ab289832 – Amyloid Beta 42 Human ELISA Kit

For the in vitro quantitative determination of Amyloid Beta 42 in human serum, plasma, tissue homogenates, cell lysate and other biological fluids.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab289832

Storage and Stability

On receipt entire assay kit should be stored at 4°C for up to 6 months.

Materials Supplied

Item	Quantity	Storage Condition
Micro ELISA Plate	8 x 12 wells	4°C
Lyophilized Standard	2 vials	4°C
Sample / Standard dilution buffer	20 mL	4°C
Biotin- detection antibody (Concentrated)	120 µL	4°C
Antibody dilution buffer	10 mL	4°C
HRP-Streptavidin Conjugate (SABC) (Avoid light)	120 µL	4°C
SABC dilution buffer	10 mL	4°C
TMB substrate (Avoid light)	10 mL	4°C
Stop Solution	10 mL	4°C
Wash buffer (25X)	30 mL	4°C
Plate sealers	5 units	4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm
- 37°C incubator
- Precision pipettes with disposable tips
- Clean eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.
- Prepare reagents within 30 minutes before the experiment.

Biotin- detection antibody working solution: Calculate the total volume of the working solution: 0.1 mL / well × quantity of wells with additional 0.1 - 0.2 mL of the total volume. Centrifuge for 1 min at 1000xg at low speed and bring down the concentrated biotin-labeled antibody to the bottom of the tube. Dilute the Biotin- detection antibody with Antibody dilution buffer at 1:100 and mix thoroughly (e.g. Add 10 µl concentrated biotin-labeled antibody into 990 µl antibody dilution buffer).

HRP-Streptavidin Conjugate (SABC): Calculate the total volume of the working solution: 0.1 mL / well × quantity of wells with additional 0.1 - 0.2 mL of the total volume. Centrifuge for 1 min at 1000xg at low speed and bring down the concentrated SABC to the bottom of tube. Dilute the SABC with SABC dilution buffer at 1:100 and mix thoroughly (e.g. Add 10 µl concentrated SABC into 990 µl SABC dilution buffer.)

Wash Buffer: Dilute 30 mL of Concentrated Wash Buffer into 750 mL of Wash Buffer with deionized or distilled water. Put unused solution back at 4°C. If crystals have formed in the concentrate, warm it with 40°C water bath and mix it gently until the crystals have completely dissolved. The solution should be cooled to room temperature before use.

Standard Preparation:

1. Centrifuge the standards tube for 1 min at 10000xg. Reconstitute the lyophilized Amyloid Beta 42 standard by adding 1 mL of Standard/Sample Dilution Buffer to make the 300 pg/mL standard stock solution, label this tube as Zero tube.
2. Tighten the tube cap and let it stand for 2 min at room temperature. Invert the tube several times to mix gently. (Or you can mix it using a low-speed vortex mixer for 3-5 seconds.)
3. Centrifuge the tubes for 1min at 1000xg, making the liquid towards the bottom of tube and removing possible bubbles.
4. Label 7 EP tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Add 0.3ml of the sample dilution buffer into each tube. Add 0.3 ml solution from zero tube into 1/2 tube and mix them thoroughly. Transfer 0.3 ml from 1/2 tube into 1/4 tube and mix them thoroughly. Transfer 0.3ml from 1/4 tube into 1/8 tube and mix them thoroughly, so on until the 1/64 tube. The blank tube should only contain 0.3 ml sample dilution buffer.
5. Suggested standard points are: 300, 150, 75, 37.5, 18.75, 9.375, 4.688, 0 pg/ml.

Sample Preparation:

- Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination. Avoid multiple freeze-thaw cycles.
- End user should estimate the concentration of the target protein in the test sample first and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit.
- Blood collection tubes should be disposable and non-endotoxin. Avoid using hemolyzed and lipemia samples.

Serum: Place whole blood samples at room temperature for 2 hours or at 2-8°C overnight. Centrifuge for 20min at 1000xg and collect the supernatant to detect immediately. Or aliquot the supernatant and store it at -20°C or -80°C for future assays.

Plasma: Collect plasma using EDTA-Na_2 as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2 - 8°C within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Or aliquot the supernatant and store it at -20°C or -80°C for future assays.

Tissue homogenates: Place the target tissue on the ice. Remove residual blood by washing tissue with pre-cooling PBS buffer (0.01M, pH=7.4). Then weigh for usage. Use lysate to grind tissue homogenates on the ice. The adding volume of lysate depends on the weight of the tissue (the volume depends on the weight of the tissue; 9 mL PBS would be appropriate for 1 g of tissue).
Some protease inhibitor is recommended to add into the PBS (e.g. 1mM PMSF). To further break the cells, sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles (ice bath for cooling is required during ultrasonic disruption; freeze-thaw cycles can be repeated twice.) Homogenates are then centrifuged for 5 minutes at 5000×g. Collect the supernatant to detect immediately. Or aliquot the supernatant and store it at -20°C or -80°C for future assays.
Note: PBS buffer or mild RIPA lysis can be used as lysates. While using RIPA lysis, make the pH=7.3. Avoid using any reagents containing NP-40 lysis buffer, Triton X-100 surfactant, or DTT due to their severe inhibition for kits working. We recommend using 50mM Tris + 0.9% NaCL + 0.1% SDS, pH 7.3.

Cell culture supernatant: Collect the supernatant: Centrifuge at 2500 rpm at 2-8°C for 5 minutes, then collect clarified cell culture supernatant to detect immediately. Or aliquot the supernatant and store it at -80°C for future assays.

Cell lysate: Suspension Cell Lysate: Centrifuge at 2500 rpm at 2-8°C for 5 minutes and collect cells. Then add precooling PBS into collected cell and mix gently. Recollect cell by repeating centrifugation. Add 0.5-1ml cell lysate and appropriate protease inhibitor (e.g. PMSF, working concentration: 1mmol/L). Lyse the cell on ice for 30min-1h or disrupt the cell by ultrasonic disruption.
Adherent Cell Lysate: Absorb supernatant and add pre-cooling PBS to wash three times. Add 0.5-1ml cell lysate and appropriate protease inhibitor (e.g. PMSF, working concentration: 1mmol/L). Scrape the adherent cell with cell scraper. Lyse the cell suspension added in the centrifuge tube on ice for 30min-1h or disrupt the cell by ultrasonic disruption. During lysate process, use the tip for pipetting or intermittently shake the centrifugal tube to completely lyse the protein. Mucilaginous product is DNA which can be disrupted by ultrasonic cell disruptor on ice. (3~5mm probe, 150-300W, 3~5 s/time, 30s intervals for 1~2s working). At the end of lysate or ultrasonic disruption, centrifuge at 10000 rpm at 2-8°C for 10 minutes. Then, the supernatant is added into EP tube to detect immediately. Or aliquot the supernatant and store it at -80°C for future assays.

Other biological fluids: Centrifuge samples for 20 min at 1000×g at 2-8°C. Collect the supernatant and carry out the assay immediately. Or aliquot the supernatant and store it at -80°C for future assays.

Assay Protocol

- Bring all reagents and samples to room temperature 30 minutes prior to the assay.
- It is recommended that all standards and samples be run at least in duplicate.
- A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in Reagent Preparation section.
2. Add 100 µL of each standards or samples into appropriate wells. Cover well and incubate for 120 minutes at room temperature.
3. Wash plate 2 times with 1X Wash Solution. Remove the cover, then absorb the liquid in the plate or tap the plate on a clean absorbent paper two or three times. Add 350ul wash buffer into each well without immersion. Discard the liquid in the well and tap on the absorbent paper again. Repeat.
4. Add 0.1 mL of Biotin-detection antibody working solution into the above wells. Seal the plate and static incubate at room temperature for 60 min.
5. Wash the plate 3 times with 1X Wash Solution and immerse for 1 min each time.
6. Add 0.1 mL of SABC working solution into each well, seal the plate and incubate at room temperature for 30 min.
7. Wash the plate 5 times with 1X Wash Solution and immerse for 1 min each time.
8. Add 90 µL of TMB substrate into each well, seal the plate and incubate at 37 °C in dark within 10-20 min.
9. Add 50 µL of Stop Solution to each well. Read result at 450 nm immediately.

Calculation

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Human Amyloid Beta 42 concentration of the samples can be interpolated from the standard curve. If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Technical Support

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