Human APP ELISA Kit

Catalog No. EK0658
Size 96T(8×12 divisible strips)

For quantitative detection of human APP in cell culture supernates.

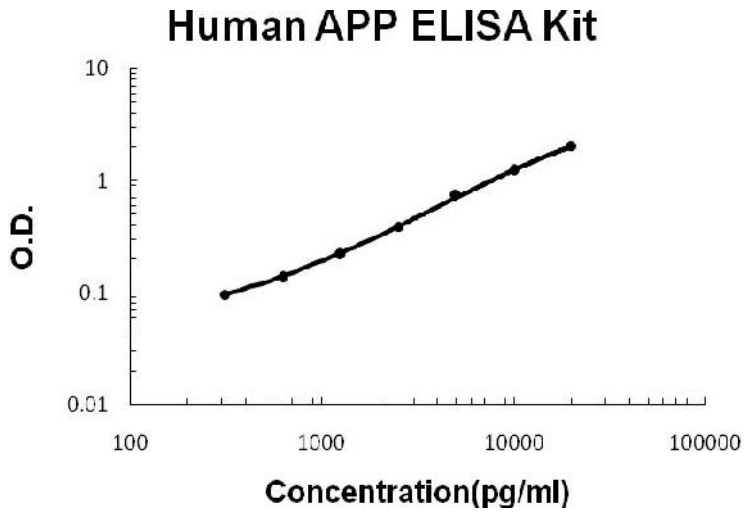
Typical Data Obtained from Human APP

(TMB reaction incubate at 37°C for 21 min)

Concentration(pg/ml)	0	312	625	1250	2500	5000	10,000	20,000
O.D	0.046	0.096	0.136	0.218	0.387	0.759	1.222	2.029

Typical Human APP ELISA Kit Standard Curve

This standard curve was generated at Boster for demonstration purpose only. A standard curve must be run with each assay.



Range	312pg/ml-20,000pg/ml
Sensitivity	< 10pg/ml
Specificity	Natural and recombinant human APP
Cross-reactivity	No detectable cross-reactivity with other relevant proteins

Storage

Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles (Shipped with wet ice.)

Principle

Boster's human APP ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. A monoclonal antibody from mouse specific for APP has been precoated onto 96-well plates. Standards(NSO,L18-L688) and test samples are added to the wells, a biotinylated detection polyclonal antibody from goat specific for APP is added subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the human APP amount of sample captured in plate.

Kit Components

Catalog number	Description	Quantity
-	96-well plate precoated with anti- human APP antibody	1
ST0000-20	Lyophilized recombinant human APP standard	20ng/tube×2
AR1107	Biotinylated anti- human APP antibody	130μl(dilution 1:100)
AR1103	Avidin-Biotin-Peroxidase Complex (ABC)	130μl(dilution 1:100)
AR1106-1	Sample diluent buffer	30 ml
AR1106-2	Antibody diluent buffer	12ml
AR1106-3	ABC diluent buffer	12ml
AR1104	TMB color developing agent	10ml
AR1105	TMB stop solution	10ml

Material Required But Not Provided

1. Microplate reader in standard size.
2. Automated plate washer.
3. Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.
4. Clean tubes and Eppendorf tubes.
5. Washing buffer (neutral PBS or TBS).
   - Preparation of 0.01M **TBS:** Add 1.2g Tris, 8.5g Nacl; 450μl of purified acetic acid or 700μl of concentrated hydrochloric acid to 1000ml H_2O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.
   - Preparation of 0.01 M **PBS:** Add 8.5g sodium chloride, 1.4g Na_2HPO_4 and 0.2g NaH_2PO_4 to 1000ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

Notice for Application of Kit

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. The TMB Color Developing agent is colorless and transparent before using, contact us freely if it is not the case.
3. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
4. Duplicate well assay is recommended for both standard and sample testing.
5. Don't let 96-well plate dry, for dry plate will inactivate active components on plate.
6. Don't reuse tips and tubes to avoid cross contamination.
7. To avoid to use the reagents from different batches together.
8. In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution will be pre-warmed in 37°C for 30 min before using.

Preparation

1. **Sample Preparation and Storage**

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

- **Cell culture supernates**: Remove particulates by centrifugation, assay immediately or aliquot and store samples at -20°C.

2. **Sample Dilution Guideline**

The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve. Dilute the sample using the provided diluent buffer. The following is a guideline for sample dilution. Several trials may be necessary in practice. **The sample must be well mixed with the diluents buffer.**

- **High target protein concentration (200-2000ng/ml).** The working dilution is 1:100. i.e. Add 1µl sample into 99 µl sample diluent buffer.
- **Medium target protein concentration (20-200ng/ml).** The working dilution is 1:10. i.e. Add 10µl sample into 90 µl sample diluent buffer.
- **Low target protein concentration (312-20,000pg/ml).** The working dilution is 1:2. i.e. Add 50µl sample to 50 µl sample diluent buffer.
- **Very Low target protein concentration (≤312pg/ml).** No dilution necessary, or the working dilution is 1:2.

3. **Reagent Preparation and Storage**

A. Reconstitution of the human APP standard：APP standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of APP standard (20ng per tube) are included in each kit. Use one tube for each experiment.

   a. 20,000pg/ml of human APP standard solution: Add 1ml sample diluent buffer into one tube, keep the tube at room temperature for 10 min and mix thoroughly.

   b. 10,000pg/ml→312pg/ml of human APP standard solutions: Label 6 Eppendorf tubes with 10,000pg/ml, 5000pg/ml, 2500pg/ml, 1250pg/ml, 625pg/ml, 312pg/ml respectively. Aliquot 0.3ml of the sample diluent buffer into each tube. Add 0.3ml of the above 20,000pg/ml APP standard solution into 1st tube and mix. Transfer 0.3ml from 1st tube to 2nd tube and mix. Transfer 0.3ml from 2nd tube to 3rd tube and mix, and so on.

Note: The standard solutions are best used within 2 hours. The 20ng/ml standard solution should be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

B. Preparation of biotinylated anti-human APP antibody working solution: The solution should be prepared no more than 2 hours prior to the experiment.

   a. The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)

   b. Biotinylated anti-human APP antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly. (i.e. Add 1µl Biotinylated anti-human APP antibody to 99µl antibody diluent buffer.)

C. Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution: The solution should be prepared no more than 1 hour prior to the experiment.

   a. The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)

   b. Avidin- Biotin-Peroxidase Complex (ABC) should be diluted in 1:100 with the ABC dilution buffer and mixed thoroughly. (i.e. Add 1µl ABC to 99µl ABC diluent buffer.)

Assay Procedure

The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 min before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard APP detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of APP amount in samples.

1. Aliquot 0.1ml per well of the 20,000pg/ml, 10,000pg/ml, 5000pg/ml, 2500pg/ml, 1250pg/ml, 625pg/ml, 312pg/ml human APP standard solutions into the precoated 96-well plate. Add 0.1ml of the sample diluent buffer into the control well (Zero well). Add 0.1ml of each properly diluted sample of human cell culture supernates to each empty well. **See "Sample Dilution Guideline" above for details.** It is recommended that each human APP standard solution and each sample be measured in duplicate.
2. Seal the plate with the cover and incubate at 37°C for 90 min.
3. Remove the cover, discard plate content, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.
4. Add 0.1ml of biotinylated anti-human APP antibody working solution into each well and incubate the plate at 37°C for 60 min.
5. Wash plate 3 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (**Plate Washing Method:** Discard the solution in the plate without touching the side walls. Blot the plate onto paper towels or other absorbent material. Soak each well with at least 0.3 ml PBS or TBS buffer for 1~2 minutes. Repeat this process two additional times for a total of THREE washes. Note: For automated washing, aspirate all wells and wash THREE times with PBS or TBS buffer, overfilling wells with PBS or TBS buffer. Blot the plate onto paper towels or other absorbent material.)
6. Add 0.1ml of prepared ABC working solution into each well and incubate the plate at 37°C for 30 min.
7. Wash plate 5 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (See Step 5 for plate washing method).
8. Add 90µl of prepared TMB color developing agent into each well and incubate plate at 37°C in dark for 20-25 min (**Note:** For reference only, the optimal incubation time should be determined by end user. And the shades of blue can be seen in the wells with the four most concentrated human APP standard solutions; the other wells show no obvious color).
9. Add 0.1ml of prepared TMB stop solution into each well. The color changes into yellow immediately.
10. Read the O.D. absorbance at 450nm in a microplate reader within 30 min after adding the stop solution.

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 APP concentration of the samples can be interpolated from the standard curve.

Note: if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Summary

1. Add samples and standards and incubate the plate at 37°C for 90 min. Do not wash.
2. Add biotinylated antibodies and incubate the plate at 37°C for 60 min. Wash plate 3 times with 0.01M TBS.
3. Add ABC working solution and incubate the plate at 37°C for 30 min. Wash plate 5 times with 0.01M TBS.
4. Add TMB color developing agent and incubate the plate at 37°C in dark for 20-25 min.
5. Add TMB stop solution and read.

Background

Amyloid precursor protein (APP) is an integral membrane protein expressed in many tissues and concentrated in the synapses of neurons. Its primary function is not known, though it has been implicated as a regulator of synapse formation, neural plasticity and iron export. APP is best known and most commonly studied as the precursor molecule whose proteolysis generates beta amyloid (Aβ), a 39- to 42-amino acid peptide whose amyloid fibrillar form is the primary component of amyloid plaques found in the brains of Alzheimer's disease patients. APP undergoes posttranslational proteolytic processing by alpha-, beta-, and gamma-secretases. Alpha-secretase generates soluble amyloid protein, while beta- and gamma-secretases generate APP components with amyloidogenic features. These 2 processing pathways are mutually exclusive.

Reference

1. PDB 1RW6; Wang Y, Ha Y (August 2004). "The X-ray structure of an antiparallel dimer of the human amyloid precursor protein E2 domain". Mol. Cell 15 (3): 343–53.
2. Priller C, Bauer T, Mitteregger G, Krebs B, Kretzschmar HA, Herms J (July 2006). "Synapse formation and function is modulated by the amyloid precursor protein". J. Neurosci. 26 (27): 7212–21.
3. Turner PR, O'Connor K, Tate WP, Abraham WC (May 2003). "Roles of amyloid precursor protein and its fragments in regulating neural activity, plasticity and memory". Prog. Neurobiol. 70 (1): 1–32.
4. Duce JA et al. (2010). "Iron-Export Ferroxidase Activity of β- Amyloid Precursor Protein Is Inhibited by Zinc in Alzheimer's Disease". Cell 142 (6): 857–67.