Human Amyloid β (1-42) ELISA Kit
Catalogue Number: OM643133
(96Tests)
Store all reagents at 2-8°C
Collect sample: serum or blood plasma
Assay range ： 1 - 100 ng/mL

FOR LABORATORY RESEARCH USE ONLY.
NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!
PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!

INTENDED USE

For research use only, not for use in diagnostic procedures.
The Human Amyloidβ (1-42) Assay Kit is a complete kit for the quantitative determination of human Aβ (1-42) in cerebrospinal fluid, cell culture media or the extract from brain tissue. If FCS etc. is contained in samples of culture supernatant, Aβ (1-42)-like substances in FCS may be measured. We recommend you to set the negative control. Aβ (1-42) in serum or plasma may be undetectable by this kit due to sensitivity.It is thought that some interference are existing in serum. Both recombinant and native forms of human Aβ (1-42) can be detected with the kit.

PRINCIPLE OF THE ASSAY

This kit is a solid phase sandwich ELISA using 2 kinds of high specific antibodies. Tetra Methyl Benzidine (TMB) is used as coloring agent (Chromogen). The strength of coloring is in proportion to the quantities of human A β (1-42).

REAGENTS PROVIDED

All reagents provided are stored at 2-8° C. Refer to the expiration date on the

label.

1. Anti- Human A β Coated plate	96 wells	1 plate
2. Biotinylated Antibody	0.12 mL	1 tube
3. A β 1-42 Standard (1000 ng/mL)	0.1 mL	1 tube
4.Streptavidin labeled HRP	0.12 mL	1 tube
5. 10x Assay Buffer	30 mL	1 tube
6. Denaturing Buffer	12 mL	1 vial
7. Sample Diluent	30 mL	1 vial
8. TMB Substrate	18 mL	1 vial
9. Stop Solution	8 mL	1 vial
10. Instruction	1 copy	

SAMPLE COLLECTION AND STORAGE

Serum – Use serum separation tubes (SST). Allow the sample to clot for 30 minutes, then centrifuge at approximately 1000 × g for 15 minutes. Assay the separated serum immediately or aliquot and store at -20°C or -80°C.

Plasma – Collect plasma using EDTA or heparin as anticoagulant. Centrifuge at 1000 × g for 15 minutes at 2-8°C within 30 minutes of collection. Store the sample at -20°C or -80°C and avoid repeated freeze-thaw cycles.

Cell Culture Supernatant & Other Biological Fluids – Centrifuge to remove particulates and assay immediately, or aliquot and store at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

Note: Serum, plasma, and cell culture supernatant samples intended for use within 7 days may be stored at 2-8°C; otherwise, they must be stored at -20°C (for ≤2 months) or -80°C (for ≤6 months) to prevent loss of bioactivity and contamination. Avoid freeze-thaw cycles. For assay, allow the samples to equilibrate to room temperature slowly.

DO NOT USE HEAT-TREATED SPECIMENS.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Precision pipettors and disposable tips to deliver 10-1000 µl. A multi-channel pipette is desirable for large assays.
2. 100 mL and 1 liter graduated cylinders.
3.Distilled or deionized water.
4. Tubes to prepare sample dilutions.
5. Microplate reader capable of measuring absorbance at 450 nm.
6. Data analysis and graphing software.

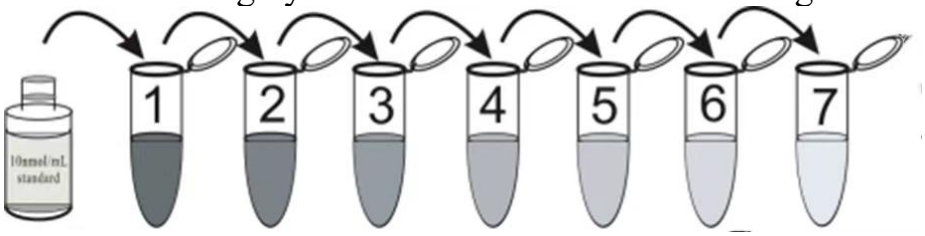
PRECAUTIONS

1. For Research or Manufacturing use only.
2. Stop reagent is 2M H_2SO4. Avoid contact with eyes, skin, and clothing. At the concentrations used in this kit, none of the other reagents are believed to be harmful.
3. This kit should only be used by qualified technicians.
4. High or low pH, detergents, urea, high salt concentrations, and organic solvents are some of the known interference factors. pH has a big impact on ELISA. So you must determine your sample pH and adjust it to 7.0-7.4.
5. If crystals have formed in all the reagents, warm to room temperature and mix gently until the crystals have completely dissolved.
6. If you have any problems of this process, you can contact Tech Department of our company.

REAGENT PREPARATION

1. Bring all kit components and samples to room temperature (20-25℃) before use.
2. Wash Solution - Dilute 30 mL of Wash Solution concentrate (10×) with 270 mL of deionized or distilled water to prepare 300 mL of Wash Solution (1×). If crystals have formed in the solution, warm to room temperature and mix gently until the crystals have been completely dissolved. The 1× wash solution is stable for 2 weeks at 2-8°C.
3. Dilution of Standards

   Label eight tubes 1# through 7#. Pipet 990 μL Sample Diluent into tube 1# , 500 μL Sample Diluent into tubes 2-7#. Add 10 μL of the 1000 μg/mL standard to tube 1#. Vortex thoroughly. Add 500 μL of tube 1# to tube 2# and vortex thoroughly. Continue this for tubes 3# through 7#.



   The concentration of Aβ 1-42 in tube 2# through 7# will be 100, 50, 25, 12.5, 6.25, 3.125, 1.56 ng/mL respectively. Label one tube as the Zero Standard(0 ng/mL). Pipet 500 μL Sample Diluent into this tube.
4. Do not dilute the other components which are ready to use.

ASSAY PROCEDURE

1. Remove the required strips from the aluminum foil bag equilibrated at room temperature for 20 minutes. Seal the remaining strips in a resealable bag and

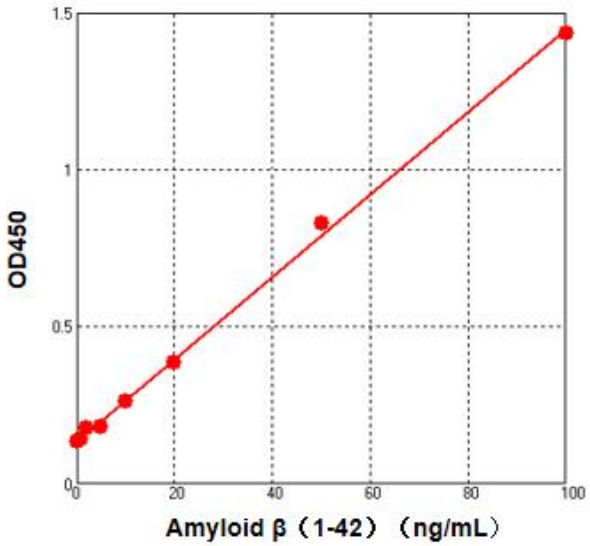
store at 4℃.

2. Set up standard wells and sample wells. Add 100 μL of standard solutions at different concentrations to the standard wells, and 100 μL of the test samples to the sample wells. Leave the blank wells empty. Incubate in a constant temperature incubator at 37℃for 60 minutes.
3. Discard the liquid, blot dry on absorbent paper, add 250 μL of wash buffer to each well, let stand for 1 minute, discard the wash buffer, and blot dry on absorbent paper. Repeat the wash process three times (an automated plate washer may also be used).
4. Add 100 μL of detection antibody to each well and incubate in a constant temperature incubator at 37℃ for 60 minutes.
5. Discard the liquid, blot dry on absorbent paper, add 250 μL of wash buffer to each well, let stand for 1 minute, discard the wash buffer, and blot dry on absorbent paper. Repeat the wash process three times (an automated plate washer may also be used).
6. Add 100 μL of 1:2000 diluted secondary antibody to each well and incubate in a constant temperature incubator at 37℃ for 60 minutes.
7. Discard the liquid, blot dry on absorbent paper, add 250 μL of wash buffer to each well, let stand for 1 minute, discard the wash buffer, and blot dry on absorbent paper. Repeat the wash process four times (an automated plate washer may also be used).
8. Add 100 μL of substrate chromogen solution (TMB) to each well and incubate at 37℃ in the dark for 15 minutes.
9. Add 50 μL of stop solution to each well. Within 15 minutes, measure the optical density (OD) of each well at a wavelength of 450 nm.

TYPICAL DATA

Subtract the absorbance of test sample blank from all data, including standards and unknown samples before plotting. Plot the subtracted absorbance of the standards against the standard concentration on log-log graph paper. Draw the best smooth curve through these points to construct the standard curve. Read the concentration for unknown samples from the standard curve.The typical standard curve is shown above. This curve can not be used to derive test results. Please run a standard curve for each assay.

Conc. (ng/ml)	Absorbance (450nm)
100	1.433
50	0.828
20	0.386
10	0.264
5	0.181
2	0.176
1	0.142
0	0.133



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