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Canadian Intellectual Property Office

CA 3017723 C 2022/09/20

(11)(21) 3 017 723

(12) BREVET CANADIEN
CANADIAN PATENT

(13) C

(86) Date de dépôt PCT/PCT Filing Date: 2017/03/16
(87) Date publication PCT/PCT Publication Date: 2017/09/21
(45) Date de délivrance/Issue Date: 2022/09/20
(85) Entrée phase nationale/National Entry: 2018/09/13
(86) N° demande PCT/PCT Application No.: KR 2017/002858
(87) N° publication PCT/PCT Publication No.: 2017/160104
(30) Priorité/Priority: 2016/03/16 (KR10-2016-0031534)

(51) Cl.Int./Int.Cl. *G01N 33/58* (2006.01), *G01N 33/50* (2006.01), *G01N 33/68* (2006.01)

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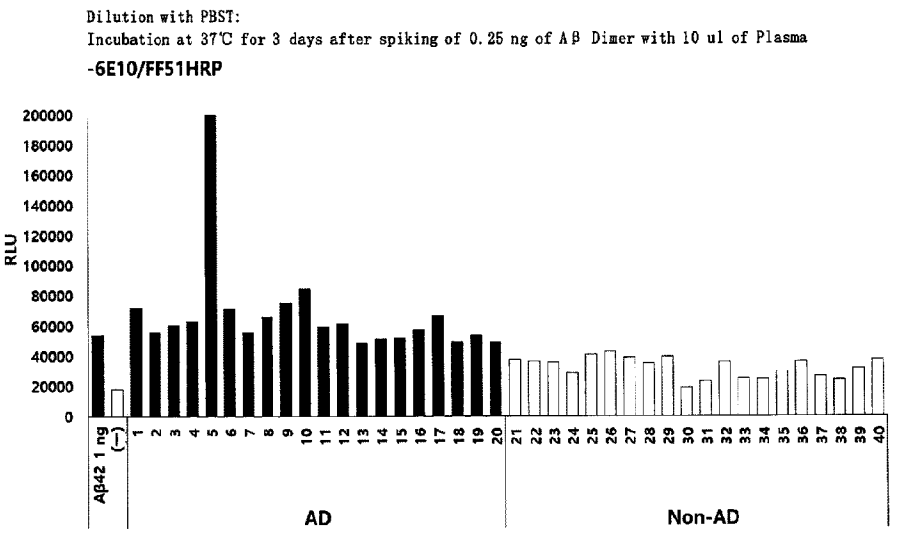
(54) Titre : PROCEDE DE DETECTION D'UN AGREGAT D'UN POLYPEPTIDE FORMANT UN AGREGAT
(54) Title: METHOD FOR DETECTING AGGREGATE OF AGGREGATE-FORMING POLYPEPTIDE



(57) Abrégé/Abstract:
The present invention relates to a method for detecting an aggregate of an aggregate-forming polypeptide of a biosample, comprising (a) a step of spiking a dimer type of the aggregate-forming polypeptide into the biosample to be analyzed; (b) a step of incubating a resultant product of step (a) to further form an aggregate of the aggregate-forming polypeptide; (c) a step of contacting a resultant product of step (b) with a binding agent-tag in which a signal-generating tag is bound to a binding agent that binds to the aggregate of the aggregate-forming polypeptide; and (d) a step of detecting a signal which is generated from the binding agent-tag bound to the aggregate of the aggregate-forming polypeptide.

Abstract

The present invention relates to a method for detecting an aggregate of an aggregate-forming polypeptide of a biosample, comprising (a) a step of spiking a dimer type of the aggregate-forming polypeptide into the biosample to be analyzed; (b) a step of incubating a resultant product of step (a) to further form an aggregate of the aggregate-forming polypeptide; (c) a step of contacting a resultant product of step (b) with a binding agent-tag in which a signal-generating tag is bound to a binding agent that binds to the aggregate of the aggregate-forming polypeptide; and (d) a step of detecting a signal which is generated from the binding agent-tag bound to the aggregate of the aggregate-forming polypeptide.

METHOD FOR DETECTING AGGREGATE OF AGGREGATE-FORMING POLYPEPTIDE

Technical Field

The present patent application claims priority to and the benefit of Korean Patent Application No. 10-2016-0031534 filed in the Korean Intellectual Property Office on March 16 2016.

The present invention relates to a method or kit for detecting an aggregate form of an aggregate-forming polypeptide in a biosample.

Background Art

First, in some cases, polypeptides constituting proteins make functional proteins by forming multimers. However, when polypeptides present as monomers in a normal state form multimers, they aggregate abnormally (e.g., being converted into a misfolded form), and cause diseases (Massimo Stefani, et al., *J. Mol. Med.* 81:678-699(2003); and Radford SE, et al., *Cell.* 97:291-298(1999)).

For example, the diseases or disorders associated with abnormal aggregation or misfolding of proteins include Alzheimer's disease, Creutzfeldt-Jakob disease, spongiform encephalopathies, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, Serpin deficiency, emphysema, cirrhosis, type II diabetes, primary systemic amyloidosis, secondary systemic amyloidosis, frontotemporal dementias, senile systemic amyloidosis, familial amyloid polyneuropathy, hereditary cerebral amyloid angiopathy, and haemodialysis-related amyloidosis.

In measuring the presence or absence or the progress of such diseases or disorders, when such

Date Reçue/Date Received 2021-05-20

measurement is difficult since the amount of the antigen is very small in the sample or the size of the antigen is very small, or when the amount of the antigen in the body is not proportional to the amount of the antigen in the sample, for example, (although the level of Aβ (amyloid-beta), which is implicated in Alzheimer's disease, is known to be higher in an abnormal person than in a normal person), when the amount of the Aβ oligomer in a blood sample is difficult to detect or the Aβ oligomer exits atypically in the blood sample, diagnosis may be difficult.

In addition, the antigen to be measured is too small in size or too small in amount, and thus, the diagnosis of diseases is often not easy by sandwich ELISA.

Accordingly, the present inventors recognized a need for the development of a method for detecting an aggregate form of an aggregate-forming polypeptide, the method maximizing the difference in diagnostic signal between a patient and a normal subject.

Detailed Description of the Invention

Technical Problem

Under the above background, the present inventors have conducted extensive research to develop a novel method for detecting an aggregate form of an aggregate-forming polypeptide, and as a result the present inventors have developed a method for detecting an aggregate form of an aggregate-forming polypeptide, the method maximizing a difference in diagnostic signal between a patient and a normal subject using a difference in the clearing system suppressing the formation of an aggregate form of a polypeptide.

Therefore, an aspect of the present invention is to

provide a method for detecting an aggregate form of an aggregate-forming polypeptide in a biosample.

Another aspect of the present invention is to provide a kit for detecting an aggregate form of an aggregate-forming polypeptide in a biosample.

Technical Solution

In accordance with an aspect of the present invention, there is provided a method for detecting an aggregate form of an aggregate-forming polypeptide in a biosample, the method including the steps of: (a) spiking a dimeric form of the aggregate-forming polypeptide with a biosample to be analyzed; (b) additionally forming an aggregate form of the aggregate-forming polypeptide by incubating a product of step (a); (c) contacting, with a product of step (b), a binder-label in which a signal generation label is conjugated to a binder binding to the aggregate form of the aggregate-forming polypeptide; and (d) detecting a signal generated from the binder-label, which is bound to the aggregate form of the aggregate-forming polypeptide, wherein the incubating in step (b) is carried out for a sufficient incubation time for multimerization of the spiked dimeric form of the aggregate-forming polypeptide by the biosample.

The present invention is directed to a method for detecting an aggregate form of an aggregate-forming polypeptide, the method maximizing the difference in diagnostic signal between a patient and a normal subject using a difference in the clearing system suppressing the formation of an aggregate form of a polypeptide.

As used herein, the term "aggregate-forming polypeptide" refers to a polypeptide capable of forming

a multimeric form (oligomeric form) or forming an aggregate form through hydrophobic interaction with monomers. In particular, the structural changes above cause various diseases. Examples thereof include Alzheimer's disease, Creutzfeldt-Jakob disease, spongiform encephalopathies, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, Serpin deficiency, emphysema, cirrhosis, type II diabetes, primary systemic amyloidosis, secondary systemic amyloidosis, frontotemporal dementias, senile systemic amyloidosis, familial amyloid polyneuropathy, hereditary cerebral amyloid angiopathy, and haemodialysis-related amyloidosis.

Generally, a non-aggregate form of the aggregate-forming polypeptide is normal, but an aggregate form thereof causes, especially, a neurodegenerative disease, such as, Alzheimer's disease, Creutzfeldt-Jakob disease, or Parkinson's disease.

According to an embodiment of the present invention, the biosample for performing the multimerization of the spiked dimeric form of the aggregate-forming polypeptide is a biosample from a human being having a disease associated with the multimeric form of the aggregate-forming polypeptide. More preferably, the sufficient incubation time to perform multimerization by the biosample refers to a time sufficient to enhance a signal generated using the biosample from the human being having a disease associated with the multimeric form of the aggregate-forming polypeptide to be 1.3-20 times greater than a signal generated using a biosample from a normal human being.

Hereinafter, the method of the present invention for detecting an aggregate form of an aggregate-forming

polypeptide in a biosample will be described in detail step by step.

(a) Step of performing spiking

First, the method of the present invention includes a step of spiking a dimeric form of the aggregate-forming polypeptide with a biosample to be analyzed.

As used herein, the term "biosample" refers to an organism-originated sample to be analyzed. The biosample refers to any cell, tissue, or biofluid from a biological source, or any other medium that can be analyzed according to the present invention, and the biosample includes a sample collected from a human being, a sample collected from an animal, and a sample collected from a food for a human being or animal. Preferably, the biosample to be analyzed is a body fluid sample including blood, serum, plasma, lymph, milk, urine, feces, ocular fluid, saliva, semen, brain extracts (e.g., brain homogenates), spinal cord fluid (SCF), appendix, spleen, and tonsillar tissue extracts. More preferably, the biosample is blood, most preferably plasma.

According to another embodiment of the present invention, the aggregate-forming polypeptide includes Aβ peptide and tau protein involved in Alzheimer's disease, prion involved in Creutzfeldt-Jakob disease and sponge foam brain disease, α-synuclein involved in Parkinson's disease, in Ig light chain involved in primary systemic amyloidosis, serum amyloid A involved in secondary systemic amyloidosis, tau protein involved in frontotemporal dementias, transthyretin involved in senile systemic amyloidosis, transthyretin involved in familial amyloid multiple neuropathy, cystatin C involved in hereditary cerebral amyloid angiopathy, β2-microglobulin involved in haemodialysis-related

amyloidosis, Huntingtin involved in Huntington's disease, superoxide dismutase involved in amyotrophic lateral sclerosis, serpin involved in serpin deficiency, pulmonary emphysema, and cirrhosis, and amylin involved in type II diabetes. More preferably, the aggregate-forming polypeptide is Aβ peptide or tau protein involved in Alzheimer's disease, or α-synuclein involved in Parkinson's disease, most preferably, Aβ peptide.

As used herein, the term "spiking" refers to an addition of a dimeric form of an aggregate-forming polypeptide to a biosample to be analyzed or a mixing after addition thereof.

As used herein, the term "multimer" also includes an oligomer.

As used herein, the term "dimer" refers to one formed by combining two monomers.

According to the present invention, in case where a dimeric form of the aggregate-forming polypeptide is spiked with a biosample to be analyzed, the difference in the diagnostic signal between a patient and a normal subject is intended to be maximized using a difference in the clearing system suppressing the formation of an aggregate form of an aggregate-forming polypeptide, that is, a biosample of the patient has a low degree of the clearing system, promoting the formation of an aggregate form of an aggregate-forming polypeptide, but a biosample of a normal subject has a high degree of the clearing system, reducing the formation of an aggregate form of a aggregate-forming polypeptide, thereby maximizing the difference in diagnostic signal.

According to still another embodiment of the present invention, the dimeric form of the aggregate-forming polypeptide is formed by disulfide bonding of two Aβ peptides each composed of the amino acid sequence

of SEQ ID NO: 1, which is a monomeric form of the aggregate-forming polypeptide, by means of the 26th Cys residues of the Aβ peptides each composed of the amino acid sequence of SEQ ID NO: 1.

According to another embodiment of the present invention, a buffer is additionally added to the product in step (a). More preferably, the buffer is added in an amount of 3-15 times (v/v) to a biosample, still more preferably, 5-13 times (v/v), yet more preferably, 7-11 times (v/v), and even more preferably 8-10 times (v/v).

For the buffer used in the present invention, various buffers known in the art may be used, but preferably, the buffer is a non-ionic surfactant-containing phosphate buffer.

For the non-ionic surfactant contained in the phosphate buffer used in the present invention, various non-ionic surfactants known in the art may be used, and preferably the non-ionic surfactant includes alkoxylated alkyl ethers, alkoxylated alkyl esters, alkyl polyglycosides, polyglyceryl esters, polysorbates, and sugar esters. More preferably, Tween-20 or Triton X-100 is used, and most preferably, Tween-20 is used.

(b) Step of additionally forming aggregate form of aggregate-forming polypeptide

Then, the method of the present invention includes (b) step of additionally forming an aggregate form of the aggregate-forming polypeptide by incubating the product of step (a).

One of the features of the present invention is that in cases where a measurement is difficult to make due to a very small amount of an aggregate form of aggregate-forming polypeptide (antigen), which is to be measured, in a sample or a very small size of an aggregate form of aggregate-forming polypeptide or when

the amount of an aggregate form of aggregate-forming polypeptide (antigen) in the body is not proportional to the amount of an aggregate form of aggregate-forming polypeptide (antigen) in the sample, the presence or absence or the progress of diseases or disorders can be measured by spiking the dimeric form of aggregate-forming polypeptide with the biosample to additionally form an aggregate form of aggregate-forming polypeptide.

According to another embodiment of the present invention, the additional forming of the aggregate form of the aggregate-forming polypeptide in step (b) is conducted by incubating the product in step (a) at a temperature of 1-50℃, more preferably 35-50℃, still more preferably 35-45℃, still more preferably 35-40℃.

In the present invention, the incubating in step (b) is conducted for a sufficient time for multimerization of the spiked dimeric form of aggregate-forming polypeptide by the biosample. More preferably, the sufficient incubation time for multimerization by the biosample is a time sufficient to enhance a signal generated using the biosample from the human being having a disease associated with the multimeric form of the aggregate-forming polypeptide to be 1.3-20 times greater than a signal generated using a biosample from a normal human being.

According to still another embodiment of the present invention, the additional forming of the aggregate form of the aggregate-forming polypeptide in step (b), in order to conduct for a time sufficient to enhance a signal generated using the biosample from a human being to be 1.3-20 times greater than a signal generated using a biosample from a normal human being, is conducted by incubating the product in step (a) for 1 to 12 days, preferably for 1 to 10 days, more preferably for 1 to 8 days, still more preferably for 1 to 6 days,

still more preferably for 1 to 6 days, still more preferably for 2 to 6 days, and most preferably for 2 to 5 days.

As used herein, the term "incubation" refers to standing (kept to stand) or shaking a biosample to be analyzed at a predetermined temperature for a predetermined period of time, and the shaking is, preferably, mild shaking.

Another of the greatest features of the present invention is that a biosample is allowed to stand (i.e., incubation) at a predetermined temperature for a predetermined period of time, so that a dimeric form of the aggregate-forming polypeptide and the aggregate-forming polypeptide, which exist in the biosample, aggregate well together, thereby maximizing the difference in diagnostic signal between a patient and a normal subject.

(c) Step of contacting, with product in step (b), binder-label binding to aggregate form of aggregate-forming polypeptide

Then, the method of the present invention includes step (c) of contacting, with a production of step (b), a binder-label in which a signal generating label is conjugated to a binder binding to the aggregate form of the aggregate-forming polypeptide.

In the present invention, the binder binding to the aggregate form of the aggregate-forming polypeptide includes an antibody, a peptide aptamer, an adnectin, an affibody (USP No. 5,831,012), an avimer (Silverman, J. et al, *Nature Biotechnology* 23(12):1556(2005)) or a Kunitz domain (Arnoux B et al., *Acta Crystallogr. D Biol. Crystallogr.* 58(Pt 7):12524(2002), and Nixon, AE, *Current opinion in drug discovery & development*

9(2):2618(2006)).

In the present invention, the signal generation label, which is conjugated to the binder binding to the aggregate form of the aggregate-forming polypeptide, includes a compound label (e.g., biotin), an enzyme label (e.g., alkaline phosphatase, peroxidase, β-galactosidase, and β-glucosidase), a radioactive label (e.g., I^{125} and C^{14}), a fluorescent label (e.g., fluorescein), a luminescent label, a chemiluminescent label, and a fluorescence resonance energy transfer (FRET) label, but is not limited thereto.

(d) Step of detecting signal generated from binder-label bound to aggregate form of aggregate-forming polypeptide

Last, the method of the present invention includes step (d) of detecting a signal generated from the binder-label bound to the aggregate form of the aggregate-forming polypeptide.

The detecting of the signal generated from the binder-label bound to the aggregate form of the aggregate-forming polypeptide may be conducted by various methods known in the art, and for example, an immunoassay method associated with an antigen-antibody response may be used.

According to still another embodiment of the present invention, steps (c) and (d) are performed by a method including the following steps: (c-1) contacting the product of step (b) with a capture antibody recognizing an epitope on the aggregate-forming polypeptide capturing the aggregate form; (c-2) contacting the captured aggregate form with a detection antibody recognizing an epitope on the aggregate-forming polypeptide; and (c-3) detecting an aggregate form-detection antibody complex.

Such a detection method employs two types of antibodies, namely, a capture antibody and a detection antibody. As used herein, the term "capture antibody" refers to an antibody that can bind to an aggregate-forming polypeptide to be detected in a biosample. The term "detection antibody" refers to an antibody that can bind to an aggregate-forming polypeptide captured by the capture antibody. The term "antibody" refers to an immunoglobulin protein that can bind to an antigen. The antibody used herein includes antibody fragments (e.g., F(ab')2, Fab', Fab, Fv) as well as a whole antibody that can bind to an epitope, an antigen, or an antigen fragment.

The detection method employs one set of a capture antibody and a detection antibody, which specifically recognize epitopes on an aggregate-forming polypeptide, and the epitopes specifically recognized by the capture antibody and the detection antibody are identical to or are overlapped with each other.

As used herein to recite the epitopes with respect to the capture antibody and the detection antibody, the term "overlapped with" encompasses epitopes having completely or partially overlapped with amino acid sequences. For example, epitopes to 6E10, FF51, and WO2 antibodies have the amino acid sequences including amino acids 3-8, 1-4, and 4-10, respectively, of the human Aβ peptide sequence. Such epitopes may be explained as completely overlapped epitopes.

According to another embodiment of the present invention, the epitopes, when expressed to recite the human Aβ peptide sequence, have the amino acid sequence of amino acids 3-8, 1-4, or 4-10.

According to still another embodiment of the

present invention, the epitope recognized by the capture antibody has a sequence that is not repeated in the aggregate-forming polypeptide, and the epitope recognized by the detection antibody has a sequence that is not repeated in the aggregate-forming polypeptide. According to the detection method of the present invention, the aggregate-forming polypeptide bound to the capture antibody cannot bind to the detection antibody any more, and the reason is that there is no additional epitope recognized by the detection antibody.

According to another embodiment of the present invention, the capture antibody and the detection antibody are identical to each other. That is, the epitopes, specifically bound to the capture antibody and the detection antibody, are preferably identical to each other.

According to still another embodiment of the present invention, the capture antibody binds to a solid substrate. Such a known material includes polystyrene, polypropylene, glass, metal, and a hydrocarbon polymer, such as a gel. The solid substrate may be present in the form of a dipstick, a microtiter plate, particle (e.g., bead), an affinity column, and an immunoblot membrane (e.g., a polyvinylidene fluoride membrane) (see, USP 5,143,825, 5,374,530, 4,908,305, and 5,498,551).

According to another embodiment of the present invention, the detection antibody has a label generating a detectable signal. The label includes a compound label (e.g., biotin), an enzyme label (e.g., alkaline phosphatase, peroxidase, β-galactosidase, and β-glucosidase), a radioactive label (e.g., I^{125} and C^{14}), a fluorescent label (e.g., fluorescein), a luminescent label, a chemiluminescent label, and a fluorescence resonance energy transfer (FRET) label, but is not limited thereto. Various labels and methods for labeling

antibodies are known in the art (Harlow and Lane, eds. *Antibodies: A Laboratory Manual* (1988) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.).

In the present invention, the antibodies that can be bound to the aggregate-forming polypeptide may be prepared using epitopes previously described as immunogens according to the prior art, such as a fusion method (Kohler and Milstein, *European Journal of Immunology*, 6:511-519(1976)), a recombinant DNA method (USP 4,816,567), or a phage antibody library method (Clackson et al, *Nature*, 352:624-628(1991) and Marks et al, *J. Mol. Biol.*, 222:58, 1-597(1991)). General methods for preparing the antibodies are described in Harlow, E. and Lane, D., *Antibodies: A Laboratory Manual*, Cold Spring Harbor Press, New York, 1988; Zola, H., *Monoclonal Antibodies: A Manual of Techniques*, CRC Press, Inc., Boca Raton, Florida, 1984; and Coligan, *CURRENT PROTOCOLS IN IMMUNOLOGY*, Wiley/Greene, NY, 1991.

The preparation of hybridoma cell lines for the production of monoclonal antibodies is conducted by the fusion of an immortal cell line and antibody-producing lymphocytes. The preparation of monoclonal antibodies may be conducted using techniques known in the art. The polyclonal antibodies may be prepared by injecting the foregoing antigen into a suitable animal, collecting anti-serum containing an antibody, and then isolating the antibody by a method for isolating an antibody through a known affinity technique.

The detection of the aggregate form-detection antibody complex may be conducted by various methods known in the art. The formation of the aggregate form-detection antibody complex shows the presence of the aggregate form in the biosample. The step above may be quantitatively or qualitatively conducted using various detectable label/substrate pairs disclosed in, for

example, *Enzyme Immunoassay*, E. T. Maggio, ed., CRC Press, Boca Raton, Florida, 1980 and Harlow and Lane, eds. *Antibodies: A Laboratory Manual*(1988) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., by the conventional methods.

In cases where the detection antibody is labeled with alkaline phosphatase, bromochloroindolylphosphate (BCIP), nitro blue tetrazolium (NBT), or ECF may be used as a substrate for a color development reaction; in cases where the detection antibody is labeled with horseradish peroxidase, chloronaphthol, aminoethyl carbazole, diaminobenzidine, D-luciferin, lucigenin (bis-N-methylacridinium nitrate), resorufin benzyl ether, luminol, Amplex Red reagent (10-acetyl-3,7-dihydroxyphenoxazine), TMB (3,3,5,5-tetramethylbenzidine), enhanced chemiluminescence (ECL), or ABTS (2,2-azine-di[3-ethylbenzthiazoline sulfonate]) may be used as a substrate.

Through such methods, the signal generated using a biosample from a human being having a disease associated with a multimeric form of an aggregate-forming polypeptide can be enhanced compared with the signal generated using a biosample from a normal human being by 1.3-20 times, preferably by 1.5-10 times, and more preferably by 1.6-10 times.

In accordance with another aspect of the present invention, there is provided a kit for detecting an aggregate form of an aggregate-forming polypeptide in a biosample containing a dimeric form of an aggregate-forming polypeptide.

The kit of the present invention uses the foregoing method for detecting an aggregate form of an aggregate-forming polypeptide in a biosample of the present

invention, and thus the description of overlapping contents therebetween will be omitted to avoid excessive complexity of the specification due to repetitive descriptions thereof.

According to another embodiment of the present invention, the kit further comprises: a capture antibody recognizing an epitope on the aggregate-forming polypeptide; and a detection antibody recognizing the epitope recognized by the capture antibody.

Advantageous Effects

Features and advantages of the present invention are summarized as follows.

(a) The present invention provides a method or kit for detecting an aggregate form of an aggregate-forming polypeptide in a biosample.

(b) In the method of the present invention, in cases where a measurement is difficult to make due to a very small amount of an aggregate form of aggregate-forming polypeptide (antigen), which is to be measured, in a sample or a very small size of an aggregate form of aggregate-forming polypeptide or when the amount of an aggregate form of aggregate-forming polypeptide (antigen) in the body is not proportional to the amount of an aggregate form of aggregate-forming polypeptide (antigen) in the sample, the difference in diagnostic signal between a patient and a normal subject is maximized by using the difference in the clearing system that suppresses the formation of an aggregate form of a polypeptide.

(c) The present invention can be carried out in a convenient and prompt manner, and can automate a method for detecting an aggregate form of an aggregate-forming polypeptide in a biosample.

Brief Description of the Drawings

FIGS. 1 and 2 show the detection results of Aβ oligomer using multimer detection system (MDS) (6E10/FF51HRP set) from samples incubated for 3 and 4 days after treatment with S26C-Beta-Amyloid (1-40) Dimer according to examples of the present invention.

FIG. 3 shows the detection results of Aβ oligomer using multimer detection system (MDS) (6E10/FF51HRP set) from samples incubated for 0, 1, 2, 3, 4, and 5 days after treatment with S26C-Beta-Amyloid (1-40) Dimer according to an example of the present invention.

FIG. 4 shows the detection results of Aβ oligomer using multimer detection system (MDS) (6E10/FF51HRP set) from samples incubated for 0 and 5 days after treatment with or without 0.25 ng of S26C-Beta-Amyloid (1-40) Dimer according to an example of the present invention.

FIGS. 5 and 6 show the detection results of Aβ oligomer using multimer detection system (MDS) (6E10/WO2HRP set) from samples incubated for 1 and 2 days after treatment with S26C-Beta-Amyloid (1-40) Dimer according to examples of the present invention.

FIG. 7 shows the detection results of Aβ oligomer using multimer detection system (MDS) (6E10/WO2HRP set) from samples incubated for 1, 2, 3, and 4 days after treatment with S26C-Beta-Amyloid (1-40) Dimer according to an example of the present invention.

Mode for Carrying Out the Invention

Hereinafter, the present invention will be described in detail with reference to examples. These examples are only for illustrating the present invention more specifically, and it will be apparent to those skilled in the art that the scope of the present invention is not limited by these examples.

EXAMPLES

Example 1: Materials

A coating buffer (Carbonate-Bicarbonate Buffer), PBST, TBST, and PBS were purchased from Sigma. Block Ace was purchased from Bio-rad. Buffer A was prepared by diluting Block Ace to 0.4% in TBST. A blocking buffer was prepared by diluting 1% Block Ace to 0.4% in TBST. HBR1 was purchased from Scantibodies Laboratory. 6E10 antibody was purchased from Biolegend. WO2-HRP antibody was purchased from Absolute Antibody. FF51-HRP was purchased from The H lab. WO2-HRP antibody was purchased from Absolute Antibody. Recombinant Aβ1-42 was purchased from Biolegend. Recombinant S26C-Beta-Amyloid (1-40) Dimer was purchased from JPT. Plasma samples were obtained from Seoul National University Bundang Hospital and Chungang University Hospital. ECL solution was purchased from Rockland. Plates were purchased from Nunc. Epitopes to 6E10, FF51, and WO2 antibodies have the amino acid sequences including amino acids 3-8, 1-4, and 4-10, respectively, of the human Aβ peptide sequence. The sequence of S26C-Beta-Amyloid (1-40) dimer is DAEFRHDSGYEVHHQKLVFFAEDVGCNKGAIIGLMVGGVV, and has a dimeric form from a disulfide bond of the 26th cysteine residues of respective monomers.

Example 2: Preparation of 6E10 plate

After 30 μg of 6E10 antibody (anti-Aβ protein, Biolegend) was diluted in 10 ml of a coating buffer (Sigma), 100 μl was dispensed into each well in a plate (Nunc), followed by reaction in a refrigerator at 4 for one day. The plate was washed three times with PBS, and 240 μl of a blocking buffer in which 1% Block Ace was dissolved in D.W. was dispensed, followed by reaction at room temperature for 2 hours or more. The plate was washed with three times with BPS, and was then dried at

room temperature for 30 minutes before.

Example 3: Preparation of control

For a positive control, 990 µl of PBST was added to 10 µl of recombinant Aβ1-42(rec. Aβ) (1 µg/ml), and 100 µl of the resulting product was used. For a negative control, 100 µl of PBS was used.

Example 4: Preparation of samples

Sample preparation was made based on two types of samples. Frozen plasma samples were dissolved in a 37℃ heat block for 15 minutes, followed by vortexing for 30 seconds before use. For samples spiked with 0.25 ng of S26C-Beta-Amyloid (1-40) Dimer, 8.08 µl of HBR1 (0.123 mg/ml), 180 µl of PBST, and 20 µl of S26C-Beta-Amyloid (1-40) Dimer(0.25 ng/10 µl) were mixed with 20 µl of plasma to prepare a total of 228.08 µl. For samples spiked with a recombinant peptide, 8.08 µl of HBR1 (0.123 mg/ml) and 200 µl of PBST were mixed with 20 µl of plasma to prepare a total of 228.08 µl.

Example 5: Incubation

The samples prepared by the treatment with S26C-Beta-Amyloid (1-40) Dimer in example 4 were incubated in a 37℃ incubator for 0, 1, 2, 3, 4, and 5 days, respectively (6E10/FF51HRP set). The samples prepared by the treatment without S26C-Beta-Amyloid (1-40) Dimer in example 4 were incubated in a 37℃ incubator for 0 and 5 days, respectively (6E10/FF51HRP set). In addition, the samples prepared by the treatment with S26C-Beta-Amyloid (1-40) Dimer in example 4 were incubated in a 37℃ incubator for 0, 1, 2, 3, 4, and 5 days, respectively (6E10/WO2HRP set).

Example 6: 6E10/FF51HRP set: Detection of Aβ

oligomer using multimer detection system (MDS) from samples incubated for 3 and 4 days after treatment with S26C-Beta-Amyloid (1-40) Dimer

The positive control, the negative control, and the samples treated with 0.25 ng of S26C-Beta-Amyloid (1-40) Dimer and incubated for 3 and 4 days were dispensed in 100 µl each on 6E10 coated plate (3 µg/ml), followed by reaction at room temperature for 1 hour. The plate was washed three times with TBST. FF51-HRP antibody was added to buffer A to reach 0.5 µg/ml, and then 100 ul each was dispensed. The plate was washed three times with TBST, and 100 µl of the ECL solution was dispensed. The plate reacted with ELC was inserted into a luminometer (PerkinElmer) to measure a luminescent signal. The results are shown in FIGS. 1 and 2.

FIGS. 1 and 2 show signal changes in AD samples and Non AD samples according to the incubation time after the addition of S26C-Beta-Amyloid (1-40) Dimer. A difference between AD and Non AD is shown in each condition of incubation for 3 and 4 days.

It is determined from FIGS. 1 and 2 that the reason why signals of the Aβ oligomer were higher in the AD patient samples compared with the Non AD patient samples is that the clearing system suppressing the formation of Aβ oligomer in the AD patient samples was less activated than that in the Non AD patient samples.

Example 7: 6E10/FF51HRP set: Detection of Aβ oligomer using multimer detection system (MDS) from samples incubated for 0 and 4 days after treatment with S26C-Beta-Amyloid (1-40) Dimer

The positive control, the negative control, and the samples treated with 0.25 g of S26C-Beta-Amyloid (1-40) Dimer and incubated for 0, 1, 2, 3, 4, and 5 days were dispensed in 100 µl each on 6E10 coated plate (3 µg/ml),

followed by reaction at room temperature for 1 hour. The plate was washed three times with TBST. FF51-HRP antibody was added to buffer A to reach 0.5 μg/ml, and then 100 ul each was dispensed. The plate was washed three times with TBST, and 100 μl of the ECL solution was dispensed. The plate reacted with ELC was inserted into a luminometer (PerkinElmer) to measure a luminescent signal. Results are shown in FIG. 3.

FIG. 3 shows a graph confirming signal changes in AD samples and Non AD samples according to the incubation time after the addition of S26C-Beta-Amyloid (1-40) Dimer. FIG. 3 shows that the signals of the AD samples were increased by 1.15 times, 1.34 times, 1.64 times, 2.14 times, 3.01 times, and 3.35 times compared with the signals of the Non AD samples.

It is determined from FIG. 3 that the reason why the signals of the Aβ oligomer were higher in the AD patient samples compared with the Non AD patient samples is that the clearing system suppressing the formation of the Aβ oligomer in the AD patient samples was less activated than that in the Non AD patient samples.

Example 8: 6E10/FF51HRP set: Detection of Aβ oligomer using multimer detection system (MDS) from samples incubated for 0 and 5 days after treatment with and without S26C-Beta-Amyloid (1-40) Dimer

The positive control, the negative control, the samples treated with or without 0.25 ng of S26C-Beta-Amyloid (1-40) Dimer and incubated for 0 and 5 days were dispensed in 100 μl each on 6E10 coated plate (0 μg/ml), followed by reaction at room temperature for 1 hour. The plate was washed three times with TBST. FF51-HRP antibody was added to buffer A to reach 0.5 μg/ml, and then 100 ul each was dispensed. The plate was washed three times with TBST, and 100 μl of the ECL solution

was dispensed. The plate reacted with ELC was inserted into a luminometer (PerkinElmer) to measure a luminescent signal. Results are shown in FIG. 4.

FIG. 4 shows the Aβ oligomer measurement data in the samples without or with the addition of S26C-Beta-Amyloid (1-40) Dimer and then incubated for 0 and 5 days. FIG. 4 shows that the signals of the AD samples were increased compared with the signals of the Non AD samples according to the time the absence and presence of spiking with Beta-Amyloid (1-40) Dimer.

The samples spiked without S26C-Beta-Amyloid (1-40) Dimer and incubated for 0 and 5 days showed 1.09-fold and 1.97-fold differences in the AD signals compared with the Non AD signals, respectively, and the variation of Aβ oligomer was a 1.8-fold increase from day 0 to day 5. Whereas, the samples spiked with S26C-Beta-Amyloid (1-40) Dimer and incubated for 0 and 5 days showed 1.1-fold and 3.47-fold differences in the AD signals compared with the Non AD signals, respectively, and the variation of Aβ oligomer was a 3.15-fold increase from day 0 to day 5.

It is determined from FIG. 4 that the reason why the signals of the Aβ oligomer were higher in the AD patient samples compared with the Non AD patient samples is that the clearing system suppressing the formation of the Aβ oligomer in the AD patient samples was less activated than that in the Non AD patient samples.

Example 9: 6E10/WO2HRP set: Detection of Aβ oligomer using multimer detection system (MDS) from samples incubated for 1 and 2 days after treatment with S26C-Beta-Amyloid (1-40) Dimer

The positive control, the negative control, and the samples treated with 0.25 ng of S26C-Beta-Amyloid (1-40) Dimer and incubated for 1 and 2 days were dispensed in

100 µl each on 6E10 coated plate (3 µg/ml), followed by reaction at room temperature for 1 hour. The plate was washed three times with TBST. WO2-HRP antibody was added to buffer A to reach 0.25 µg/ml, and then 100 ul each was dispensed. The plate was washed three times with TBST, and 100 µl of the ECL solution was dispensed. The plate reacted with ELC was inserted into a luminometer (PerkinElmer) to measure a luminescent signal. The results are shown in FIGS. 5 and 6.

FIGS. 5 and 6 show signal changes in AD samples and Non AD samples according to the incubation time after the addition of S26C-Beta-Amyloid (4-40) Dimer. A difference between AD and Non AD is shown in each condition of incubation for 1 and 2 days.

Considering FIGS. 5 and 6, the reason why signals of the Aβ oligomer were higher in the AD patient samples compared with the Non AD patient samples is considered to be that the clearing system suppressing the formation of Aβ oligomer in the AD patient samples was less activated than that in the Non AD patient samples.

Example 10: 6E10/WO2HRP set: Detection of Aβ oligomer using multimer detection system (MDS) from samples incubated for 1 and 2 days after treatment with S26C-Beta-Amyloid (1-40) Dimer

The positive control, the negative control, and the samples treated with 0.25 ng of S26C-Beta-Amyloid (1-40) Dimer and incubated for 3 and 4 days were dispensed in 100 µl each on 6E10 coated plate (3 µg/ml), followed by reaction at room temperature for 1 hour. The plate was washed three times with TBST. WO2-HRP antibody was added to buffer A to reach 0.25 µg/ml, and then 100 ul each was dispensed. The plate was washed three times with TBST, and 100 µl of the ECL solution was dispensed. The plate reacted with ELC was inserted into a luminometer

(PerkinElmer) to measure a luminescent signal. Results are shown in FIG. 7.

FIG. 7 shows a graph confirming signal changes in AD samples and Non AD samples according to the incubation time after the addition of S26C-Beta-Amyloid (5-40) Dimer. As a result, on day 1 of incubation, the average signal of AD samples was 1.19-fold greater than that of Non AD samples, and the sample signals were increased overall. Whereas, on days 2, 3, and 4, the sample signal values, which have been high overall, was dropped and thus the AD samples showed 1.69-fold, 1.50-fold, and 1.41-fold differences compared with the Non AD samples.

It is determined from FIG. 7 that the reason why the signals of the Aβ oligomer were higher in the AD patient samples compared with the Non AD patient samples is that the clearing system suppressing the formation of the Aβ oligomer in the AD patient samples was less activated than that in the Non AD patient samples.

Claims

1. A method for detecting an aggregate form of an aggregate-forming polypeptide in a biosample, the method comprising the steps of:

(a) spiking a dimeric form of the aggregate-forming polypeptide with the biosample to be analyzed to form a product;

(b) forming the aggregate form of the aggregate-forming polypeptide by incubating the product of step (a);

(c) contacting, with the aggregate form of the aggregate-forming polypeptide of step (b), a binder-label in which a signal generating label is conjugated to a binder binding to the aggregate form of the aggregate-forming polypeptide; and

(d) detecting a signal generated from the binder-label bound to the aggregate form of the aggregate-forming polypeptide,

wherein the incubating in step (b) is carried out for a sufficient time for multimerization of the spiked dimeric form of the aggregate-forming polypeptide by the biosample.

2. The method of claim 1, wherein the biosample multimerizing the spiked dimeric form of the aggregate-forming polypeptide is a biosample from a human being having a disease associated with the multimeric form of the aggregate-forming polypeptide.

3. The method of claim 2, wherein the sufficient incubation time for multimerization by the biosample is a time sufficient to enhance a signal generated using the biosample from the human being having a disease associated with the multimeric form of

the aggregate-forming polypeptide to be 1.3-20 times greater than a signal generated using a biosample from a normal human being.

4. The method of any one of claims 1 to 3, wherein the biosample is blood.

5. The method of any one of claims 1 to 4, wherein the biosample is plasma.

6. The method of any one of claims 1 to 5, wherein the aggregate-forming polypeptide is Aβ peptide, tau protein, prion, α-synuclein, Ig light chain, serum amyloid A, transthyretin, cystatin C, β2-microglobulin, Huntingtin, superoxide dismutase, serpin, or amylin.

7. The method of any one of claims 1 to 6, wherein the aggregate-forming polypeptide is Aβ peptide.

8. The method of claim 7, wherein the dimeric form of the aggregate-forming polypeptide is a dimeric form formed by disulfide bonding between Cys residues at the 26^{th} amino acid position of each Aβ peptide, each Aβ peptide comprising the amino acid sequences of SEQ ID NO: 1.

9. The method of any one of claims 1 to 8, wherein a buffer is additionally added to the product of step (a).

10. The method of claim 9, wherein the buffer is added in an amount of 3-15 times (v/v) relative to an amount of the biosample.

11. The method of claim 9 or 10, wherein the

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buffer is a non-ionic surfactant-containing phosphate buffer.

12. The method of any one of claims 1 to 11, wherein the forming of the aggregate form of the aggregate-forming polypeptide in step (b) is carried out by incubating the product of step (a) at a temperature of 1-50°C.

13. The method of any one of claims 1 to 12, wherein steps (c) and (d) are performed by the following steps:

(c-1) contacting the aggregate form of the aggregate-forming polypeptide of step (b) with a capture antibody recognizing an epitope on the aggregate-forming polypeptide, thereby capturing the aggregate form;

(c-2) contacting the captured aggregate form of step (c-1) with the binder-label recognizing an epitope on the aggregate-forming polypeptide, wherein the binder is selected from the group consisting of an antibody, a peptide aptamer, an adnectin, an affibody, an avimer, and a Kunitz domain; and

(d-1) detecting an aggregate form-detection antibody complex.

14. The method of claim 13, wherein the binder is a detection antibody recognizing an epitope identical to or overlapped with the epitope in step (c-1).

15. The method of claim 13, wherein the capture antibody is bound to a solid substrate.

16. The method of claim 13, wherein the label generates a detectable signal.

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17. The method of claim 16, wherein the label conjugated to the detection antibody includes a compound label, an enzyme label, a radioactive label, a fluorescent label, a luminescent label, a chemiluminescent label, or a FRET label.

18. A kit for detecting an aggregate form of an aggregate-forming polypeptide in a biosample, the kit comprising a dimeric form of an aggregate-forming polypeptide and instructions for use.

19. The kit of claim 18, wherein the biosample is blood.

20. The kit of claim 18 or 19, wherein the biosample is plasma.

21. The kit of any one of claims 18 to 20, wherein the aggregate-forming polypeptide is: Aβ peptide, tau protein, prion, α-synuclein, Ig light chain, serum amyloid A, transthyretin, cystatin C, β2-microglobulin, Huntingtin, superoxide dismutase, serpin, or amylin.

22. The kit of any one of claims 18 to 21, wherein the aggregate-forming polypeptide is Aβ peptide.

23. The kit of claim 22, wherein the dimeric form of the aggregate-forming polypeptide is a dimeric form formed by disulfide bonding between Cys residues at the 26^{th} amino acid position of each Aβ peptide, each Aβ peptide comprising the amino acid sequence of SEQ ID NO: 1.

24. The kit of any one of claims 18 to 23,

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wherein the kit further comprises a buffer.

25. The kit of claim 24, wherein the buffer is a non-ionic surfactant-containing phosphate buffer.

26. The kit of any one of claims 18 to 25, wherein the kit further comprising: a capture antibody recognizing an epitope on the aggregate-forming polypeptide; and a detection antibody recognizing the epitope recognized by the capture antibody.

27. The kit of claim 26, wherein the detection antibody is a detection antibody recognizing an epitope identical to or overlapped with the epitope recognized by the capture antibody.

28. The kit of claim 26, wherein the capture antibody is bound to a solid substrate.

29. The kit of claim 26, wherein the detection antibody has a label generating a detectable signal.

30. The kit of claim 29, wherein the label bound to the detection antibody includes a compound label, an enzyme label, a radioactive label, a fluorescent label, a luminescent label, a chemiluminescent label, or a FRET label.

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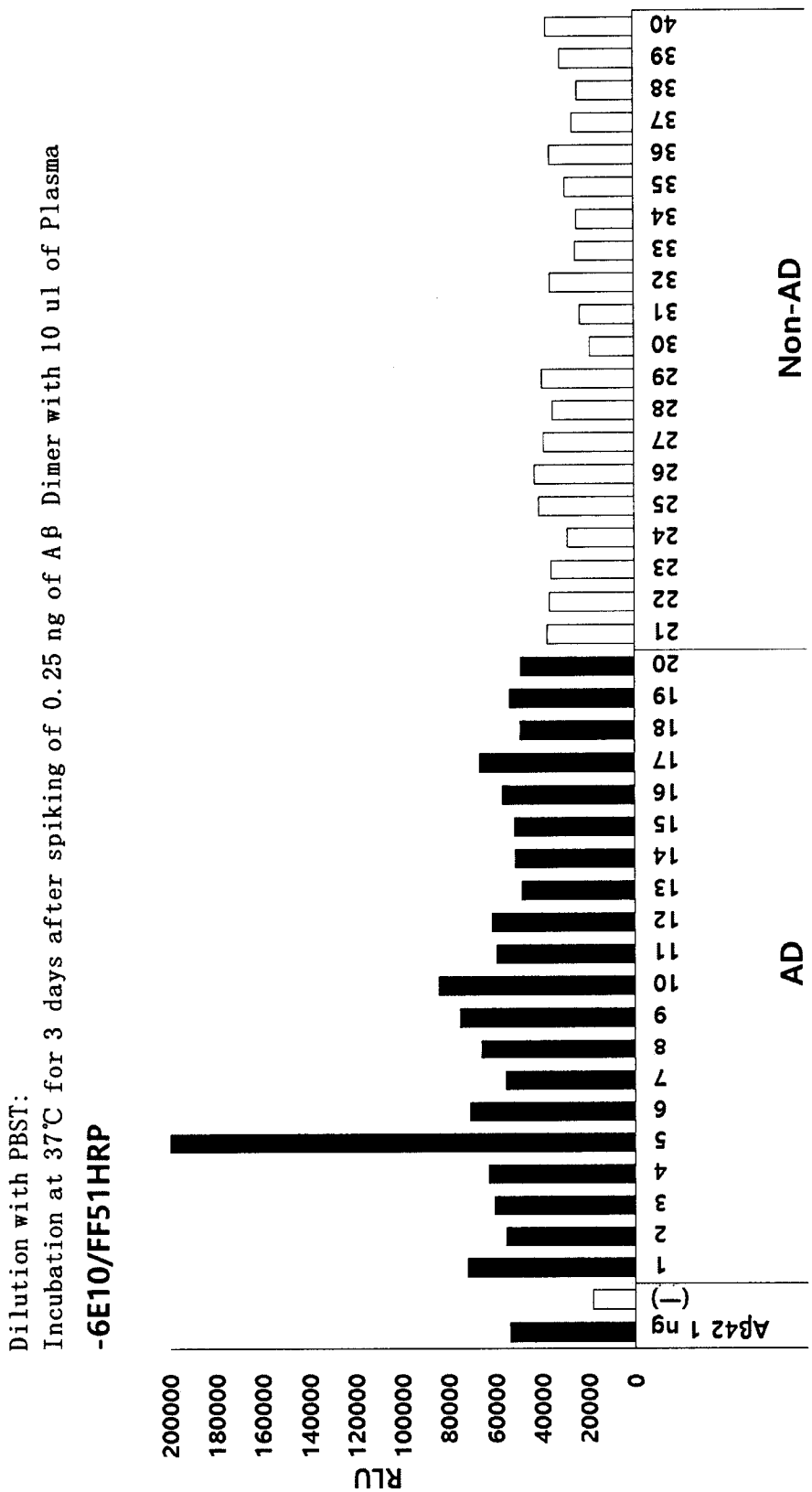
FIG 1)


Dilution with PBST:
Incubation at 37℃ for 3 days after spiking of 0.25 ng of Aβ Dimer with 10 ul of Plasma
-6E10/FF51HRP
RLU
200000
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
Aβ42 1 ng
(-)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
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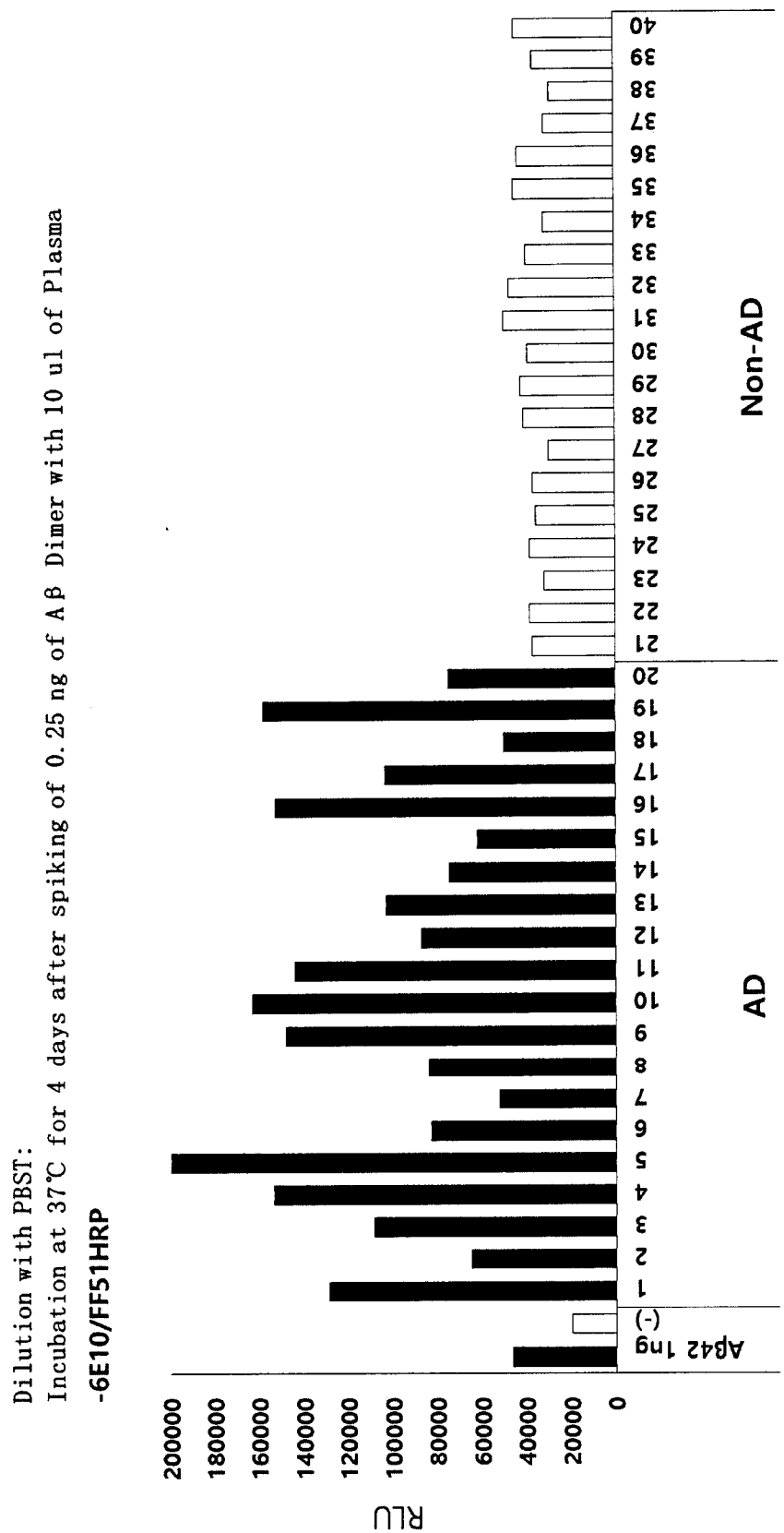
30
31
32
33
34
35
36
37
38
39
40
AD
Non-AD

FIG 2)


Dilution with PBST:
Incubation at 37℃ for 4 days after spiking of 0.25 ng of Aβ Dimer with 10 ul of Plasma
-6E10/FF51HRP
RLU
200000
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
Aβ42 1ng
(-)
AD
Non-AD
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
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30
31
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37
38
39
40

FIG 3)

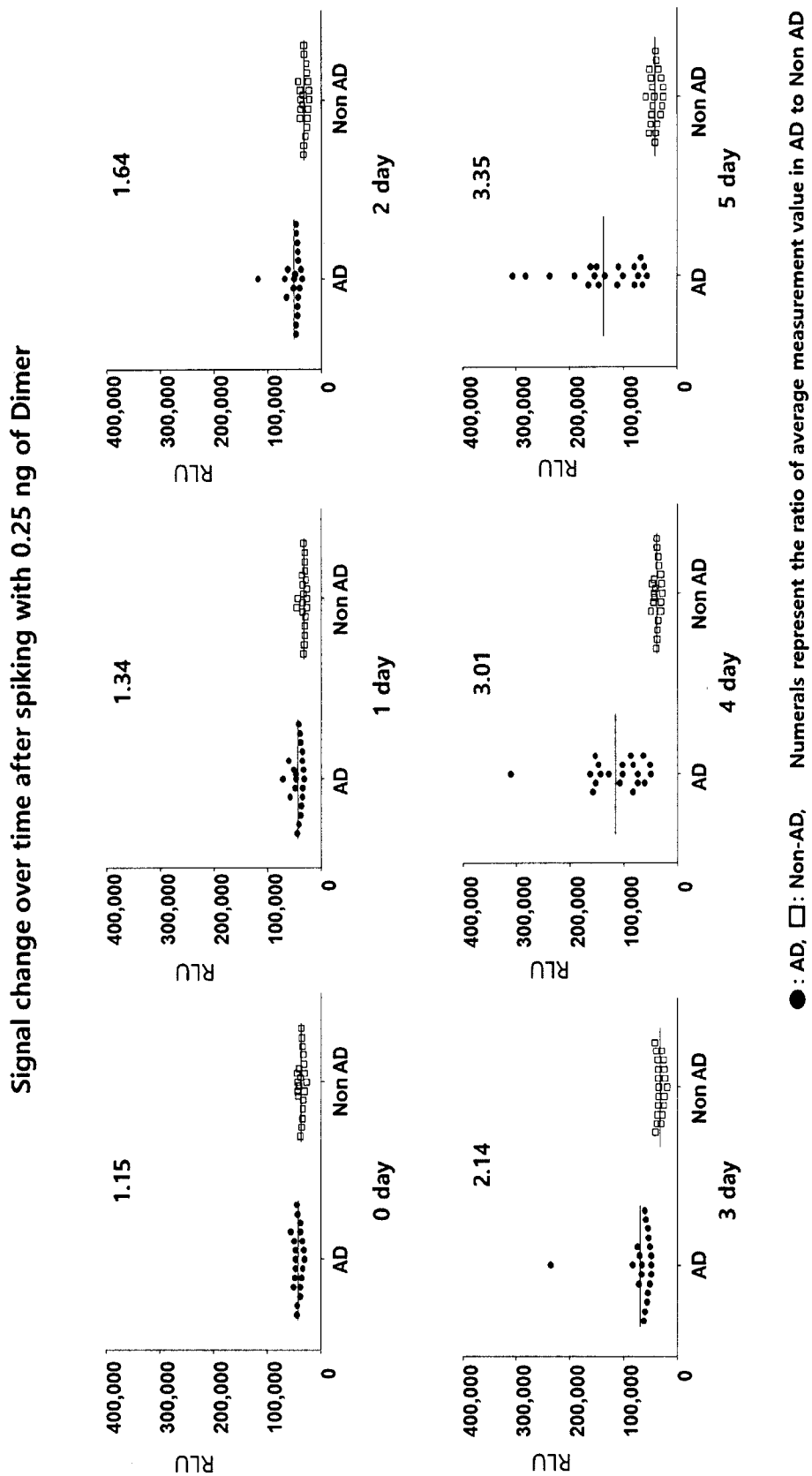

Signal change over time after spiking with 0.25 ng of Dimer
RLU
400,000
300,000
200,000
100,000
0
AD
Non AD
1.15
0 day
1.34
1 day
1.64
2 day
2.14
3 day
3.01
4 day
3.35
5 day
●: AD, □: Non-AD, Numerals represent the ratio of average measurement value in AD to Non AD

FIG 4)

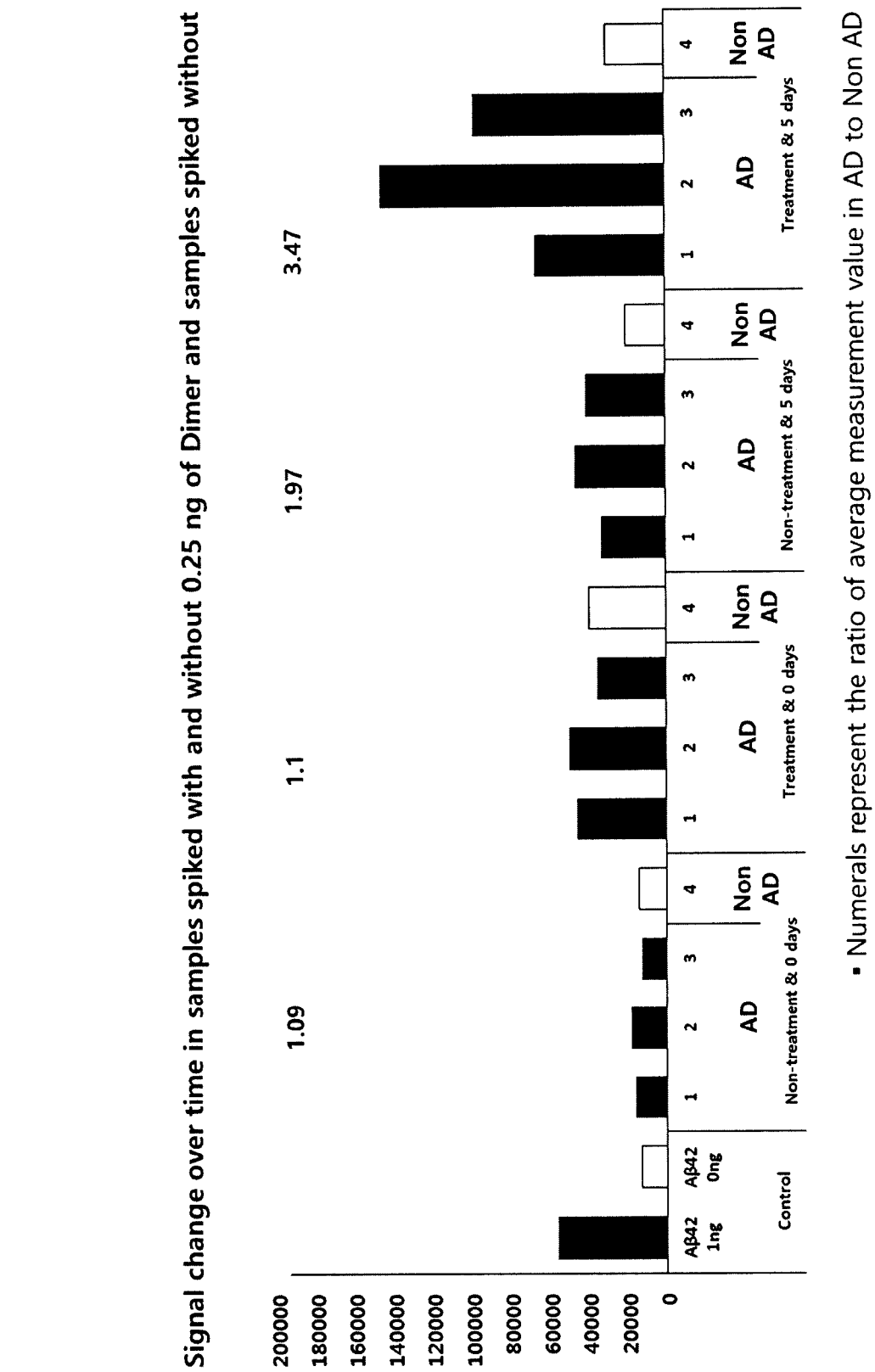

Signal change over time in samples spiked with and without 0.25 ng of Dimer and samples spiked without
RLU
200000
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
1.09
1.1
1.97
3.47
Aβ42 1ng
Aβ42 0ng
Control
1
2
3
4
AD
Non AD
Non-treatment & 0 days
Treatment & 0 days
Non-treatment & 5 days
Treatment & 5 days
▪ Numerals represent the ratio of average measurement value in AD to Non AD

FIG 5)

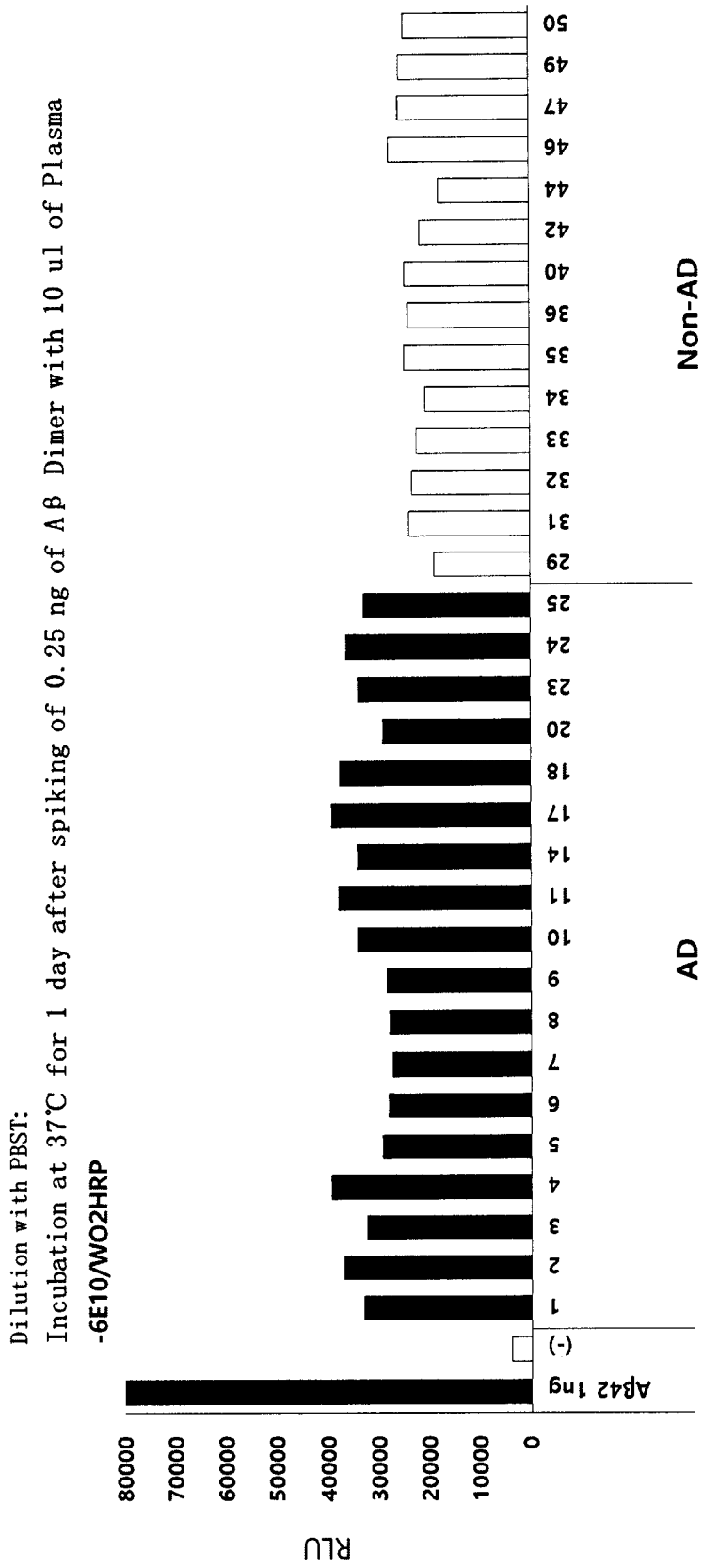

Dilution with PBST:
Incubation at 37℃ for 1 day after spiking of 0.25 ng of Aβ Dimer with 10 ul of Plasma
-6E10/WO2HRP
RLU
80000
70000
60000
50000
40000
30000
20000
10000
0
Aβ42 1ng
(-)
1
2
3
4
5
6
7
8
9
10
11
14
17
18
20
23
24
25
AD
29
31
32
33
34
35
36
40
42
44
46
47
49
50
Non-AD

FIG 6)

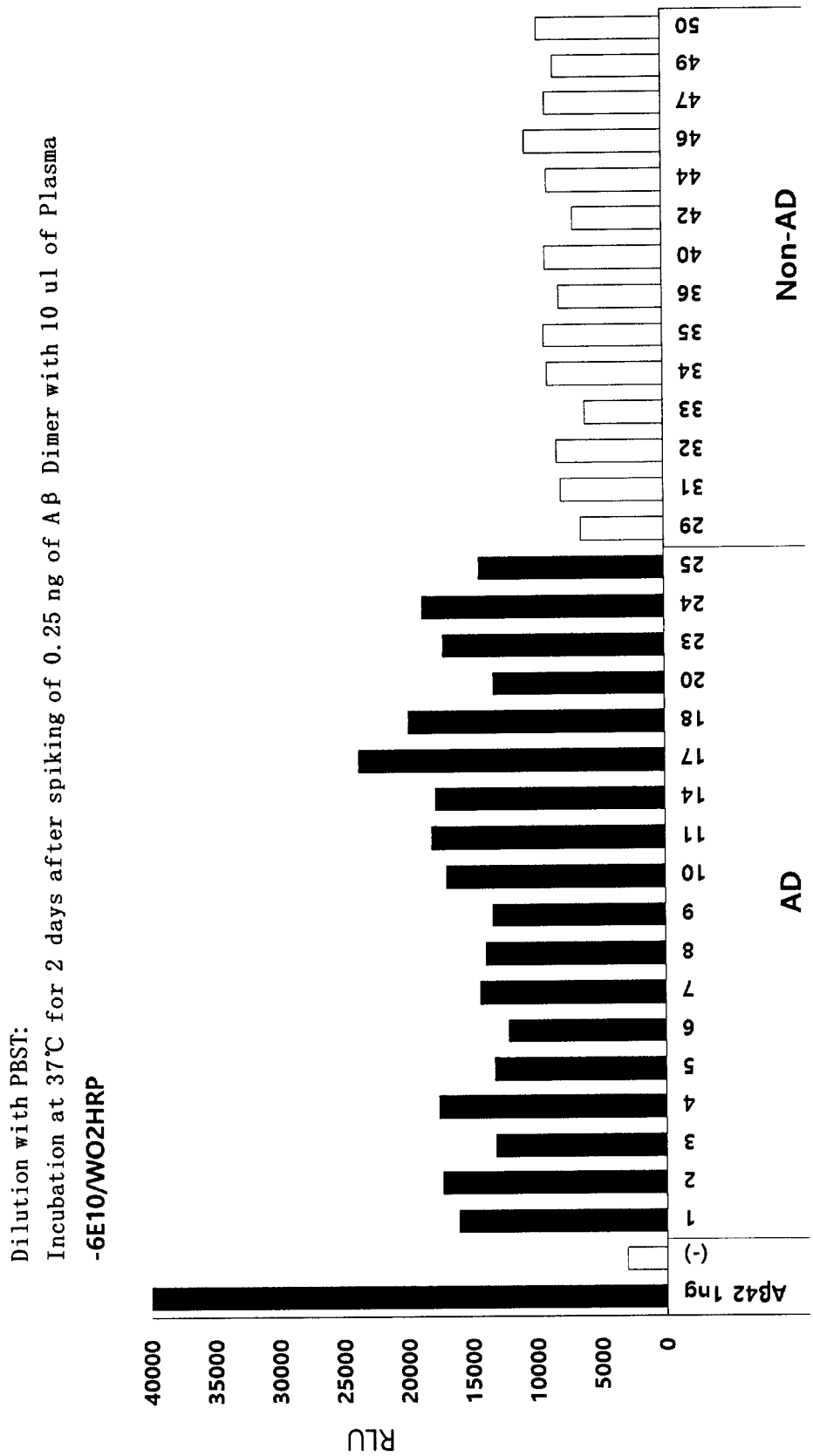
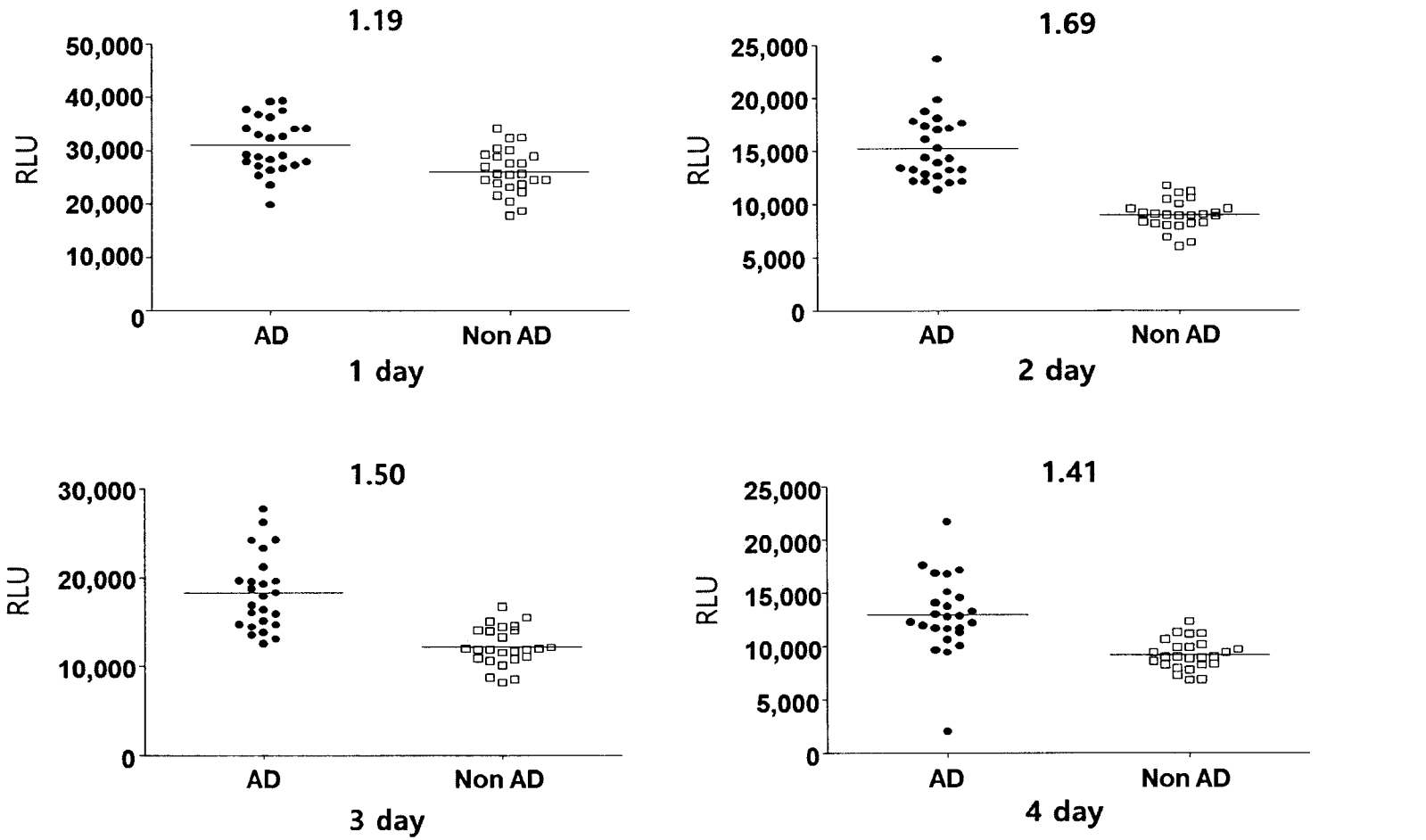

Dilution with PBST:
Incubation at 37℃ for 2 days after spiking of 0.25 ng of Aβ Dimer with 10 ul of Plasma
-6E10/WO2HRP
RLU
40000
35000
30000
25000
20000
15000
10000
5000
0
Aβ42 1ng
(-)
1
2
3
4
5
6
7
8
9
10
11
14
17
18
20
23
24
25
AD
29
31
32
33
34
35
36
40
42
44
46
47
49
50
Non-AD

FIG 7)



Dilution with PBST:
Incubation at 37℃ for 3 days after spiking of 0.25 ng of Aβ Dimer with 10 ul of Plasma

-6E10/FF51HRP