Bioplex manager 4.1 software (Bio-Rad Laboratories, Hercules, CA). The standard curve generated from the phospho-IGF-R1 intra-standard curve is shown in Figure 6A. The phospho-IGF1R concentration in each of the PBMC lysate samples using the intra-assay 4-parameter logistic curve fit is shown in Figure 6B.

EXAMPLE 5 - CHARACTERIZATION OF Aβ PEPTIDES

Peptides

[00102] All peptides were received as lyophilized powder. Modified peptides were obtained from GenScript (GS) and Anaspec (AN). These peptides were synthesized using solid phase methods known to those skilled in the art (*See*, for example, Barany, G. et al., *The Peptides: Analysis, Synthesis, Biology - Special Methods in Peptide Synthesis, Part A*, Vol. 2, pp. 3-284, Gross, E. et al., eds., Academic Press, New York, publ. (1980); and in Stewart, J.M. et al., *Solid-Phase Peptide Synthesis*, 2nd Edition, Pierce Chemical Co., Rockford, IL, publ. (1984)).

[00103] GS1-6 and AN7 peptides were received and reconstituted in ddH20 to a concentration of 1mg/ml. These were then aliquoted out into 100 µl aliquots in the1.4 ml blank tubes PP, round Matrix (Thermo Scientific, cat#4249, lot 1030509). The full length $A\beta_{42}$ peptide was purchase from MSD (lot # T03080X1). This peptide was diluted in DMSO to make a solution of 0.1mg/ml. This was then aliquoted out in 100 µl aliquots in the 1.4 ml blank tubes. These peptides were kept frozen at -70 °C for storage. Figure 13

shows the structures of the polyethylene glycol spacers incorporated into the modified $A\beta_{(1-42)}$ peptides.

TABLE 5: List of Aβ Peptides

GS#	Peptide Sequence	% Purity (HPLC)	MW
GS 1	DAEFRHDSGYEVHHQK-{PEG(20-atoms}3-GGVVIA (SEQ ID NO:12)	95	3406.71
GS 2	DAEFRHDSGYEVHHQK-{PEG(9-atoms}6-MVGGVVIA (SEQ ID NO:13)	95	3553.02
GS 3	DAEFRHDSGYEVHHQK-PEG(9-ATOMS)5-IGLMVGGVVIA (SEQ ID NO:14)	95	3691.15
GS 4	DAEFRHDSGYEVHHQKEERPIGLMVGGVVIA (SEQ ID NO:6)	96	3476.91
GS 5	DAEFRHDSGYEVHHQKDREPNRIGLMVGGVVIA (SEQ ID NO:8)	97	3733.17
GS 6	DAEFRHDSGYEVHHQKIGLMVGGVVIA (SEQ ID NO:4)	98	2965.37
AN 7	DAEFRHDSGYEVHMVGGVVIA (SEQ ID NO:2)	90	2288.49
FL	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIADAEFRHDSGYEVHMVGGVVIA (SEQ ID NO:19)		

Dynamic Light Scattering

[00104] The novel peptides (GenScript) were received as dry powders, and two sets of stock samples were prepared by weighing small amounts (~0.5 mg each) in 1.8 ml polypropylene tubes and dissolving in a simple phosphate buffer (10 mM Na_2HPO_4, pH 7.4, 10 mM NaCl, prepared in 99.9% D_2O; filtered through 0.2 μm filter). One set was prepared at 1.0 mg/ml, the second set was prepared at 0.10 mg/ml, and stored at room

temperature. Peptides were dissolved by vortexing for 1 min, and centrifuged in a microcentrifuge for 5 min at 14,000 rpm, at room temperature. For $A\beta_{42}$ samples (MSD), vials containing 0.1 mg peptide were suspended in 1.0 ml buffer, and treated as above. A volume (200 µl) of each centrifuged 1.0 mg/ml stock was transferred to a 0.5 ml polypropylene tube, and provided for NMR analysis.

[00105] The absorbance spectra of all samples were recorded (220-750 nm) using a NANODROP® ND-1000 instrument, and blanked against the buffer. Dynamic light scattering analysis was conducted on the peptides in a 384 well polystyrene plate (CORNING®, type 3540) with a Wyatt DLS DynaPro plate reader. Data collection and analysis was completed using software from the manufacturer (DYNAMICS, versions 7.0.0.94 and 7.0.1.12). Each peptide was evaluated in triplicate with 30 µl loaded per well, and overlaid with 5 µl mineral oil, to minimize evaporation. Buffer blanks were also included in the analysis for comparison. After loading the plate, a transparent adhesive sealing tape cover was applied to the plate and the plate was centrifuged for 2 min at 1000 rpm. Each sample on the plate was read 30 times, 5 sec per acquisition, maintaining a temperature of 25 °C. Data sets were collected on both the 1.0 mg/ml and 0.10 mg/ml samples over the course of 27 days. The adhesive cover was removed to read the samples in the plate reader, and used to cover the plate between readings over the 27 day time course.

[00106] Figures 7A through 7F show dynamic light scattering data. In Figure 7, the full length $A\beta_{42}$ peptide was subjected to DLS analysis and compared to four representative modified peptides. The full length peptide was shown to be aggregated with results that calculated the radius of the different aggregate species to be between 82 and 231nm in length. This adds up to large molecular weight aggregates in the solution. However, the modified peptides remain monomeric as determined by both molecular weights as well as radius. GS#3 was contrasted with the other peptides because it did retain some aggregation (Figure 7E). These results demonstrate that the modified peptides which are lacking an aggregation domain, do in fact remain monomeric in solution. Figure 7a represents the raw data accumulated for all five peptides. Shown is the intensity autocorrelation versus time. The higher the intensity autocorrelation number is represents larger diameters of the peptides in solution. Figures 7b-7f depict the percent mass versus the radius of the peptides. Monomeric peptides would be represented by a

larger percent mass at the smaller radius size. The larger aggregated peptides have smaller percent masses at a higher radius size.

TABLE 6: Summary of DLS data

	Item	Radius (nm)	%Pd	Mw-R (kDa)	%Intensity	% Mass
GS#1	Peak1	1.2	4.6	5.0	6.3	99.9
	Peak 2	48.4	3.9	29512.0	93.7	0.1
GS#2	Peak 1	0.9	4.3	3.0	1.6	98.0
	Peak 2	4.6	1.9	117.0	3.4	1.8
	Peak 3	36.9	3.8	15580.0	76.1	0.1
	Peak 4	172.0	4.5	572266.0	18.9	0.1
GS#3	Peak 1	4.5	0.0	119.0	0.4	57.9
	Peak 2	15.7	4.5	2101.0	4.8	19.7
	Peak 3	61.6	4.5	51794.0	94.8	22.4
GS#4	Peak 1	1.3	3.2	6.0	3.8	97.7
	Peak 2	6.0	0.0	225.0	0.6	0.2
	Peak 3	27.0	4.5	7527.0	5.2	0.0
	Peak 4	127.0	4.3	283287.0	25.1	0.2
FL Aβ1-42	Peak 1	82.9	4.4	103812.0	50.4	26.1
	Peak 2	231.0	3.2	1149570.0	49.6	73.9

Circular Dichroism

[00107] Circular dichroism (CD) spectra were collected using an Aviv Model 202 Circular dichroism spectrometer. Samples were pipetted in 1 mm path length quartz cuvettes, and scanned from 260-185 nm. Parameters for collecting data include spectral bandwidth of 1.00 nm, a step size of 1.0 nm, an averaging time of 20 sec per point, and

temperature set at 25 °C. Samples were stored in the quartz cuvettes at room temperature, and scanned over a time course of 23 days. Raw data was corrected for blank buffer contributions, and presented in the format of mdeg versus wavelength.

[00108] Figure 8 shows the circular dichroism analysis. Circular dichroism analysis was performed on the full length $A\beta_{42}$ and four representative peptides to determine the order and secondary structures of these peptides. As shown in Figure 8, the full length peptide shows more of an ordered structure than the other peptides. The four representative peptides contain an unordered CD spectra when compared to the full length peptide. These data suggest that the modified peptides do not aggregate or form any higher order structures unlike that of the full length peptide.

MSD ELISA

[00109] Biotin labeled anti-C-terminal $A\beta_{42}$ antibody (565-20 µg/ml) was immobilized to a 96 well MSD streptavidin coated plate by adding 30 µl/well. The plate was then covered and incubated overnight at 25 °C, shaking at 500rpm. The plates were then washed 3x with 300 µl/well of wash buffer (R & D system cat# WA126). After washing, 225µl/well of blocking buffer (1%BSA + 0.1% Tween-20 in PBS) was added to the plate and incubated for 30 minutes at room temperature, shaking at 500 rpm. Once the blocking step was done, the plates were washed as described previously. Reference standard $A\beta_{42}$ peptides (full length or modified peptides) were added to the wells. $A\beta_{42}$ peptides were captured by the immobilized capture antibody. A second ruthenium tagged (Ru) antibody to the N-terminus of $A\beta_{42}$ (26D6) was added thereby completing the sandwich. The complex was detected using an MSD sector 6000 instrument using ECL. The raw fluorescence units (RFU) measured by the instrument were fit to a 4-parameter logistic model to create a standard curve.

[00110] Figure 9 shows peptide stability data. The full length $A\beta_{42}$ and seven modified peptides were subjected to stability studies at different temperatures for up to 40 days (Figures 9A-9F). These peptides were kept at 4 °C, 25 °C, and 37 °C for the specified length of time and then frozen until the assay was run. Peptides were then collected and run on an MSD Elisa format. Results are shown as a percent of the baseline signal for each peptide measured at time zero. Indeed, short term incubation at 37 °C has been used to estimate the long term stability of molecules at 4 °C and room temperature (Anderson

et al., *Clinical Chemistry*, 37(3): 398-402 (1991)). Therefore, incubation of the peptides at 37 °C for short term analysis can be used to assess the long term stability of the peptides. As shown in Figure 9, the full length $A\beta_{42}$ peptide signal drops dramatically starting at 16 hr incubation at 37 °C. In contrast, the modified peptides remain stable at all temperatures measured. This data would suggest that these modified peptides are more stable than the full length $A\beta_{42}$.

[00111] Figures 10A-10I show the full length $A\beta_{42}$ and seven modified peptides were subjected to stability studies at different temperatures for up to 60 days. These peptides were kept at 25 °C and 37 °C for the specified length of time and then frozen until the assay was run. Peptides were then collected and run on an MSD Elisa format. Results are shown as a standard curve, plotting signal versus peptide concentration. As shown in Figure 10, the full length peptide gave decreasing standard curves at 25 °C and 37 °C. In comparison, all seven peptides analyzed gave similar curves across all time points and temperature ranges (only 37 °C shown). The results suggest that the modified peptides will give a more consistent signal than the full length $A\beta_{42}$peptide when used as a standard for assay reproducibility.

[00112] Figure 11 shows the standard curve analysis of full length versus modified peptides. Full length $A\beta_{42}$ and seven modified peptides were diluted out for an eight point calibration curve and ran on the MSD Elisa format. As shown in Figure 11, all of the peptides produced almost identical standard curves. This result suggests that the modified peptides can be used in the place of the full length peptide for the calculation of Aβ in sample matrixes.

[00113] Figure 12 shows a CSF sample analysis using full length versus modified peptides. Full length $A\beta_{42}$and seven modified peptides were diluted out for an eight point calibration curve and ran on the MSD Elisa format with 13 CSF samples. The concentration of Aβ in each CSF sample was calculated based on the standard curve for each peptide. As shown in Figure 12, the back calculated Aβ concentrations in the CSF samples were equivalent and was independent of which Aβ peptide used for the standard curve. This suggests that these modified peptides can be used in place of the full length $A\beta_{42}$ peptide in these assays without affecting the calculations of the Aβ levels.

WHAT IS CLAIMED IS:

1. A composition comprising an N-terminal immunoreactive region, a C-terminal immunoreactive region and a linker region.

2. A composition comprising an N-terminal immunoreactive region, a C-terminal immunoreactive region and a linker region, wherein the composition is used as a reference standard in an immunoassay.

3. The composition of claim 2 wherein the immunoassay is selected from the group consisting of a sandwich immunoassay, a single antibody assay, a double sandwich immunoassay and a competition assay.

4. The composition according to claims 1 or 2, wherein the N-terminal immunoreactive region binds $A\beta_{42}$.

5. The composition according to claims 1 or 2, wherein the C-terminal immunoreactive region binds $A\beta_{42}$.

6. The composition according to claims 1 or 2, wherein the linker region is a non-immunoreactive domain.

7. The composition of claim 6, wherein the linker region comprises a linker selected from the group consisting of polyethylene glycol, a glutamine residue, an alanine residue, a lysine residue, a lipid, a globular protein, a nucleic acid and an alkyl chain.

8. An isolated modified peptide molecule having an amino acid sequence selected from the group consisting of SEQ ID NO:1, 2, 3, 4, 5, 6, 7, 8, 9. 10, 11, 12, 13, 14, 15, 17, 19, 21, 22, 23, 24, 25, 26 and 27.

9. A method of measuring the quantity of an analyte in a biological sample, the method comprising:

(a) attaching a reference standard to at least two beads thereby forming a first bead set and a second bead set, wherein the reference standard comprises an epitope recognized by a first detection antibody and wherein each bead set comprises a different concentration of the reference standard;

(b) attaching a capture antibody specific to the analyte to a third bead set;

(c) mixing all of the bead sets together to form a suspension array;

(d) applying the biological sample to the suspension array whereby the analyte binds to the capture antibody on the third bead set;

(e) adding a first detection antibody to the suspension array, wherein the first detection antibody binds the reference standard and analyte bound to the capture antibody;

(f) measuring a first signal from the first detection antibody bound to the reference standard in the first bead set;

(g) measuring a second signal from the first detection antibody bound to the reference standard in the second bead set;

(h) generating a standard curve based upon the first and second signals; and

(i) quantitating the amount of the analyte in the third bead set by measuring a third signal from the first detection antibody and comparing the third signal to the first and second signal measurements on the standard curve.

10. The method of claim 9, wherein the reference standard comprises the composition according to claims 1, 2 or 8.

11. The method of claim 9, wherein the biological sample is selected from the group consisting of blood, serum, plasma, peripheral blood mononuclear cells, peripheral blood lymphocytes, tissue, cerebrospinal fluid and cells.

12. The method of claim 9, wherein the analyte is selected from the group consisting of $A\beta_{42}$, $A\beta_{40}$, $A\beta_{38}$, tau and insulin growth factor receptor 1.

13. The method of claim 9, wherein the method is performed in a multi-well plate, nitrocellulose filter, glass fiber or on a glass slide.

14. The method of claim 9, wherein the first signal and second signal is a signal selected from the group consisting of phycoerythrin, alexa 532, streptavidin-phycoerythrin and streptavidin-Alexa 532.

15. A kit for conducting an immunoassay to detect $A\beta_{42}$ peptide, the kit comprising a composition according to claims 1, 2 or 8.

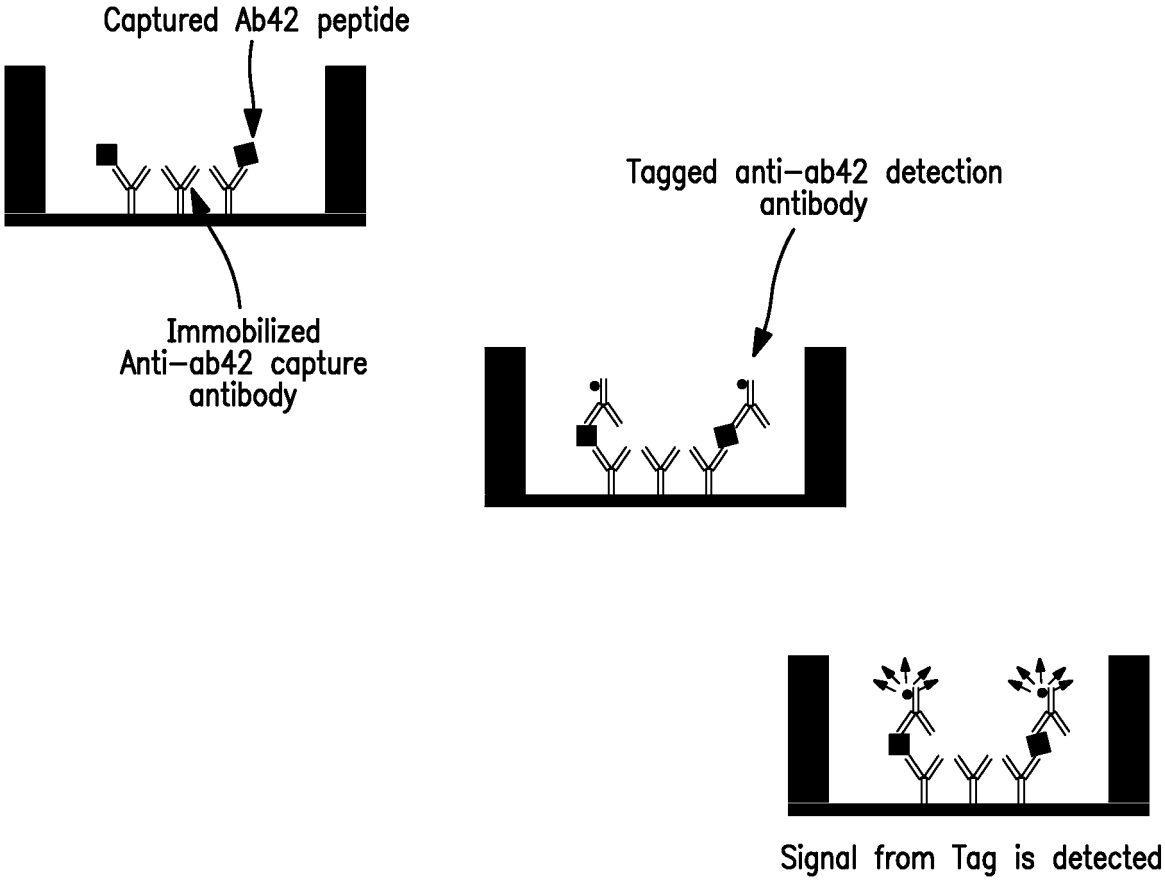

Captured Ab42 peptide
Immobilized
Anti-ab42 capture
antibody
Tagged anti-ab42 detection
antibody
Signal from Tag is detected

FIG. 1

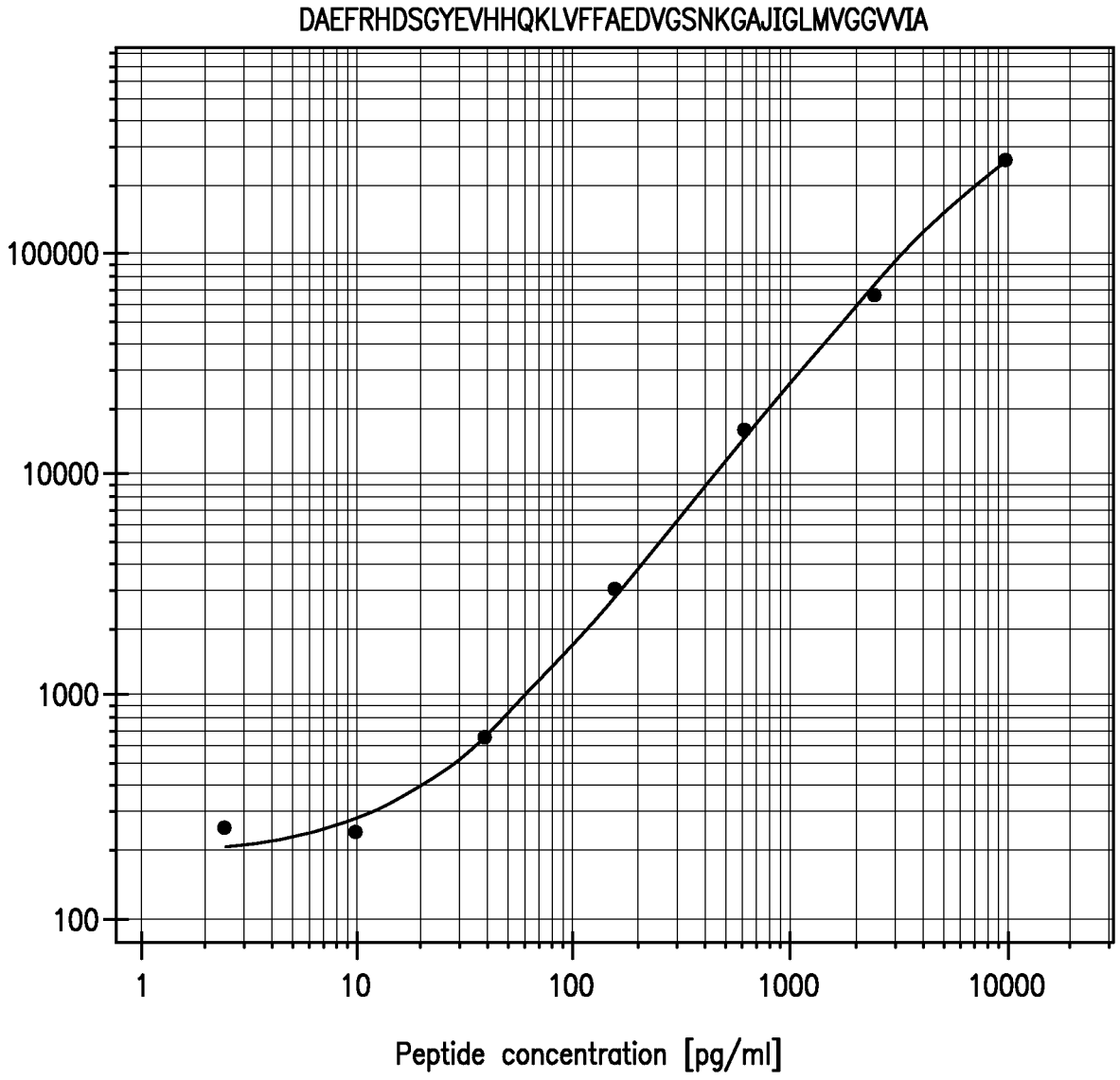
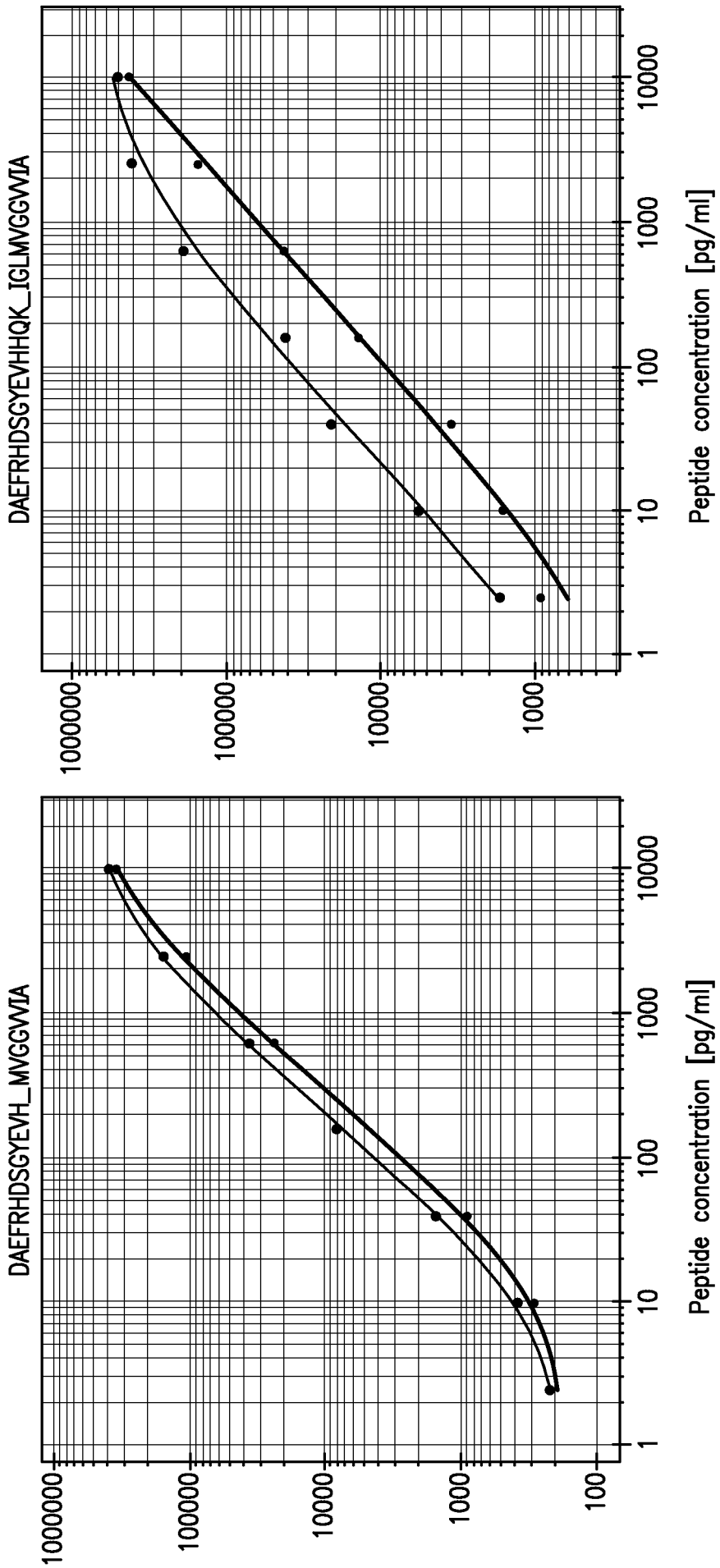

DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAJIGLMVGGVVIA
100000
10000
1000
100
1
10
100
1000
10000
Peptide concentration [pg/ml]

FIG. 2


DAEFRHDSGYEVHHQK_IGLMVGGVVIA
Peptide concentration [pg/ml]
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100000
10000
1000
1
10
100
1000
10000
DAEFRHDSGYEVH_MVGGVVIA
Peptide concentration [pg/ml]
1000000
100000
10000
1000
100
1
10
100
1000
10000

FIG. 3A

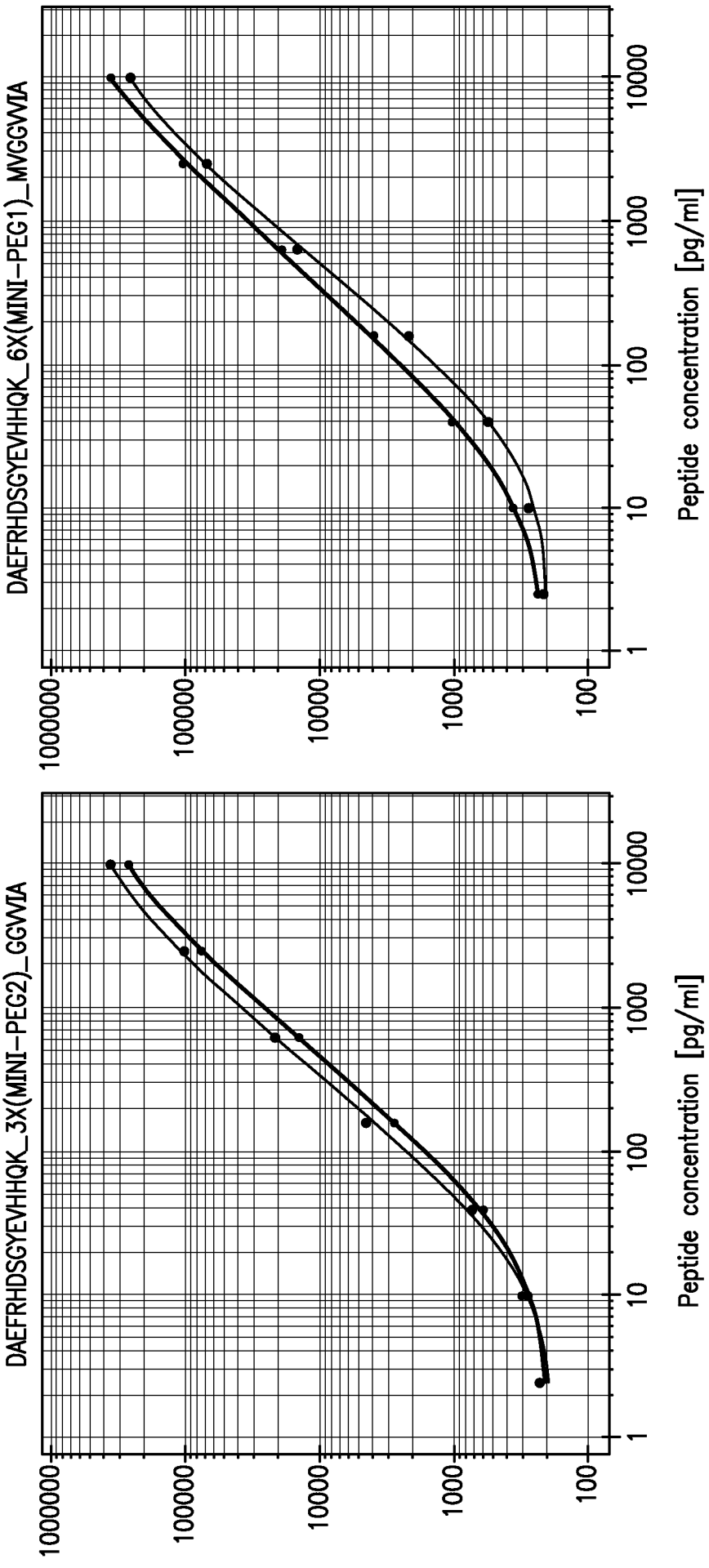

DAEFRHDSGYEVHHQK_6X(MINI-PEG1)_MVGGVVIA
DAEFRHDSGYEVHHQK_3X(MINI-PEG2)_GGVVIA
Peptide concentration [pg/ml]
Peptide concentration [pg/ml]
1000000
100000
10000
1000
100
1
10
100
1000
10000

FIG. 3B

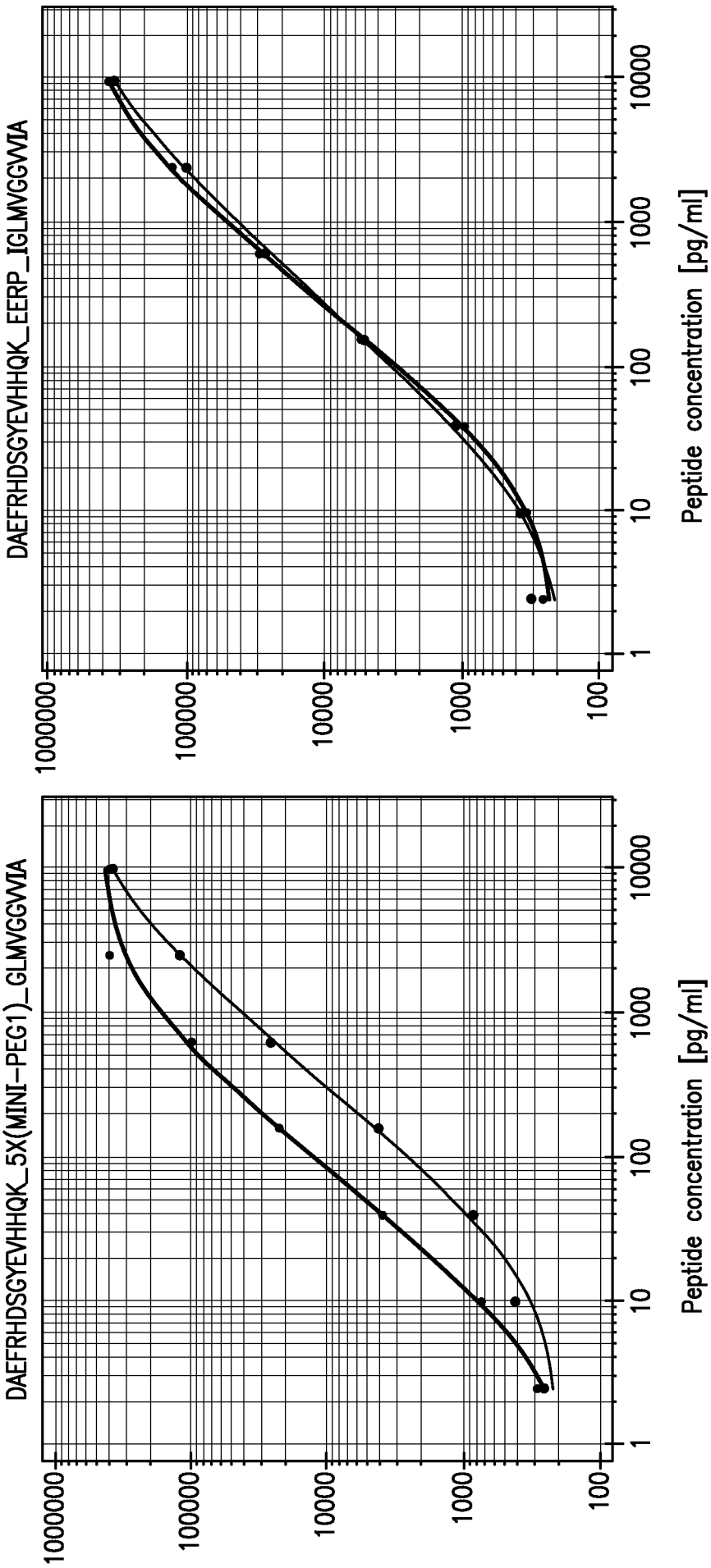

DAEFRHDSGYEVHHQK_EERP_IGLMVGGVVIA
DAEFRHDSGYEVHHQK_5X(MINI-PEG1)_GLMVGGVVIA
Peptide concentration [pg/ml]
Peptide concentration [pg/ml]
1000000
100000
10000
1000
100
1
10
100
1000
10000

FIG. 3C

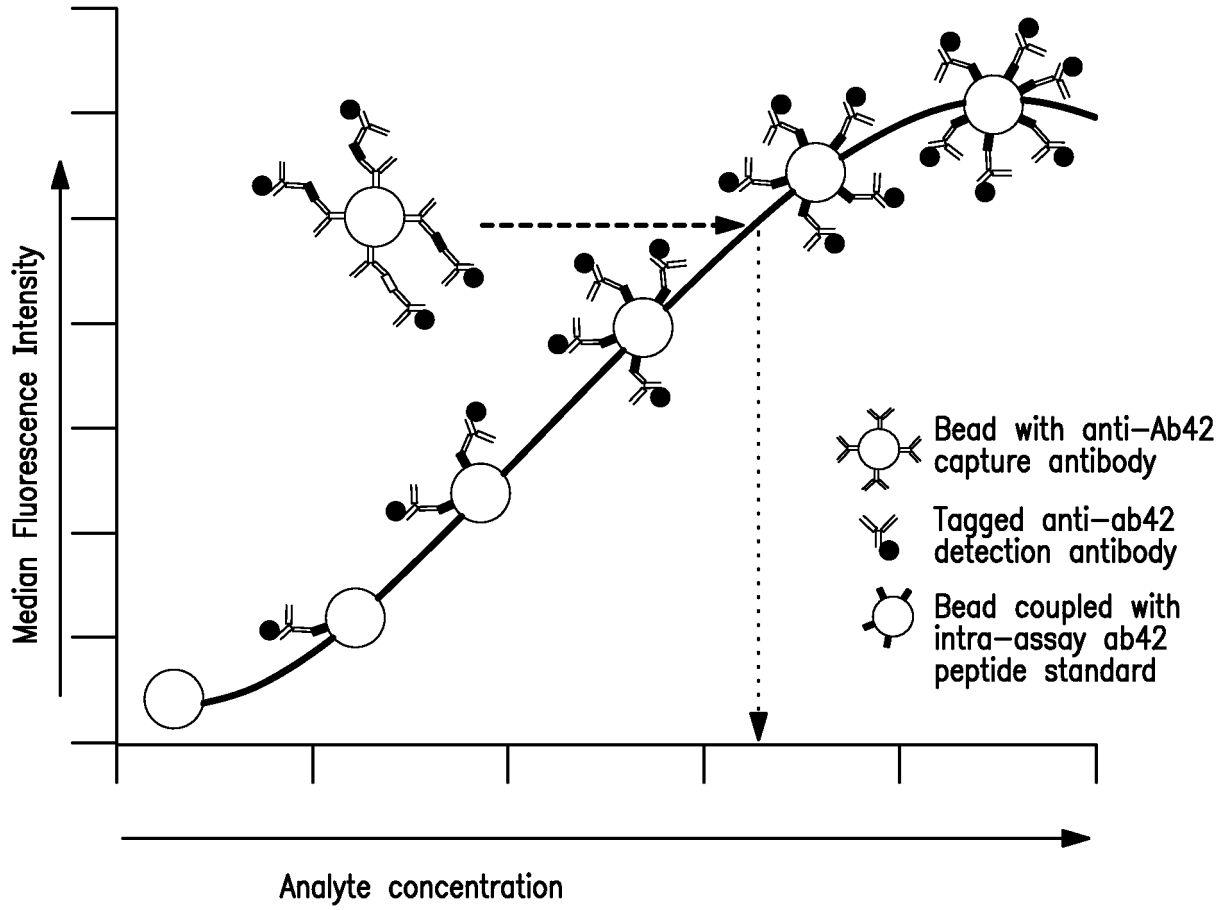

Median Fluorescence Intensity
Analyte concentration
Bead with anti-Ab42 capture antibody
Tagged anti-ab42 detection antibody
Bead coupled with intra-assay ab42 peptide standard

FIG. 4

Selected Bead Set #	$A\beta_{40}$ peptide coupling concentration [μg/35ml]	Median Fluorescence Intensity (MFI)
5	0	14
10	1	28
20	10	151
40	100	782
70	500	1944
90	5000	7869

FIG. 5A

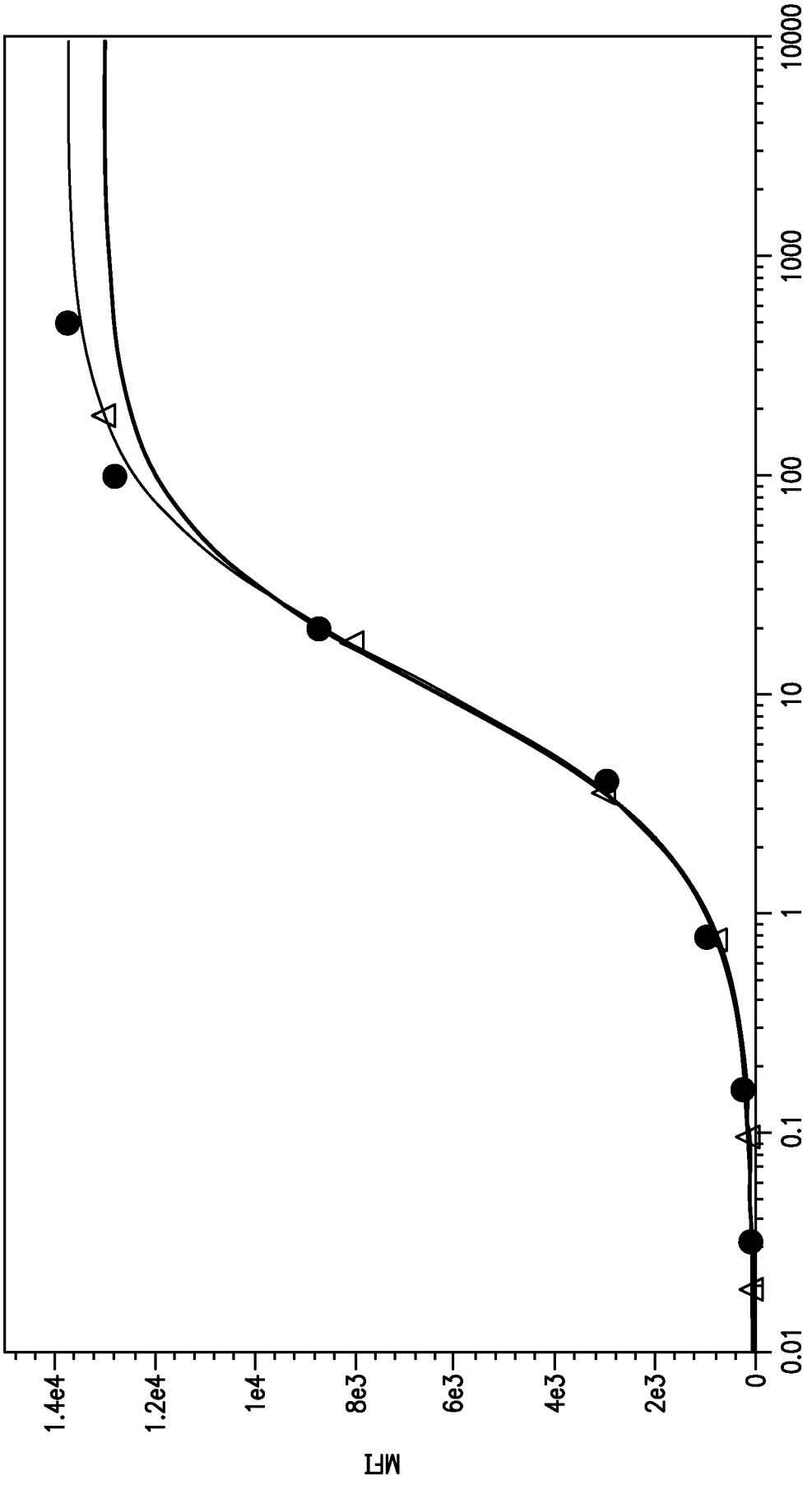

MFI
1.4e4
1.2e4
1e4
8e3
6e3
4e3
2e3
0
0.01
0.1
1
10
100
1000
10000
Concentration Abeta (ng/mL)

FIG. 5B

Sample	Results generated from Intra-assay $A\beta_{40}$ std curves (circles)	Results generated from $A\beta_{42}$ soluble std curves (triangles)
CSF-1	781 pg/ml	781 pg/ml
CSF-2	213 pg/ml	212 pg/ml
CSF-3	152 pg/ml	150 pg/ml
CSF-4	224 pg/ml	223 pg/ml

FIG. 5C

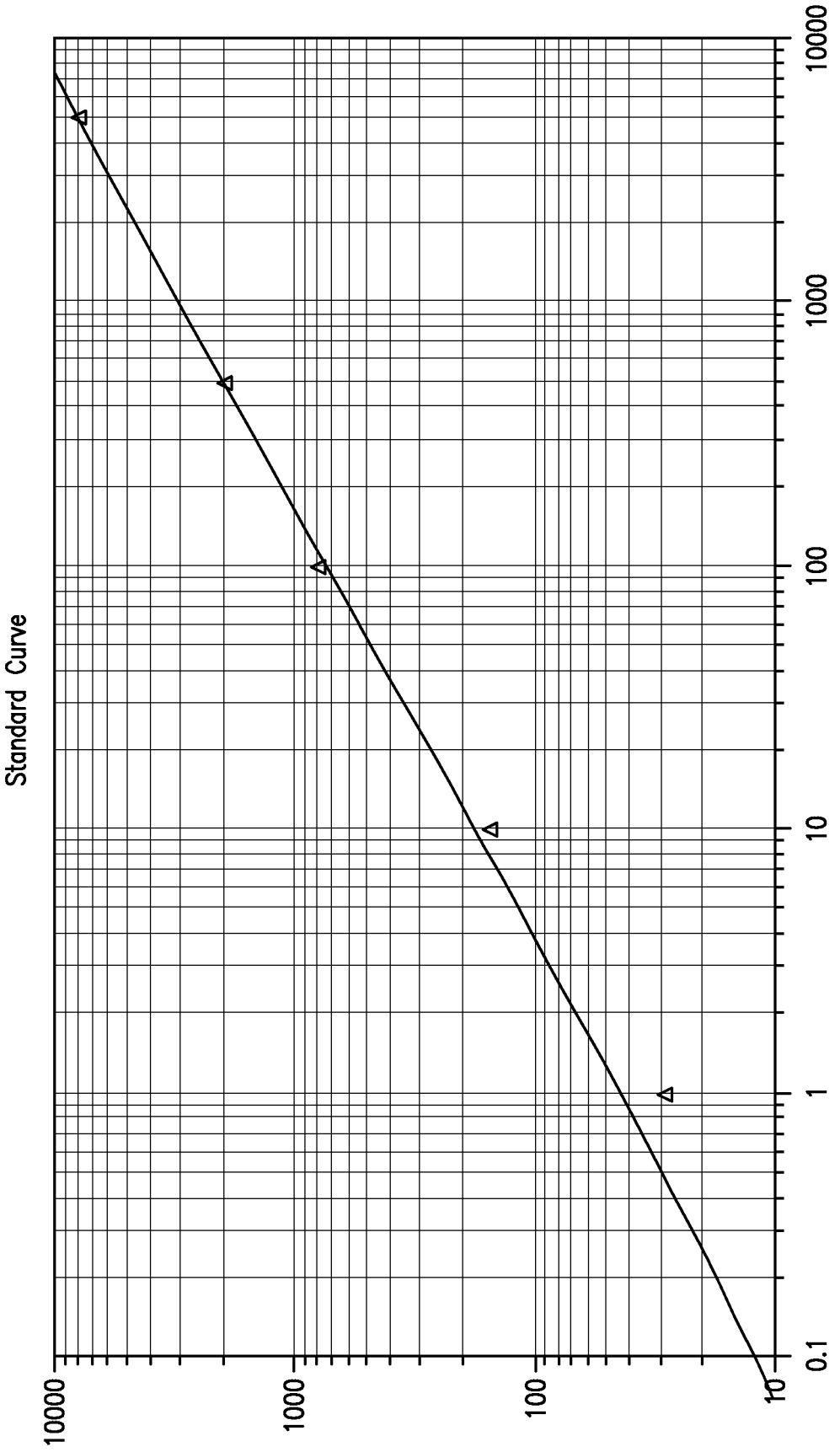

Standard Curve
Concentration
10000
1000
100
10
1
0.1
10000
1000
100
10

FIG. 6A

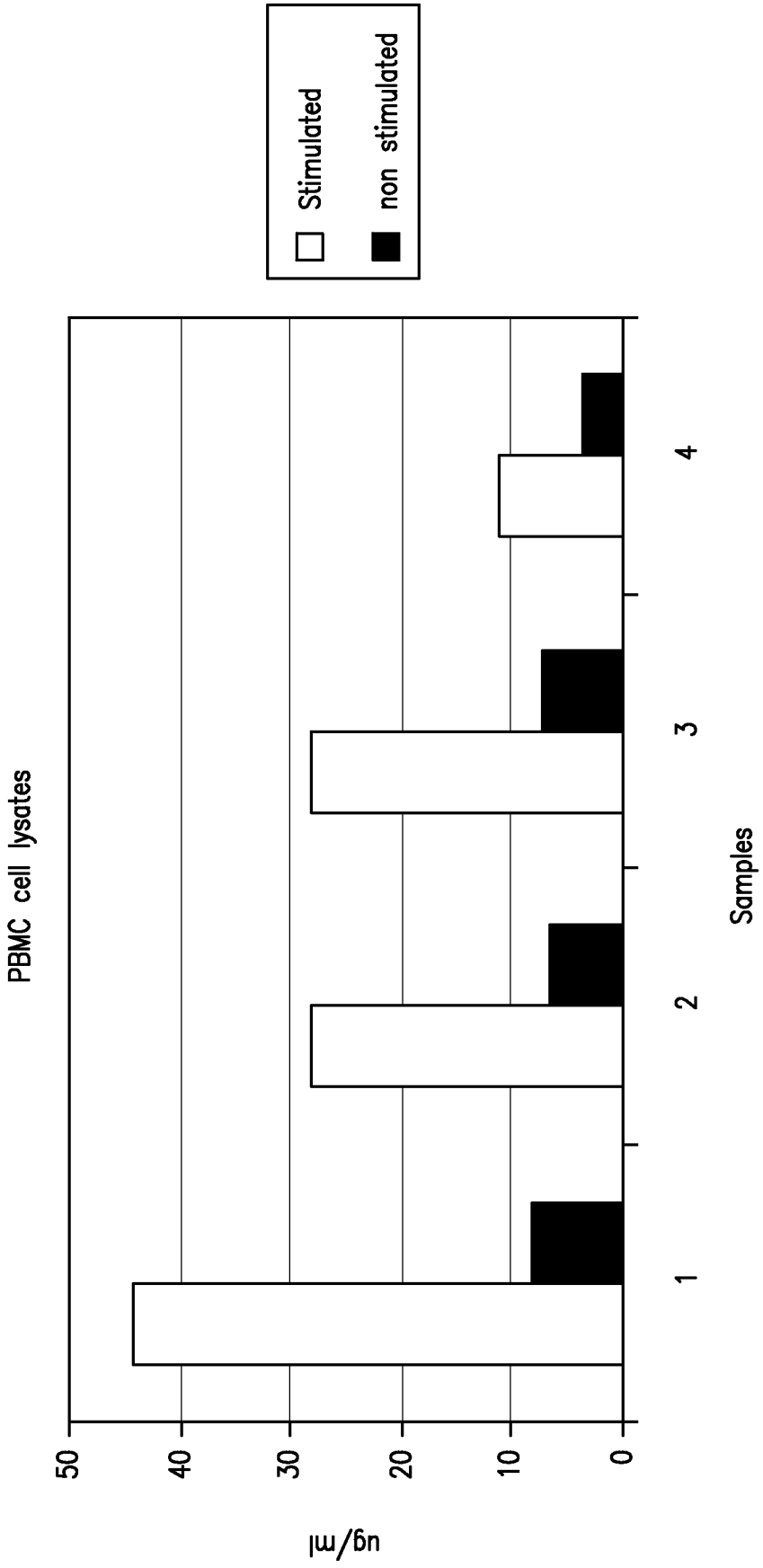

Stimulated
non stimulated
PBMC cell lysates
Samples
1
2
3
4
0
10
20
30
40
50
ug/ml

FIG. 6B

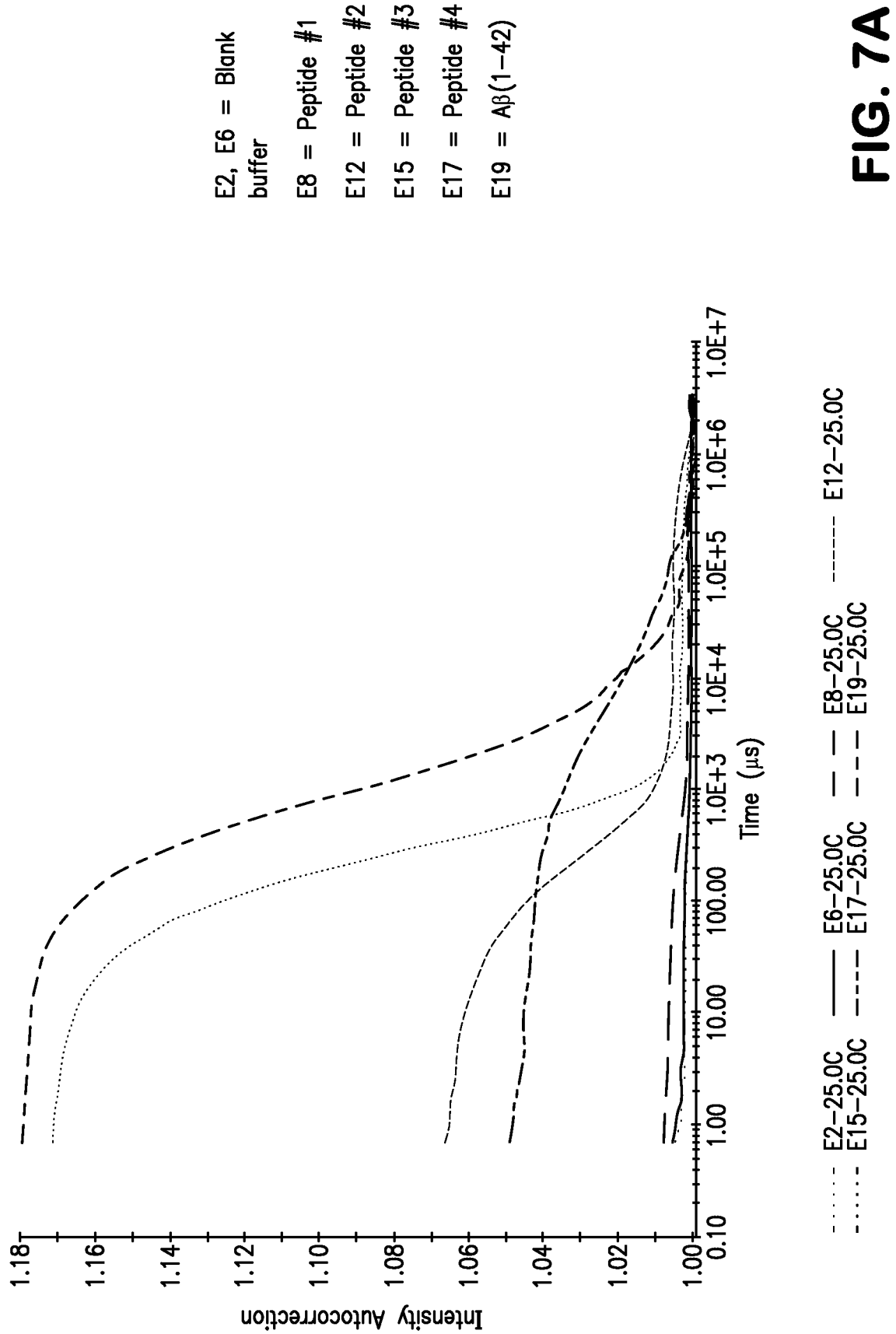

E2, E6 = Blank buffer
E8 = Peptide #1
E12 = Peptide #2
E15 = Peptide #3
E17 = Peptide #4
E19 = Aβ(1–42)
Intensity Autocorrection
1.18
1.16
1.14
1.12
1.10
1.08
1.06
1.04
1.02
1.00
0.10
1.00
10.00
100.00
1.0E+3
1.0E+4
1.0E+5
1.0E+6
1.0E+7
Time (µs)
E2–25.0C
E15–25.0C
E6–25.0C
E17–25.0C
E8–25.0C
E19–25.0C
E12–25.0C

FIG. 7A

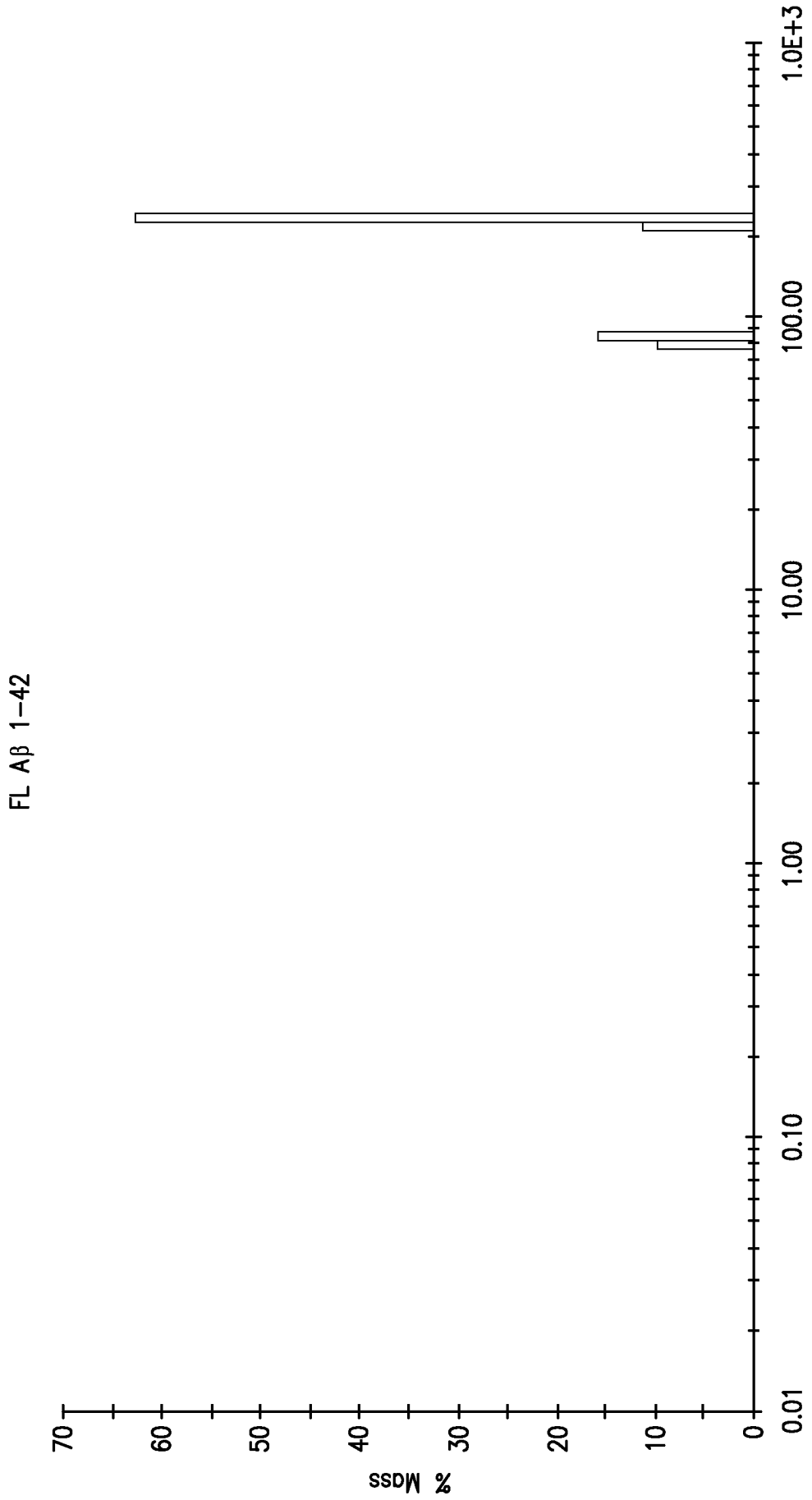

FL Aβ 1−42
% Mass
70
60
50
40
30
20
10
0
0.01
0.10
1.00
10.00
100.00
1.0E+3
Radius(nm)

FIG. 7B

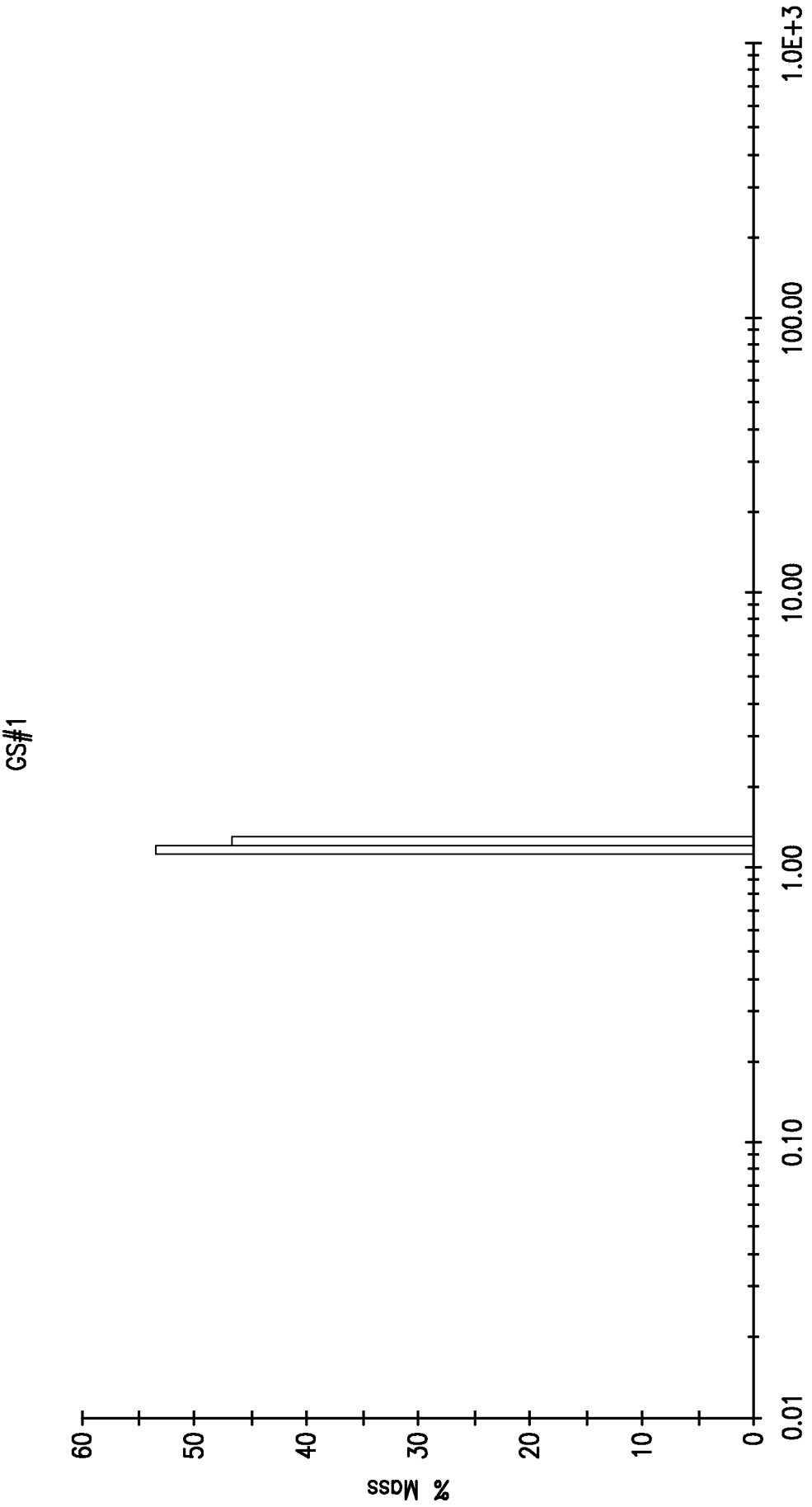

GS#1
% Mass
60
50
40
30
20
10
0
0.01
0.10
1.00
10.00
100.00
1.0E+3
Radius(nm)

FIG. 7C

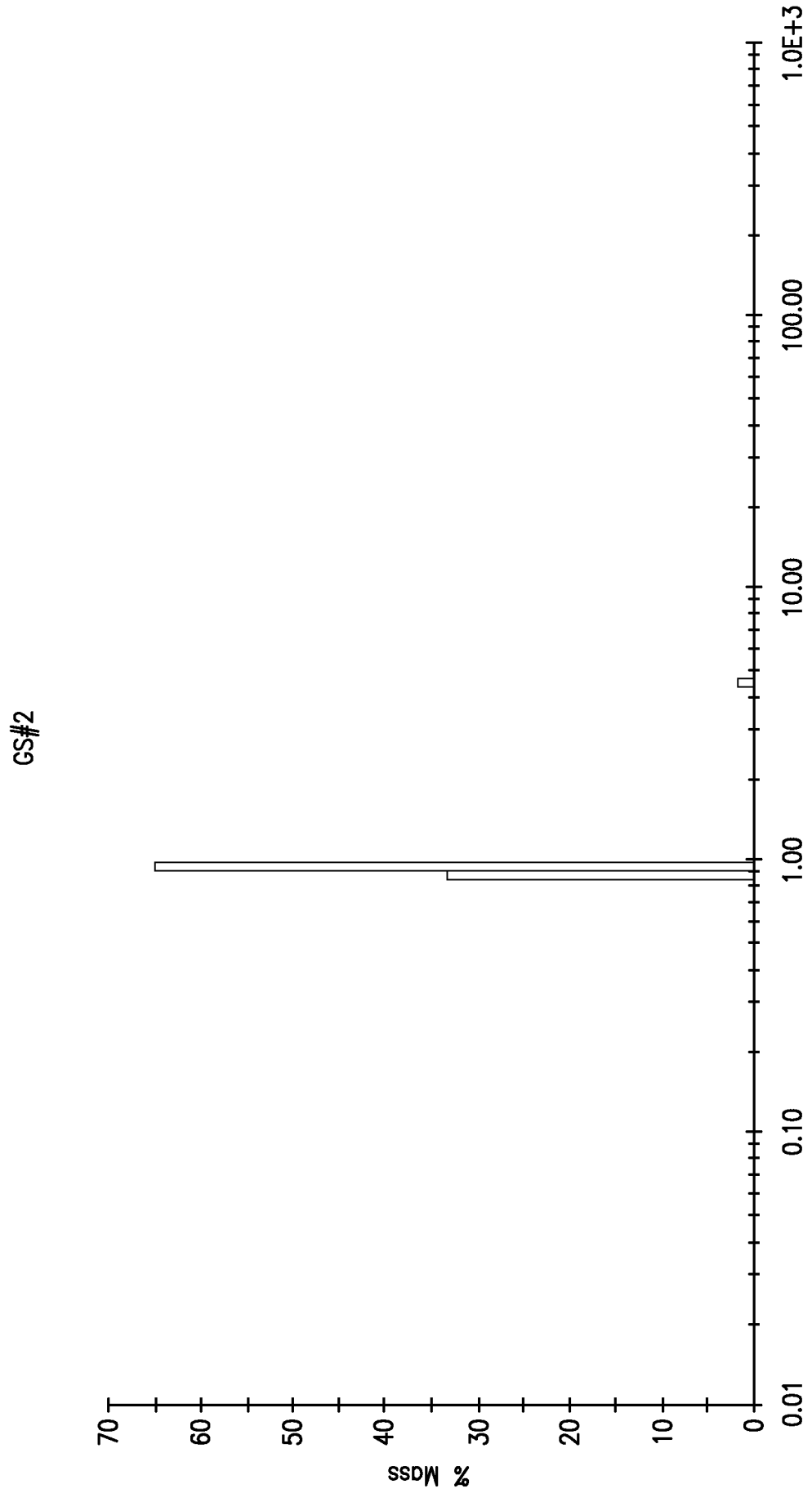

GS#2
% MASS
70
60
50
40
30
20
10
0
0.01
0.10
1.00
10.00
100.00
1.0E+3
Radius(nm)

FIG. 7D

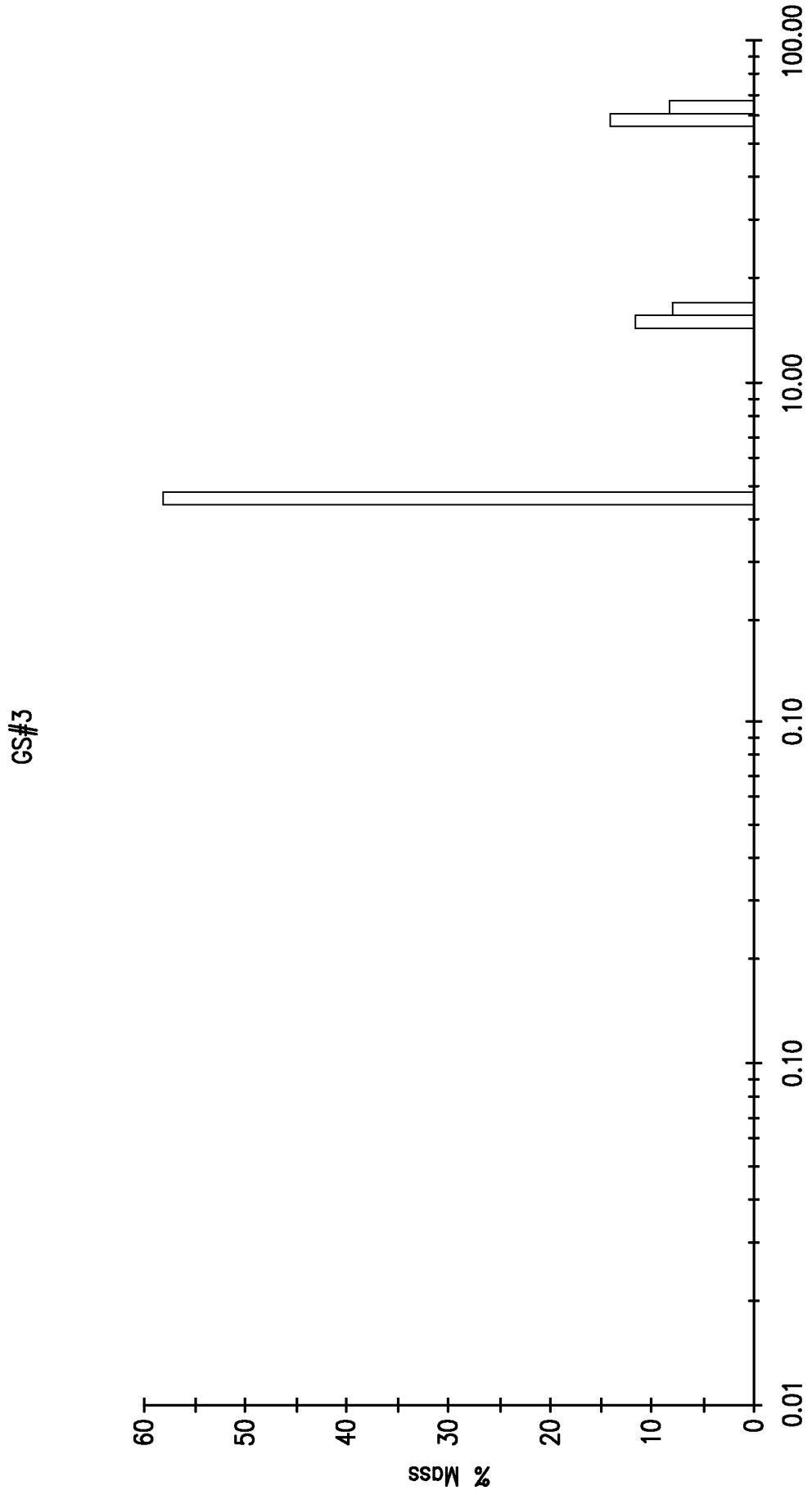


FIG. 7E

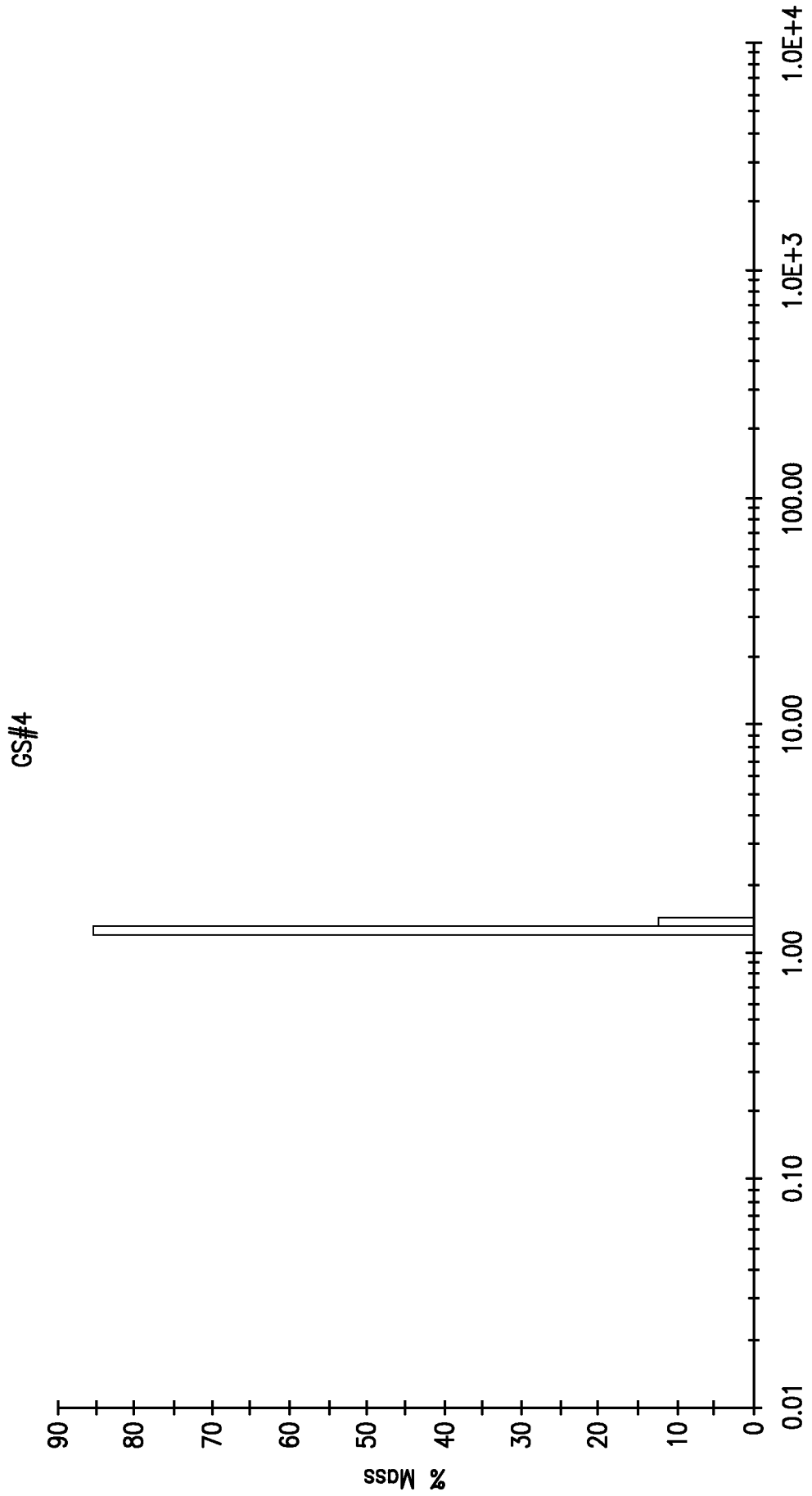

GS#4
% MASS
90
80
70
60
50
40
30
20
10
0
0.01
0.10
1.00
10.00
100.00
1.0E+3
1.0E+4
Radius(nm)

FIG. 7F

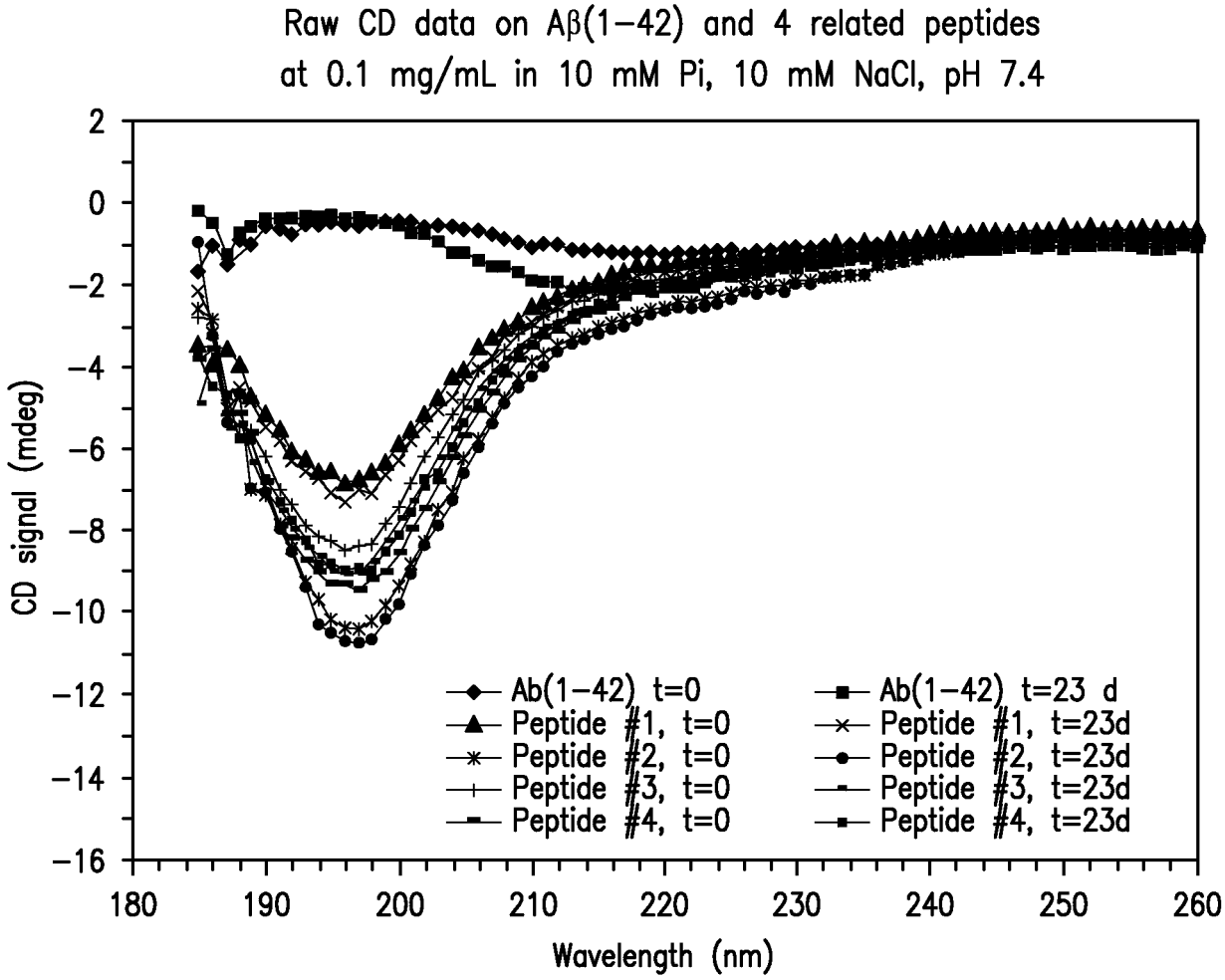

Raw CD data on Aβ(1−42) and 4 related peptides
at 0.1 mg/mL in 10 mM Pi, 10 mM NaCl, pH 7.4
CD signal (mdeg)
Wavelength (nm)
2
0
−2
−4
−6
−8
−10
−12
−14
−16
180
190
200
210
220
230
240
250
260
Ab(1−42) t=0
Peptide #1, t=0
Peptide #2, t=0
Peptide #3, t=0
Peptide #4, t=0
Ab(1−42) t=23 d
Peptide #1, t=23d
Peptide #2, t=23d
Peptide #3, t=23d
Peptide #4, t=23d

FIG. 8

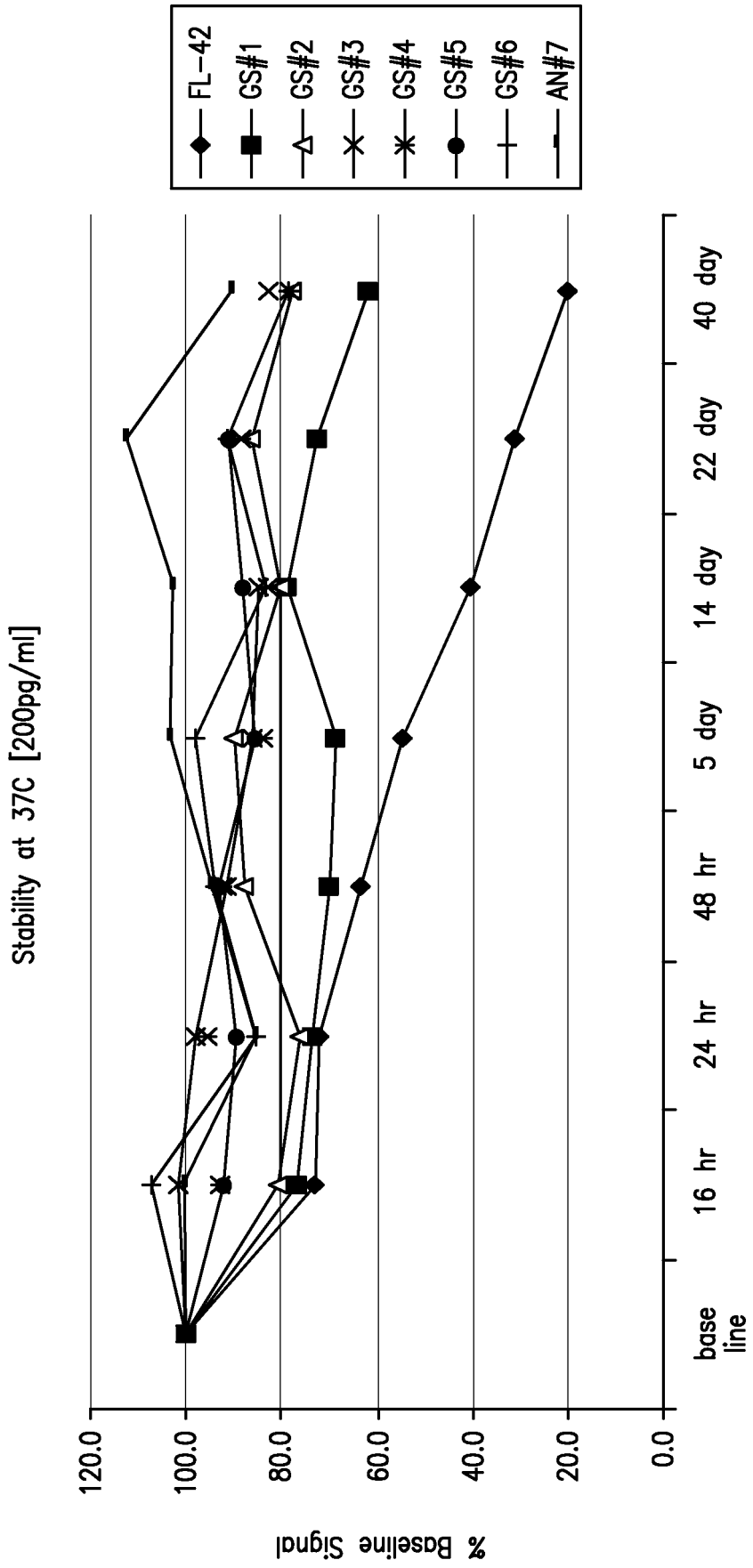

Stability at 37C [200pg/ml]
% Baseline Signal
120.0
100.0
80.0
60.0
40.0
20.0
0.0
base line
16 hr
24 hr
48 hr
5 day
14 day
22 day
40 day
FL-42
GS#1
GS#2
GS#3
GS#4
GS#5
GS#6
AN#7

FIG. 9A

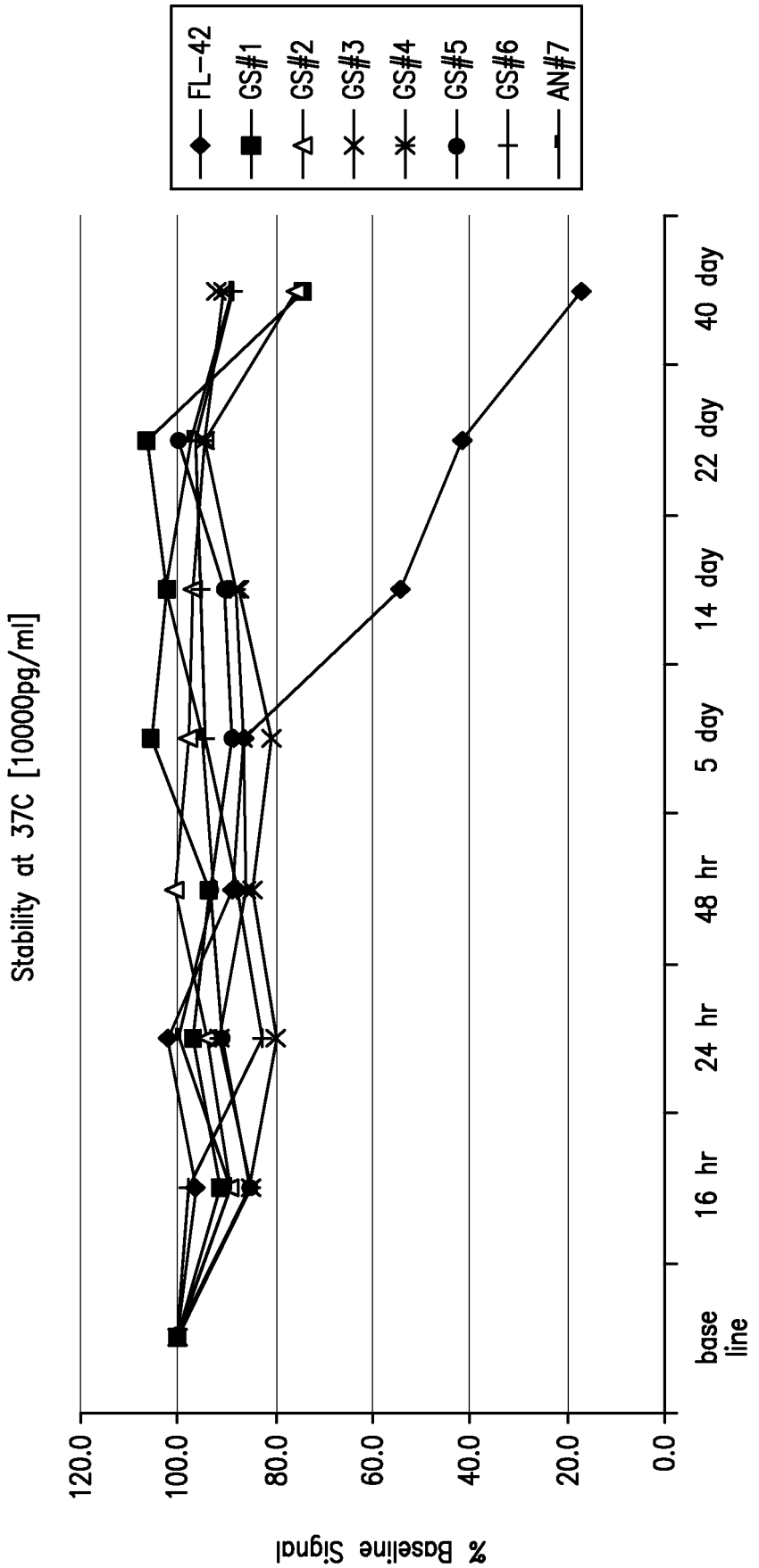

Stability at 37C [10000pg/ml]
% Baseline Signal
120.0
100.0
80.0
60.0
40.0
20.0
0.0
base line
16 hr
24 hr
48 hr
5 day
14 day
22 day
40 day
FL-42
GS#1
GS#2
GS#3
GS#4
GS#5
GS#6
AN#7

FIG. 9B

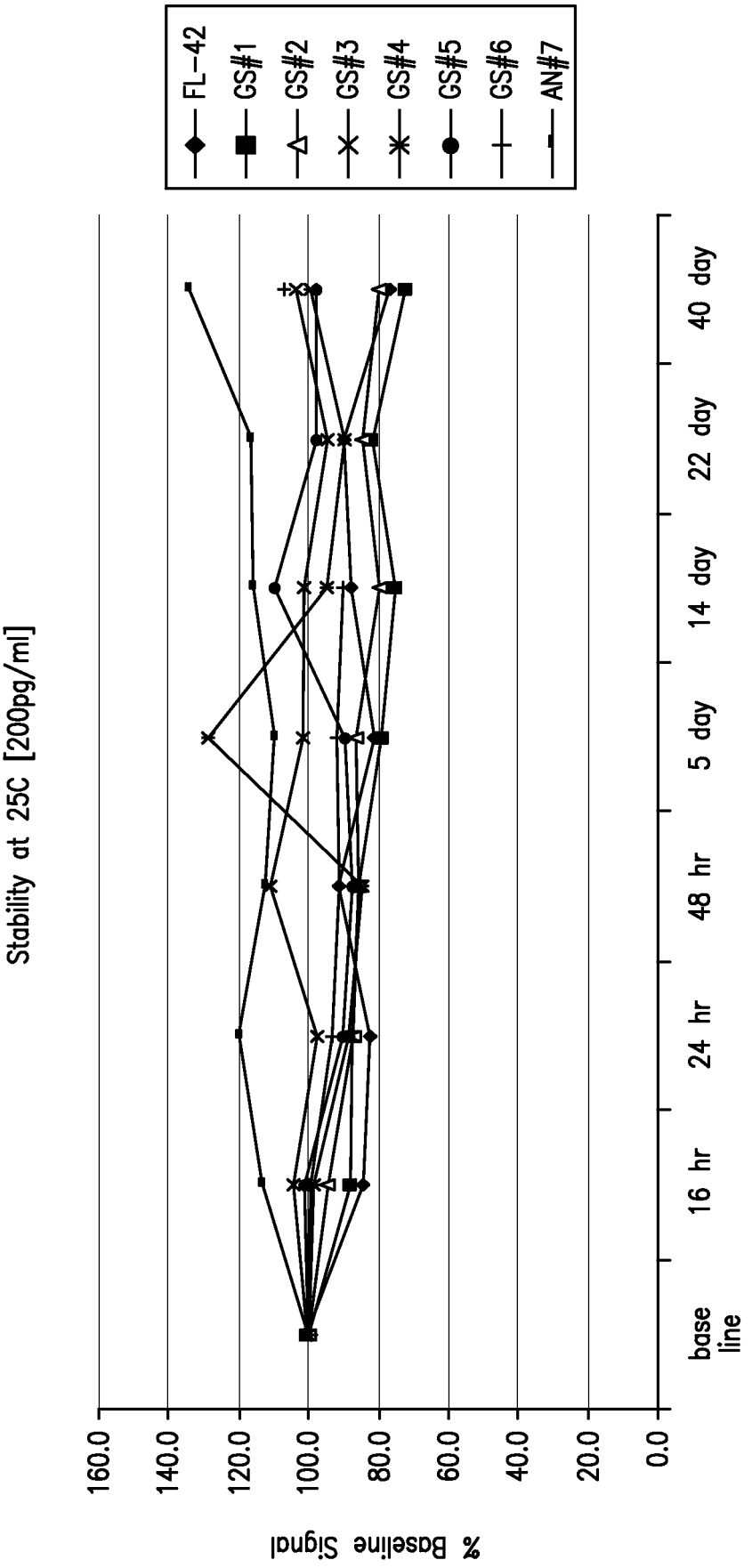

Stability at 25C [200pg/ml]
% Baseline Signal
160.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
base line
16 hr
24 hr
48 hr
5 day
14 day
22 day
40 day
FL-42
GS#1
GS#2
GS#3
GS#4
GS#5
GS#6
AN#7

FIG. 9C

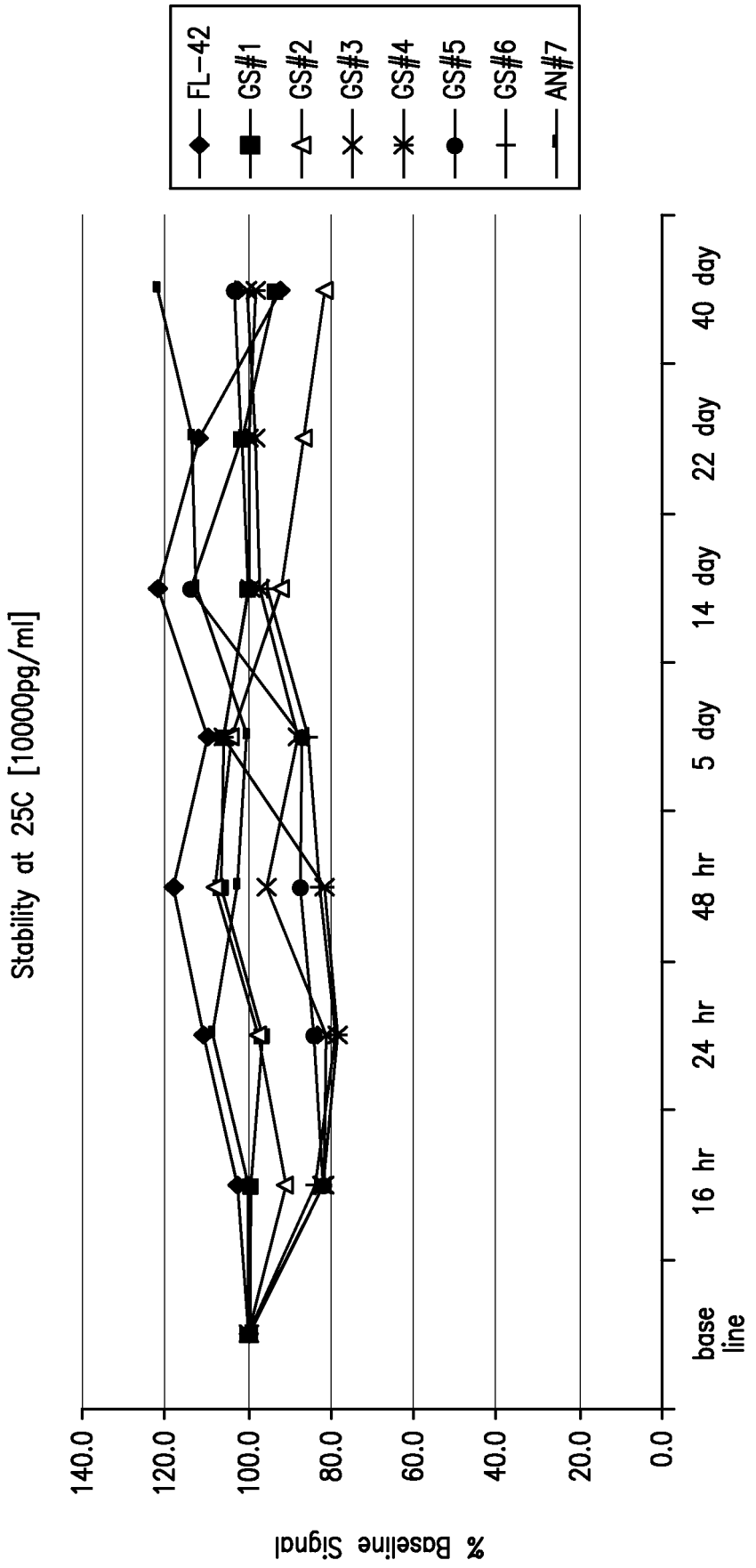

Stability at 25C [10000pg/ml]
% Baseline Signal
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
base line
16 hr
24 hr
48 hr
5 day
14 day
22 day
40 day
FL-42
GS#1
GS#2
GS#3
GS#4
GS#5
GS#6
AN#7

FIG. 9D

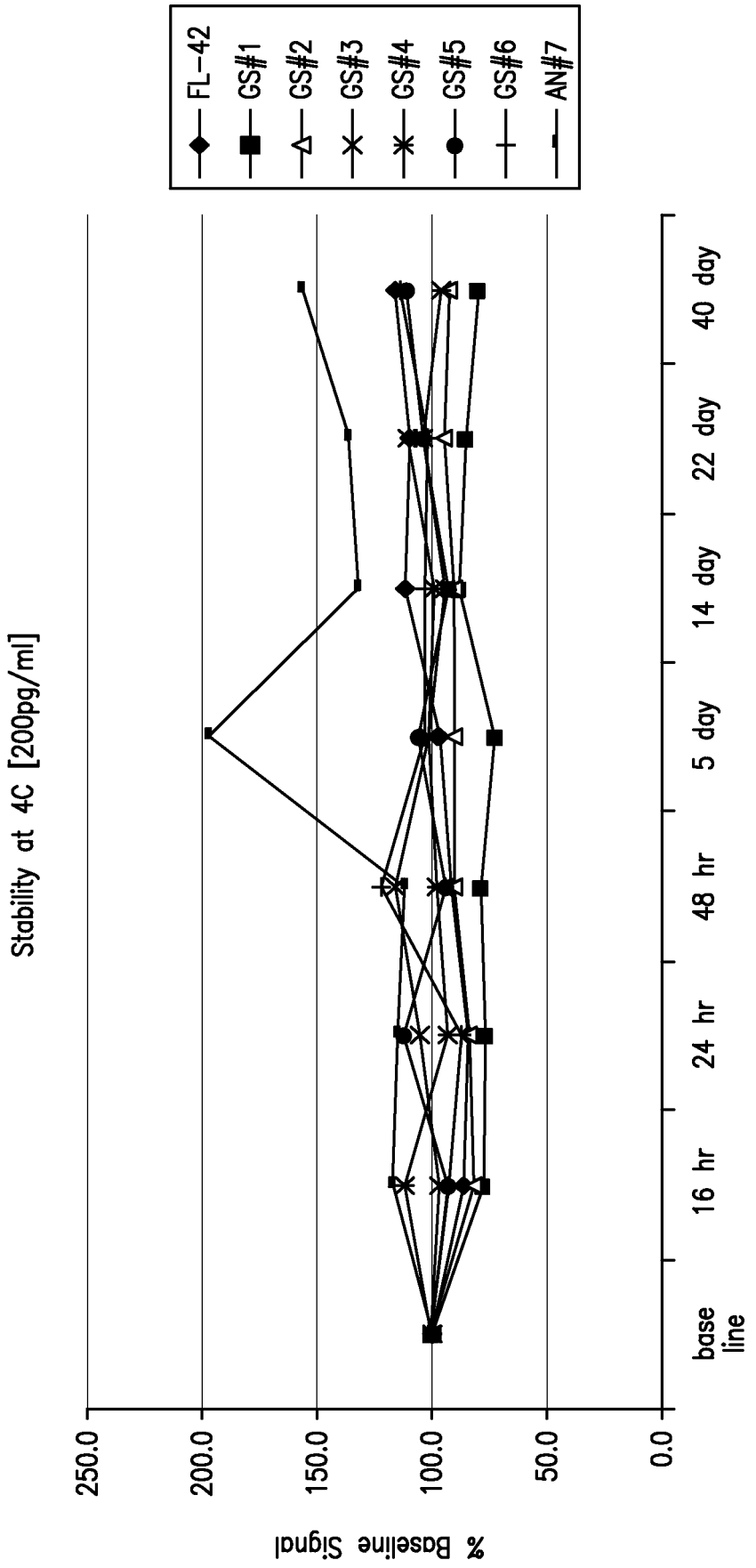

Stability at 4C [200pg/ml]
% Baseline Signal
250.0
200.0
150.0
100.0
50.0
0.0
base line
16 hr
24 hr
48 hr
5 day
14 day
22 day
40 day
FL-42
GS#1
GS#2
GS#3
GS#4
GS#5
GS#6
AN#7

FIG. 9E

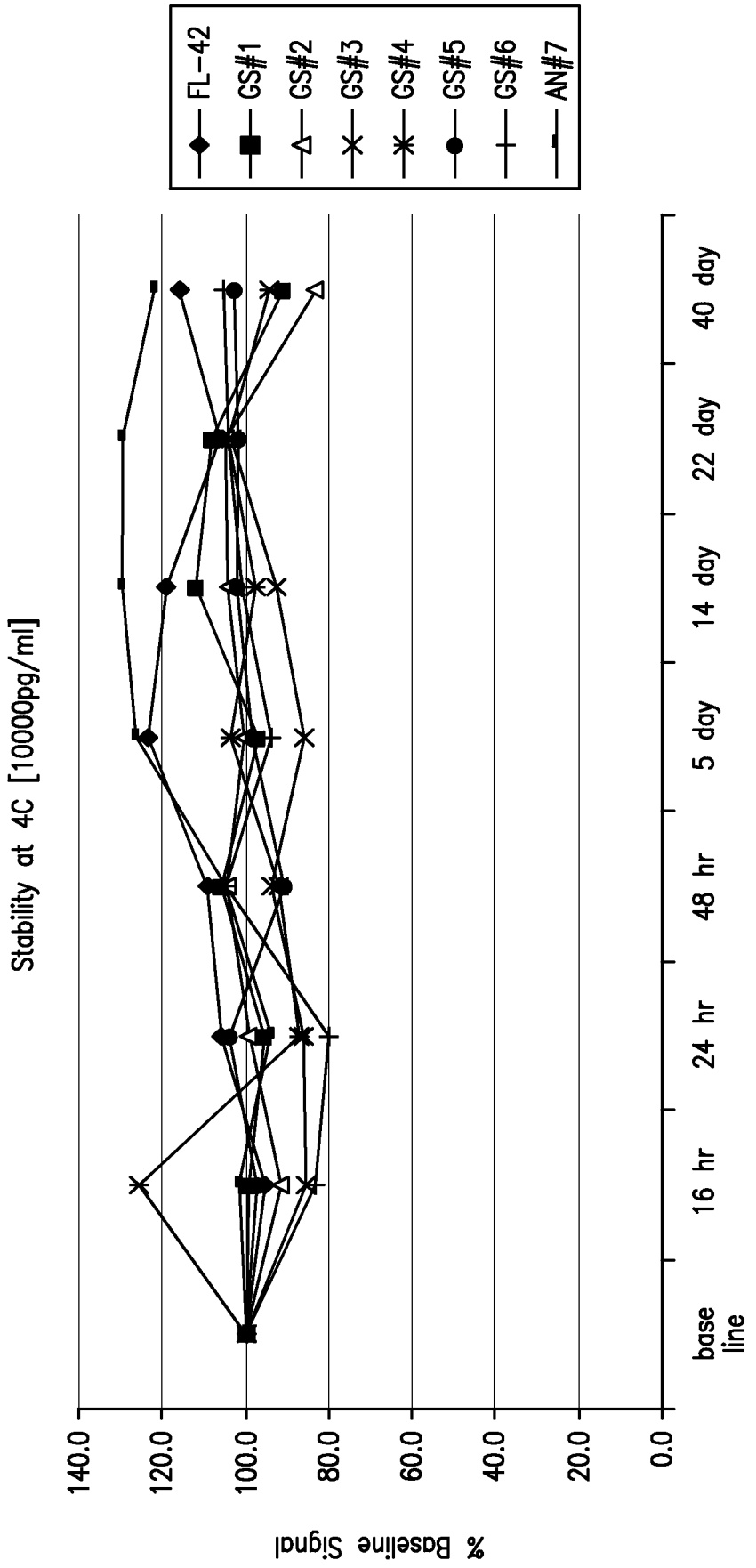

Stability at 4C [10000pg/ml]
% Baseline Signal
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
base line
16 hr
24 hr
48 hr
5 day
14 day
22 day
40 day
FL-42
GS#1
GS#2
GS#3
GS#4
GS#5
GS#6
AN#7

FIG. 9F

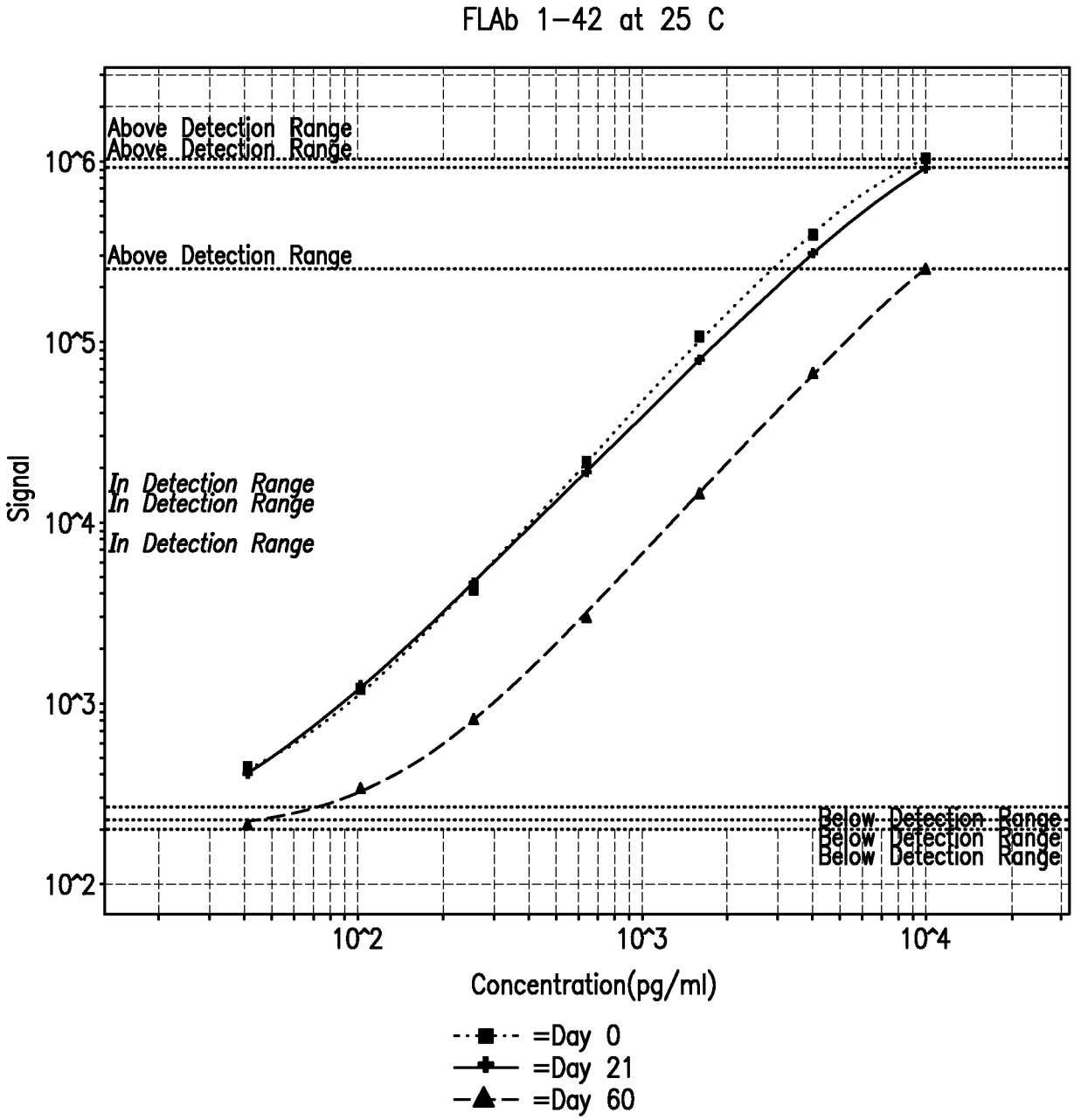

FLAb 1-42 at 25 C
Above Detection Range
Above Detection Range
Above Detection Range
In Detection Range
In Detection Range
In Detection Range
Below Detection Range
Below Detection Range
Below Detection Range
10^6
10^5
10^4
10^3
10^2
Signal
10^2
10^3
10^4
Concentration(pg/ml)
=Day 0
=Day 21
=Day 60

FIG. 10A

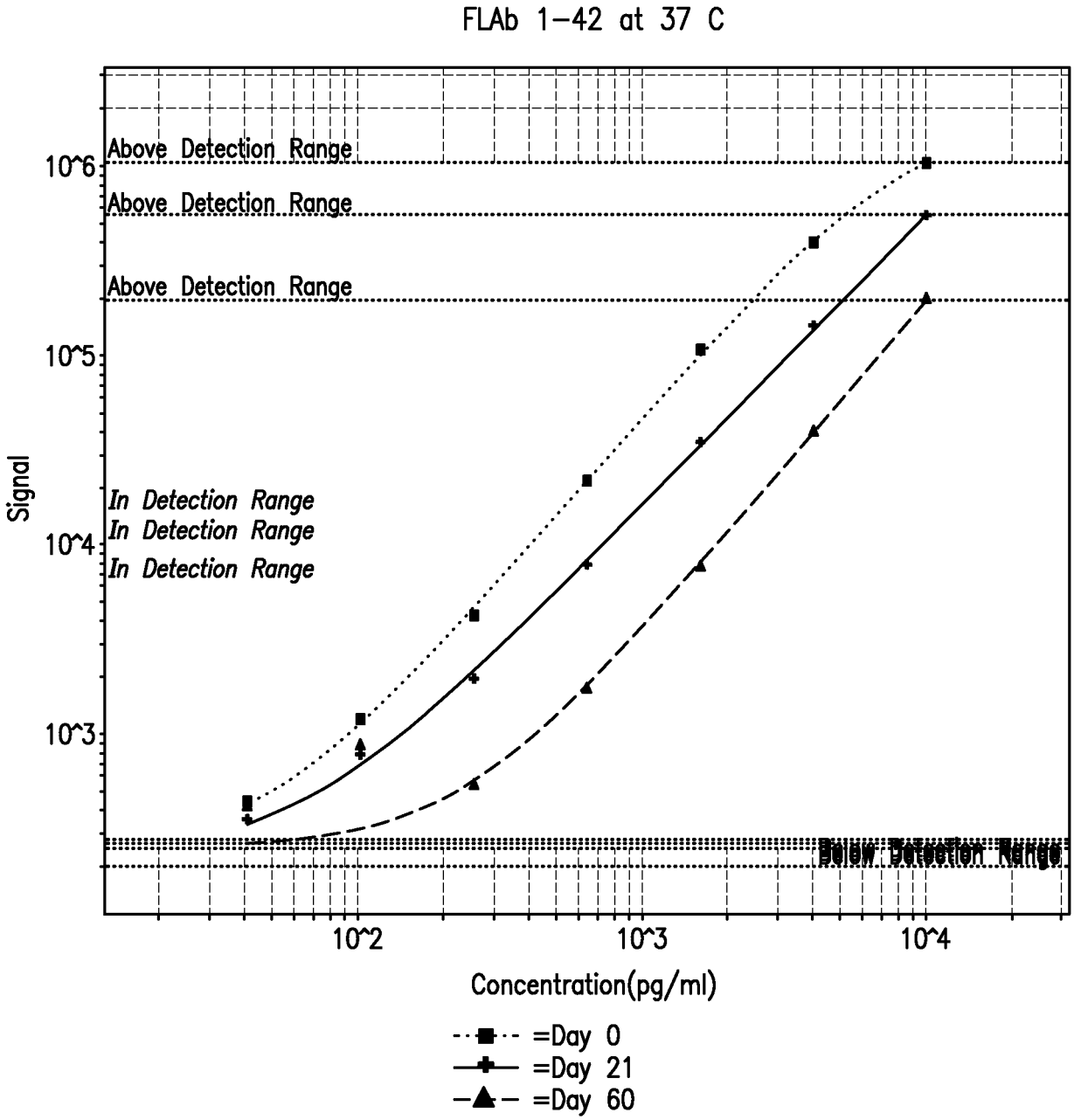

FLAb 1-42 at 37 C
Above Detection Range
Above Detection Range
Above Detection Range
In Detection Range
In Detection Range
In Detection Range
Below Detection Range
10^6
10^5
10^4
10^3
Signal
10^2
10^3
10^4
Concentration(pg/ml)
=Day 0
=Day 21
=Day 60

FIG. 10B

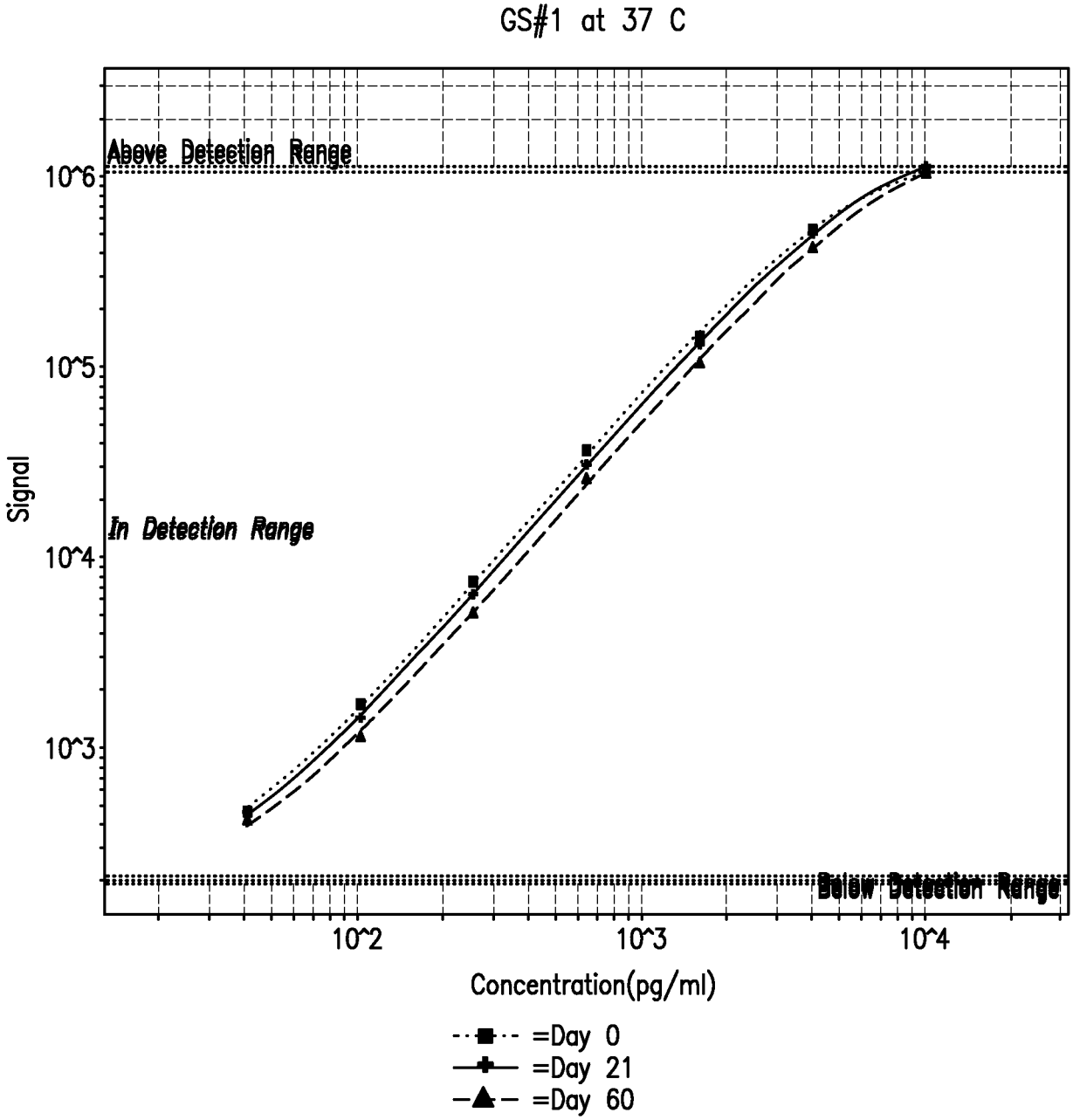

GS#1 at 37 C
Above Detection Range
10^6
10^5
10^4
10^3
Signal
In Detection Range
Below Detection Range
10^2
10^3
10^4
Concentration(pg/ml)
=Day 0
=Day 21
=Day 60

FIG. 10C

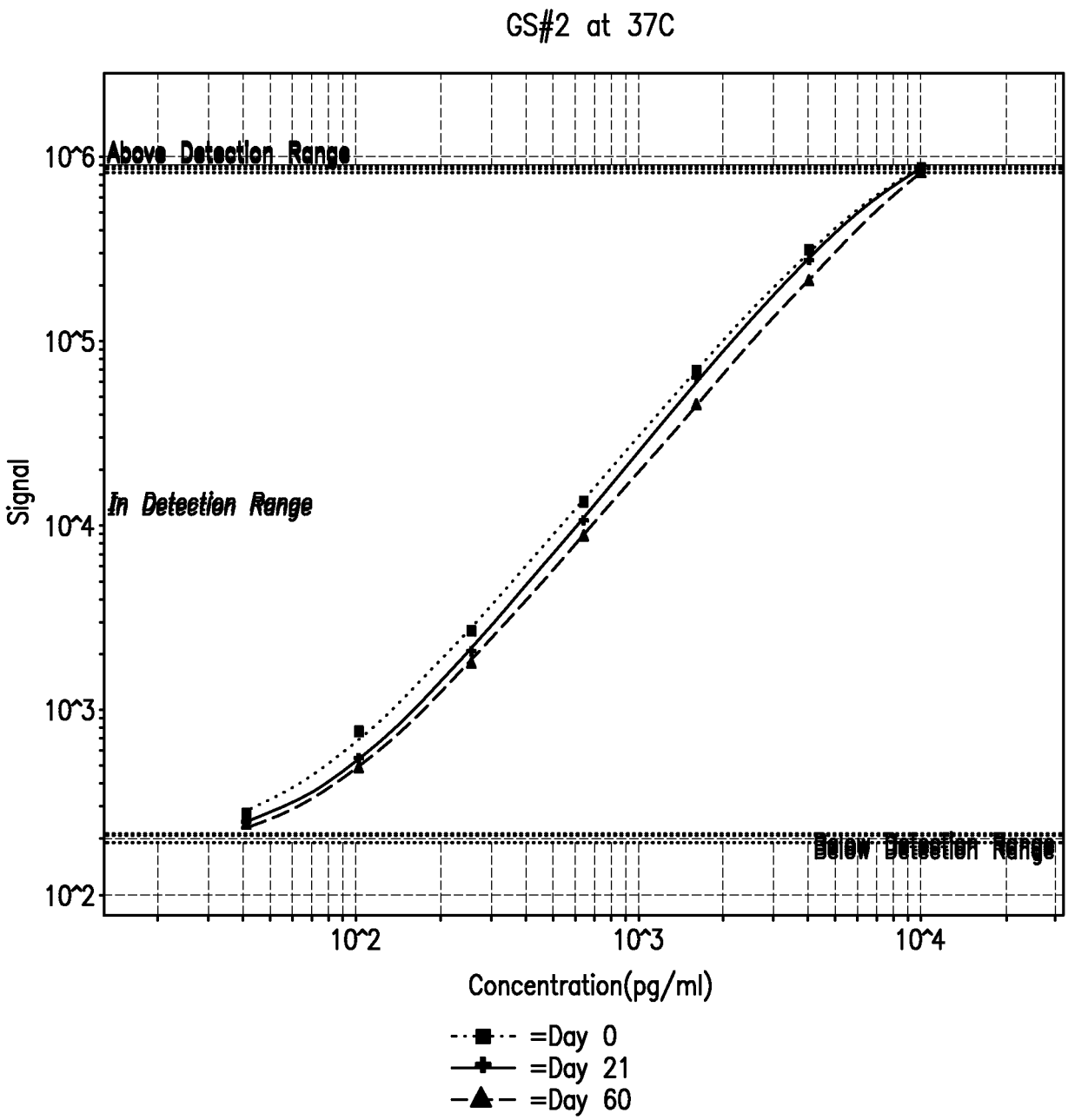

GS#2 at 37C
Above Detection Range
In Detection Range
Below Detection Range
Signal
10^6
10^5
10^4
10^3
10^2
10^2
10^3
10^4
Concentration(pg/ml)
=Day 0
=Day 21
=Day 60

FIG. 10D

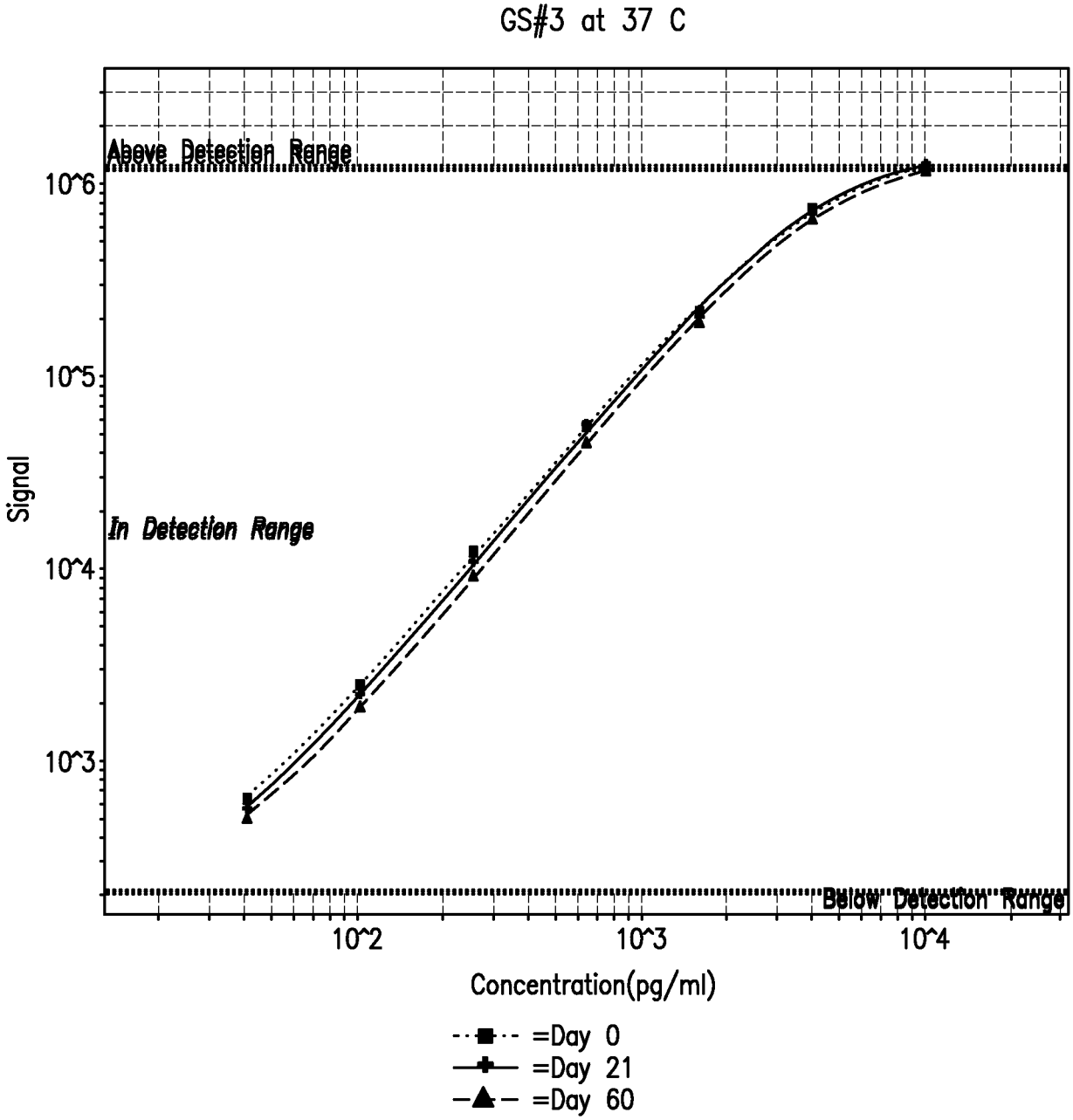

GS#3 at 37 C
Above Detection Range
In Detection Range
Below Detection Range
Signal
10^6
10^5
10^4
10^3
10^2
10^3
10^4
Concentration(pg/ml)
=Day 0
=Day 21
=Day 60

FIG. 10E

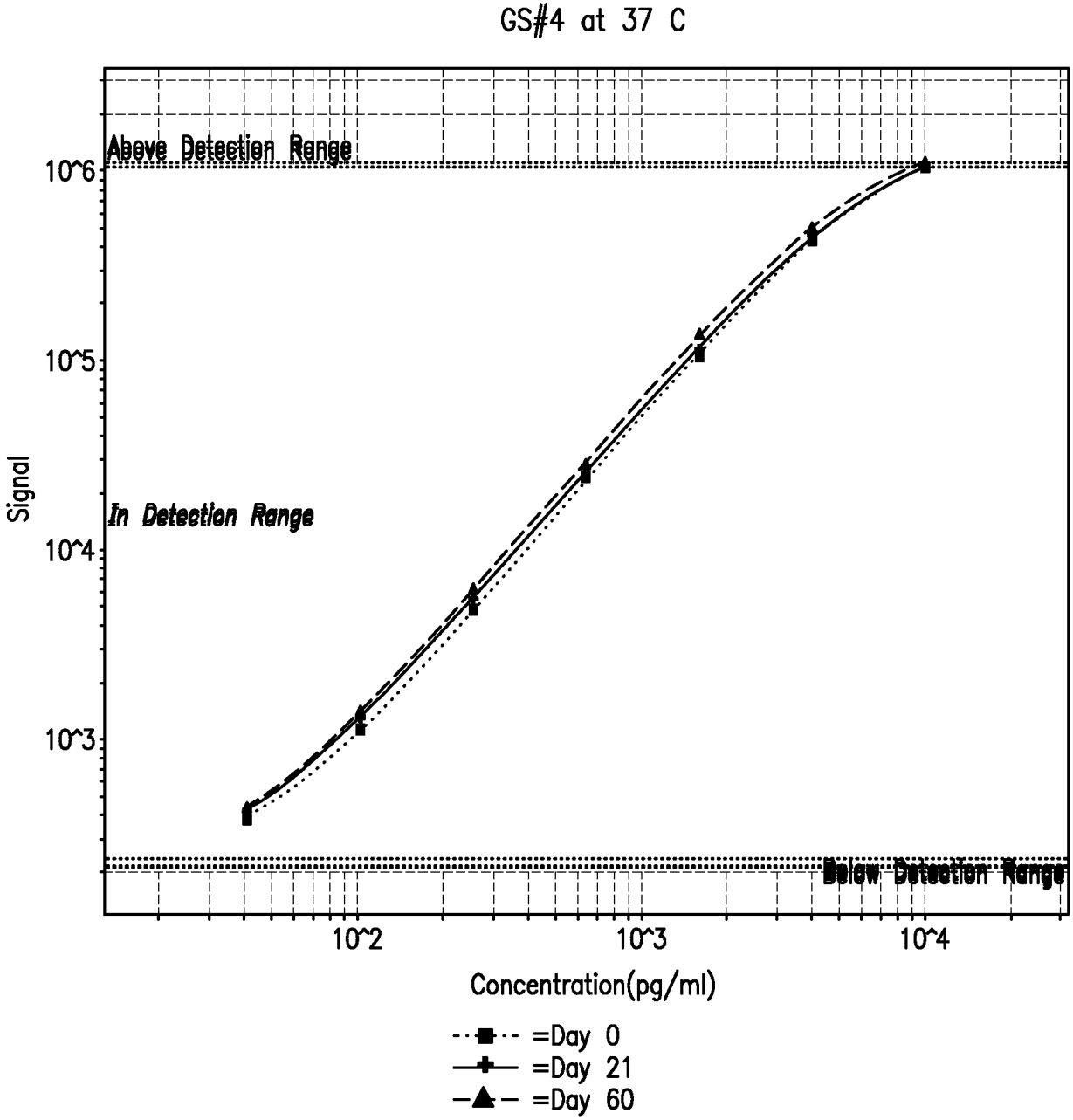

GS#4 at 37 C
Above Detection Range
10^6
10^5
10^4
10^3
Signal
In Detection Range
Below Detection Range
10^2
10^3
10^4
Concentration(pg/ml)
=Day 0
=Day 21
=Day 60

FIG. 10F

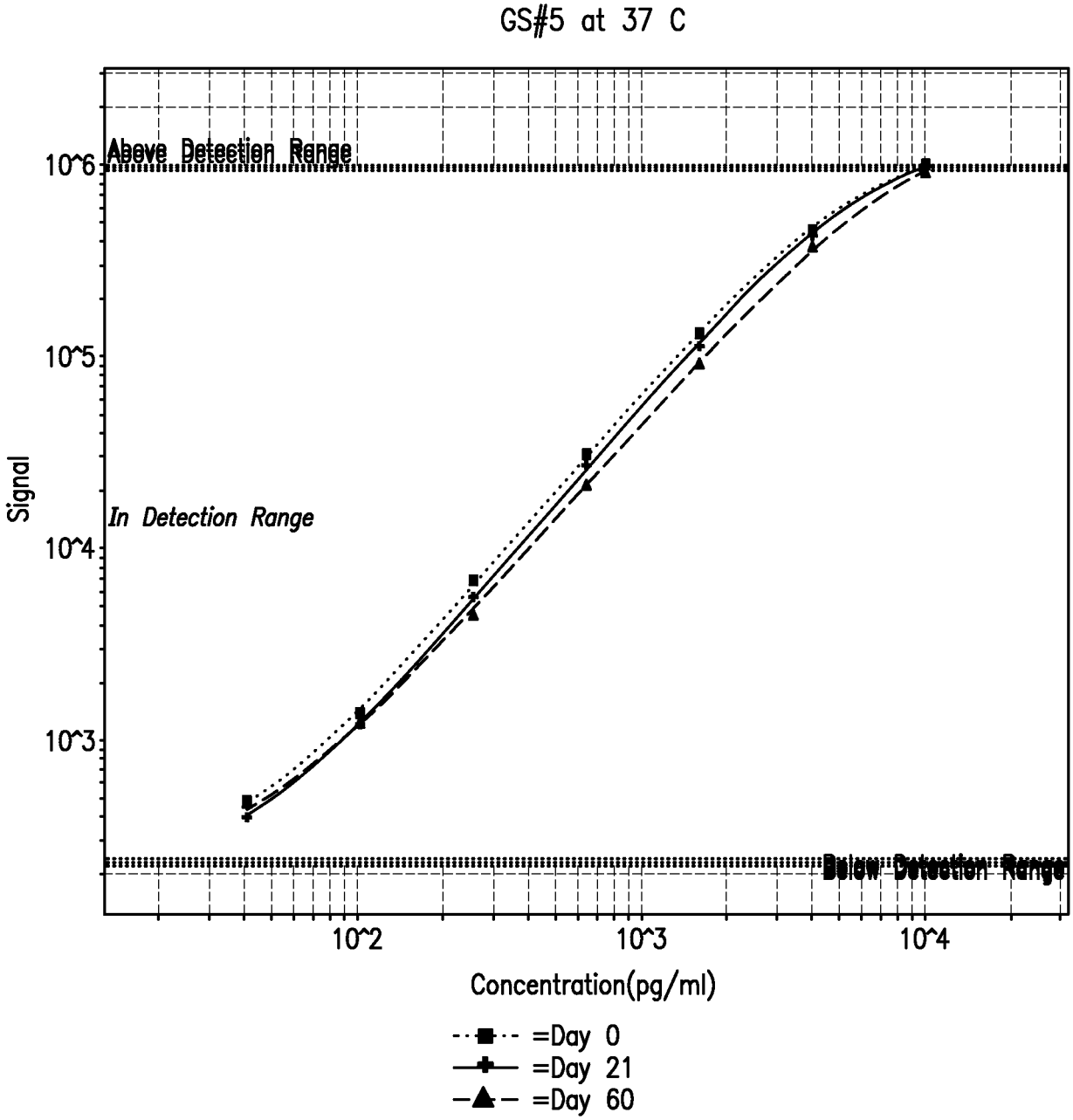

GS#5 at 37 C
Above Detection Range
10^6
10^5
10^4
10^3
Signal
In Detection Range
Below Detection Range
10^2
10^3
10^4
Concentration(pg/ml)
=Day 0
=Day 21
=Day 60

FIG. 10G

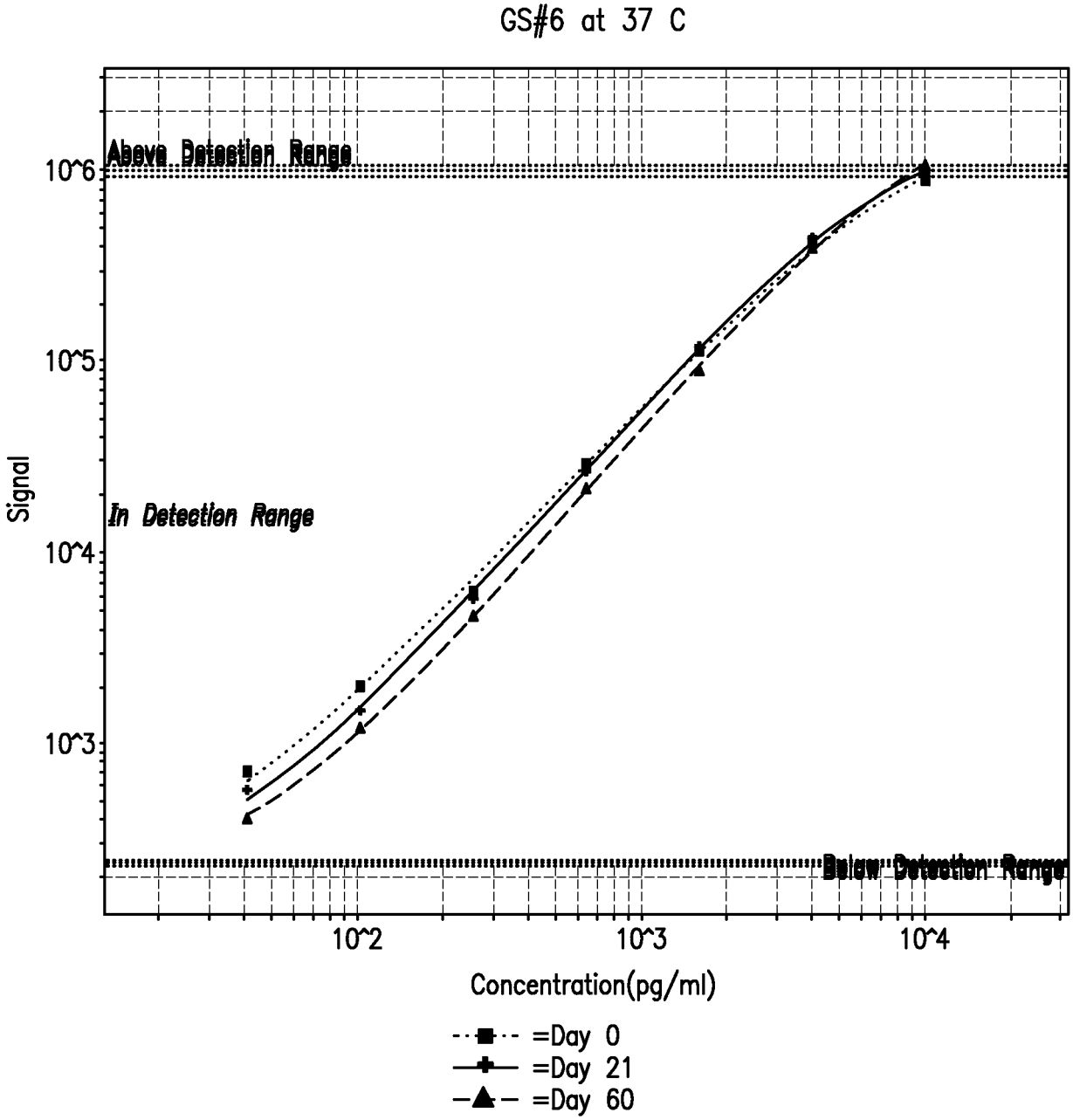

GS#6 at 37 C
Above Detection Range
In Detection Range
Below Detection Range
Signal
10^6
10^5
10^4
10^3
10^2
10^3
10^4
Concentration(pg/ml)
=Day 0
=Day 21
=Day 60

FIG. 10H

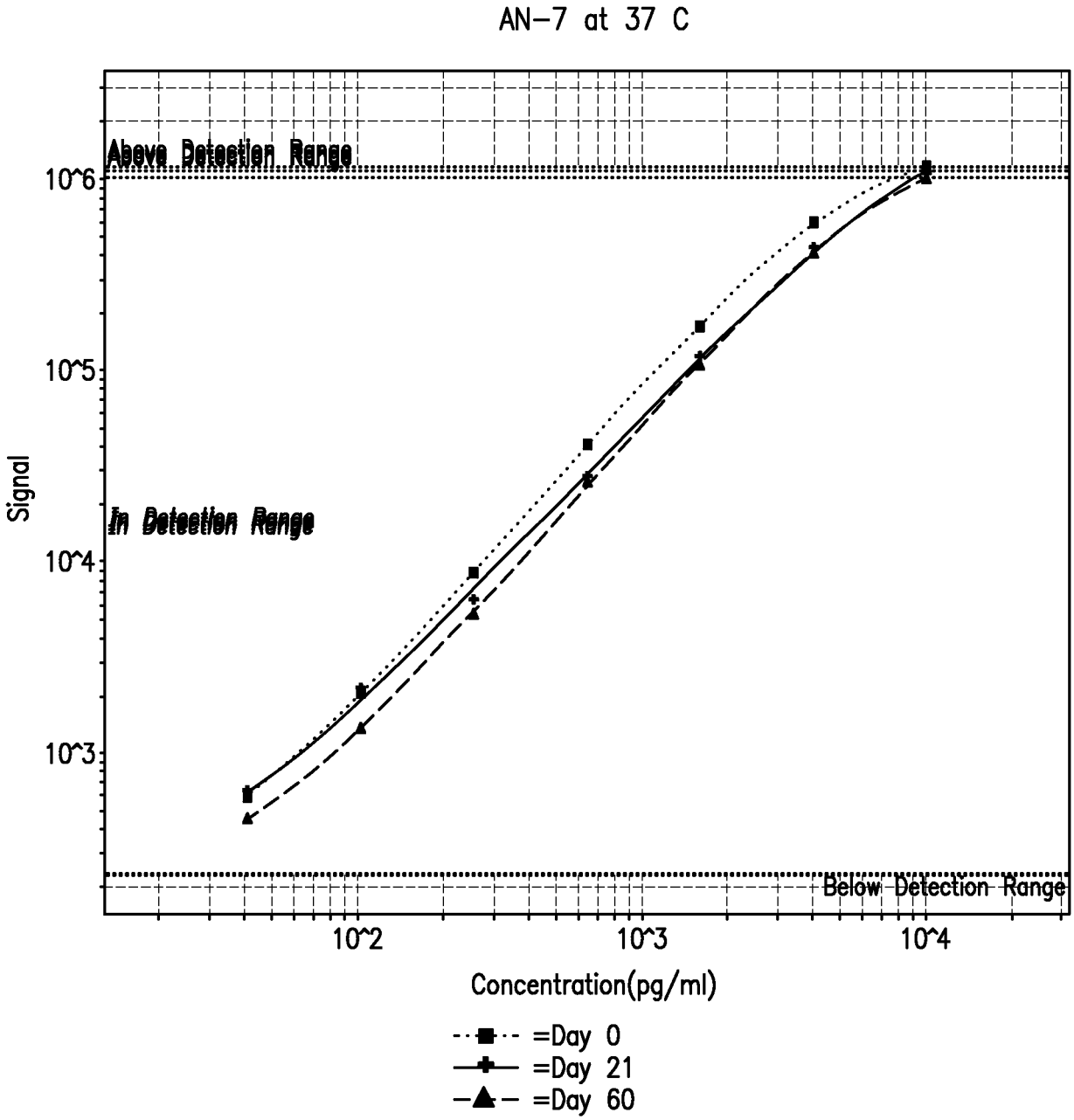

AN-7 at 37 C
Above Detection Range
In Detection Range
Below Detection Range
Signal
10^6
10^5
10^4
10^3
10^2
10^3
10^4
Concentration(pg/ml)
=Day 0
=Day 21
=Day 60

FIG. 10I

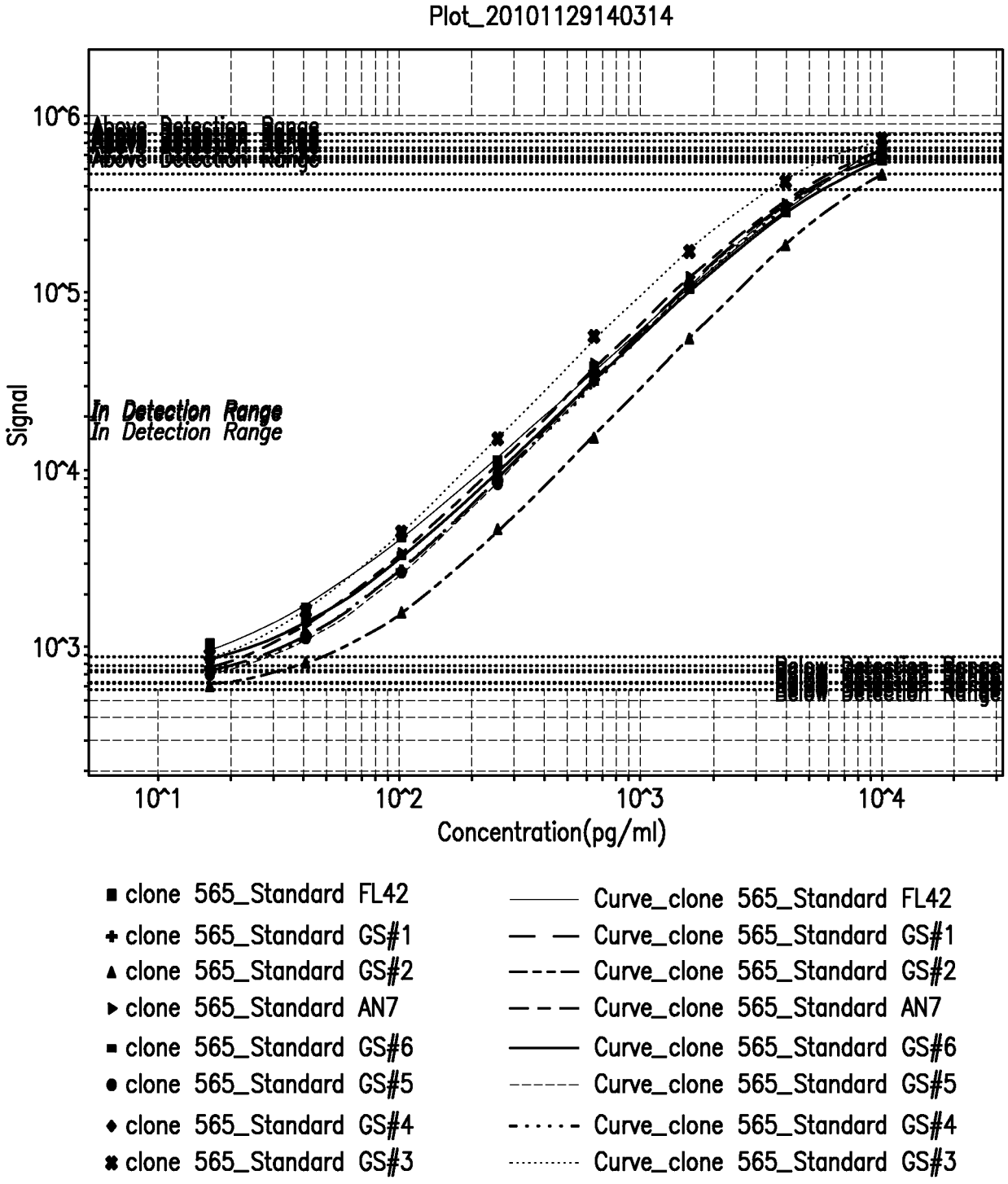

Plot_20101129140314
10^6
10^5
10^4
10^3
Signal
In Detection Range
10^1
10^2
10^3
10^4
Concentration(pg/ml)
clone 565_Standard FL42
clone 565_Standard GS#1
clone 565_Standard GS#2
clone 565_Standard AN7
clone 565_Standard GS#6
clone 565_Standard GS#5
clone 565_Standard GS#4
clone 565_Standard GS#3
Curve_clone 565_Standard FL42
Curve_clone 565_Standard GS#1
Curve_clone 565_Standard GS#2
Curve_clone 565_Standard AN7
Curve_clone 565_Standard GS#6
Curve_clone 565_Standard GS#5
Curve_clone 565_Standard GS#4
Curve_clone 565_Standard GS#3

FIG. 11

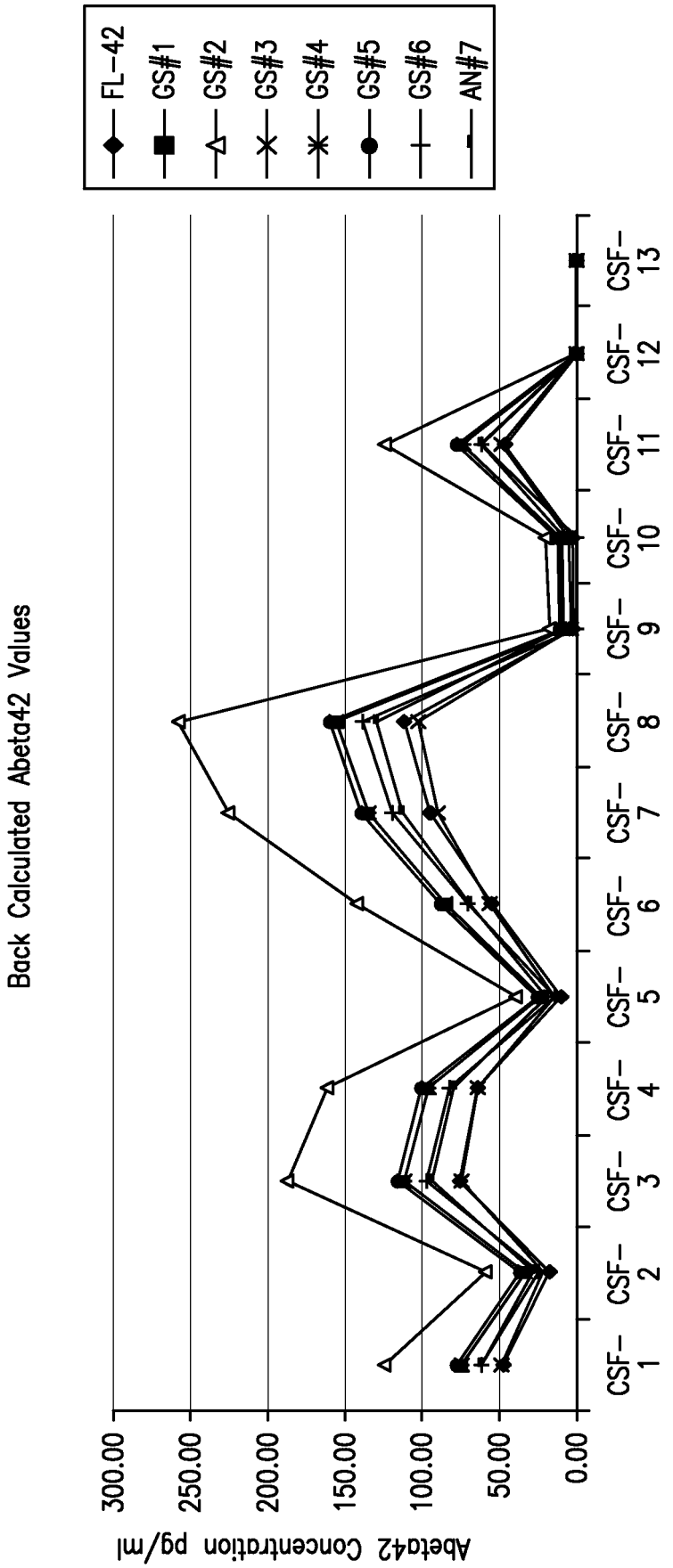
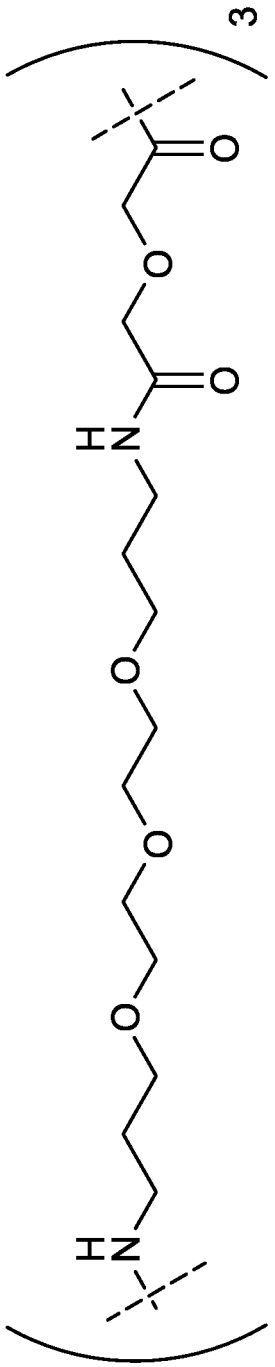
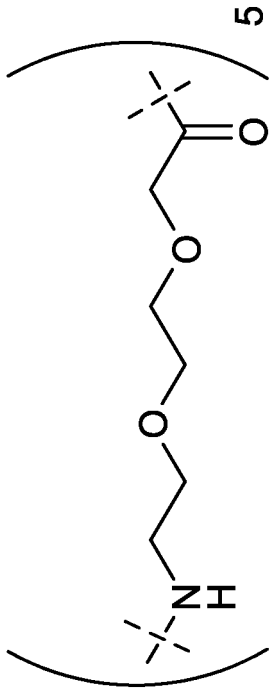

Back Calculated Abeta42 Values
Abeta42 Concentration pg/ml
300.00
250.00
200.00
150.00
100.00
50.00
0.00
CSF- 1
CSF- 2
CSF- 3
CSF- 4
CSF- 5
CSF- 6
CSF- 7
CSF- 8
CSF- 9
CSF- 10
CSF- 11
CSF- 12
CSF- 13
FL-42
GS#1
GS#2
GS#3
GS#4
GS#5
GS#6
AN#7

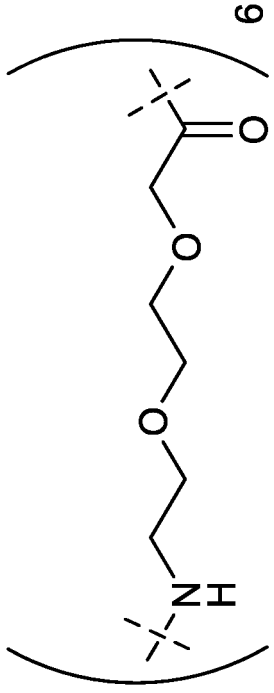
FIG. 12



PEG(20-ATOMS)$_3$



PEG(9-ATOMS)$_5$



PEG(9-ATOMS)$_6$

FIG. 13

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2011/024151

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/68 G01N33/531 C07K19/00
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 C07K

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 practical, search terms used)
EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, INSPEC, 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 2007/090126 A2 (INVITROGEN CORP [US]; BEERNICK HANS T [US]; BRODEY MARY M [US]) 9 August 2007 (2007-08-09) paragraph [0044] paragraph [0050] table 1 examples 1-5 claims 1-42 ----- -/--	1-15

[X] Further documents are listed in the continuation of Box C.

[X] 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 document 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
25 March 2011	29/07/2011

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	Bayer, Martin

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2011/024151

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WURTH C ET AL: "Mutations that Reduce Aggregation of the Alzheimer's Abeta42 Peptide: an Unbiased Search for the Sequence Determinants of Abeta Amyloidogenesis", JOURNAL OF MOLECULAR BIOLOGY, LONDON, GB, vol. 319, no. 5, 21 June 2002 (2002-06-21) , pages 1279-1290, XP004449702, ISSN: 0022-2836, DOI: DOI:10.1016/S0022-2836(02)00399-6 the whole document --- US 2002/182660 A1 (FONG KEI-LAI L [US]) 5 December 2002 (2002-12-05) the whole document ---	1-15 1-15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2011/024151

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

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

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

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

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

see additional sheet

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

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

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

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

1-15(partially)

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-15(partially)

Concerns a composition comprising an N-terminal immunoreactive region, a C-terminal immunoreactive region and a linker region; and related subject-matter.

2-24. claims: 1-15(partially)

Concern SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9. 10, 11, 12, 13, 14, 15, 17, 19, 21, 22, 23, 24, 25, 26 and 27; and related subject-matter.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2011/024151

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2007090126 A2	09-08-2007	EP 1987361 A2	05-11-2008
		US 2010167320 A1	01-07-2010
		US 2008038761 A1	14-02-2008
US 2002182660 A1	05-12-2002	NONE	

专利名称(译)	使用批内校准标准的免疫测定标准和临床生物标志物的测量		
公开(公告)号	EP2534490A1	公开(公告)日	2012-12-19
申请号	EP2011704514	申请日	2011-02-09
[标]申请(专利权)人(译)	百时美施贵宝公司		
申请(专利权)人(译)	施贵宝公司		
当前申请(专利权)人(译)	施贵宝公司		
[标]发明人	RHYNE PAUL MAPELLI CLAUDIO WONG OITAK BERISHA FLORA NEELY ROBERT JOHN		
发明人	RHYNE, PAUL MAPELLI, CLAUDIO WONG, OITAK BERISHA, FLORA NEELY, ROBERT, JOHN		
IPC分类号	G01N33/68 G01N33/531 C07K19/00		
CPC分类号	G01N33/531 G01N33/6896 C07K14/4711 C07K16/28 G01N33/54393 G01N2333/4709 G01N2496/00		
优先权	61/302835 2010-02-09 US		
其他公开文献	EP2534490B1		
外部链接	Espacenet		

摘要(译)

本发明提供了创建定量标准以校准分析物的新型组合物和方法。这些组合物和方法能够产生用于分析分析物和测量临床生物标志物的标准物和校准物。还提供了包含用于测定法，例如夹心免疫测定法的新型组合物的试剂盒。