Version 01-08/24

User's Manual

Mouse Appbp2 (Amyloid protein-binding protein 2) ELISA Kit

REF **DEIA-JY2431**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

In vitro quantitative determination of target concentrations in serum, plasma, cell culture supernatant and other biological samples.

Principles of Testing

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Anti Appbp2 antibody was pre-coated onto the 96-well plate. The biotin conjugated anti Appbp2 antibody was used as the detection antibody. The standards and pilot samples were added to the wells subsequently. After incubation, unbound conjugates were removed by wash buffer. Then, biotinylated detection antibody was added to bind with Appbp2 conjugated on coated antibody. After washing off unbound conjugates, HRP-Streptavidin was added. After a third washing, TMB substrates were added to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that turned yellow after adding a stop solution. Read the O.D. absorbance at 450nm in a microplate reader. The concentration of Appbp2 in the sample was calculated by drawing a standard curve. The concentration of the target substance is proportional to the OD450 value.

Reagents And Materials Provided

1. ELISA Microplate: 8×12, 2-8°C/-20°C
2. Lyophilized Standard: 2 vial, 2-8°C/-20°C
3. Sample Dilution Buffer: 20 ml, 2-8°C
4. Biotin-labeled Antibody (Concentrated, 100×): 1 vial, 120 µl, 2-8°C (Avoid Direct Light)
5. Antibody Dilution Buffer: 10 ml, 2-8°C
6. HRP-Streptavidin Conjugate (SABC 100×): 120 µl, 2-8°C (Avoid Direct Light)
7. SABC Dilution Buffer: 10 ml, 2-8°C
8. TMB Substrate: 10 ml, 2-8°C (Avoid Direct Light)
9. Stop Solution: 10 ml, 2-8°C
10. Wash Buffer (25×): 30 ml, 2-8°C
11. Plate Sealer: 5 pieces
12. Product Description: 1 copy

Materials Required But Not Supplied

1. Microplate reader (wavelength: 450nm)
2. 37°C incubator (CO_2 incubator for cell culture is not recommenced.)
3. Automated plate washer or multi-channel pipette/5ml pipettor (for manual washing purpose)
4. Precision single (0.5-10µL, 5-50µL, 20-200µL, 200-1000µL) and multi-channel pipette with disposable tips(Calibration is required before use.)

5. Sterile tubes and Eppendorf tubes with disposable tips
6. Absorbent paper and loading slot
7. Deionized or distilled water

Storage

2-8°C (Sealed), Don't cryopreserve.

Specimen Collection And Preparation

1. **Serum**

Place whole blood sample at room temperature for 2 hours or at 2-8°C overnight. Centrifuge for 20 min at 1000 ×g and collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -20°C or -80°C for future's assay.

2. **Plasma**

EDTA-Na_2/K_2 is recommended as the anticoagulant. Centrifuge samples for 15 minutes at 1000 ×g 2-8°C within 30 minutes after collection. Collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -20°C or -80°C for future's assay. For other anticoagulant types and uses, please refer to the sample preparation guideline.

3. **Tissue Sample**

Generally tissue samples are required to be made into homogenization. Protocol is as below:

3.1. 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.

3.2. Use lysate to grind tissue homogenates on the ice. The adding volume of lysate depends on the weight of the tissue. Usually, 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitors are recommended to add into the PBS (e.g. 1mM PMSF).

3.3. Do further process using ultrasonic disruption or freeze-thaw cycles (Ice bath for cooling is required during ultrasonic disruption; Freeze-thaw cycles can be repeated twice.) to get the homogenates.

3.4. Homogenates are then centrifuged for 5 minutes at 5000 ×g. Collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -20°C or -80°C for future's assay.

3.5. Determine total protein concentration by BCA kit for further data analysis. Usually, total protein concentration for Elisa assay should be within 1-3mg/ml. Some tissue samples such as liver, kidney, pancreas which containing a higher endogenous peroxidase concentration may react with TMB substrate causing false positivity. In that case, try to use 1% H_2O_2 for 15 min inactivation and perform the assay again.

Notes: PBS buffer or the 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 50 mM Tris+0.9% NaCL+0.1% SDS, PH7.3. You can prepare by yourself or contact us for purchasing.

4. **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 you can aliquot the supernatant and store it at -80°C for future's assay.

5. **Cell Lysate**

   5.1. 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.

   5.2. 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.

   5.3. 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).

   5.4. At the end of lysate or ultrasonic disruption, centrifuge at 10000rpm at 2-8°C for 10 minutes. Then, the supernatant is added into EP tube to detect immediately. Or you can aliquot the supernatant and store it at -80°C for future's assay.

Notes: Read notes in tissue sample.

6. **Other Biological Sample**

   Centrifuge samples for 15 minutes at 1000 ×g at 2-8°C. Collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -80°C for future's assay.

Notes for Samples

1. Blood collection tubes should be disposable and non-endotoxin. Avoid to use hemolyzed and lipemia samples.
2. The best sample storage condition: less than 5 days at 2-8°C; within 6 months at -20°C; within 2 years at -80°C. Stored in liquid nitrogen for a longer storage. When melting frozen samples, rapid water bath at 15 - 25°C can decrease the effect of ice crystal (0°C) on the sample. After melting, centrifuge to remove the precipitate, and then mix well.
3. The detection range of this kit is not equivalent to the concentration of analyze in the sample. For analyses with higher or lower concentration, please properly dilute or concentrate the sample.
4. Pretest is recommended for special samples without reference data to validate the validity.

Recommended Sample Dilution Ratio

If other dilution ratio for your sample model is required, please refer to the universal dilution ratio below. (The ratio is suitable for single-well assay. For duplicate assay, please follow the calculation: volume of sample and diluent x number of duplicate well).

For 2 fold dilution (1/2): One step dilution. Add 60µL sample into 60µL sample diluent and mix gently.

For 5 fold dilution (1/5): One step dilution. Add 24µL sample into 96µL sample diluent and mix gently.

For 10 fold dilution (1/10): One step dilution. Add 12µL sample into 108µL sample diluent and mix gently.

For 20 fold dilution (1/20): One step dilution. Add 6µL sample into 114µL sample diluent and mix gently.

For 50 fold dilution (1/50): One step dilution. Add 3µL sample and 47µL normal saline (0.9% NaCl) into 100µ

L sample diluent and mix gently.

For 100 fold dilution (1/100): One step dilution. Add 3μL sample and 177μL normal saline into 120μL sample diluent and mix gently.

For 1000 fold dilution (1/1000): Two step dilution. Create a 50-fold dilution first (normal saline is used throughout the dilution). Then, create a 20-fold dilution and mix gently.

For 10000 fold dilution (1/10000): Two step dilution. Create a 100-fold dilution first (normal saline is used throughout the dilution). Then, create the same dilution again and mix gently.

For 100000 fold dilution (1/100000): Three step dilution. Create a 50-fold dilution and 20-fold dilution respectively (normal saline is used in the first two steps.) Finally, create a 100-fold dilution and mix gently.

Notes: The volume in each dilution is not less than 3μL. Dilution factor should be within 100 fold. Mixing during dilution is required to avoid foaming.

Reagent Preparation

Take the Elisa kit from the fridge around 20 minutes earlier and equilibrate to room temperature(18-25°C). For repeated assays, please just take the strips and standards required for the current assay, store the rest materials according to the relevant condition.

1. **Wash Buffer**

Dilute 30 ml concentrated wash buffer to 750 ml wash buffer with deionized or distilled water and mix well. (The recommended resistivity of ultrapure water is 18 MΩ.) Alternatively, take appropriate amount of concentrated wash buffer according to the assay requirement, then create a 25-fold dilution and mix well. Store the rest solution at 2-8°C. Crystals formed in the concentrated wash buffer can be heated by water bath at 40°C till complete dissolution. (Heating temperature should be below 50°C.) Mix well for the next step. It's better to use up the prepared wash buffer in one day. Store the rest buffer at 2-8°C within 48h.

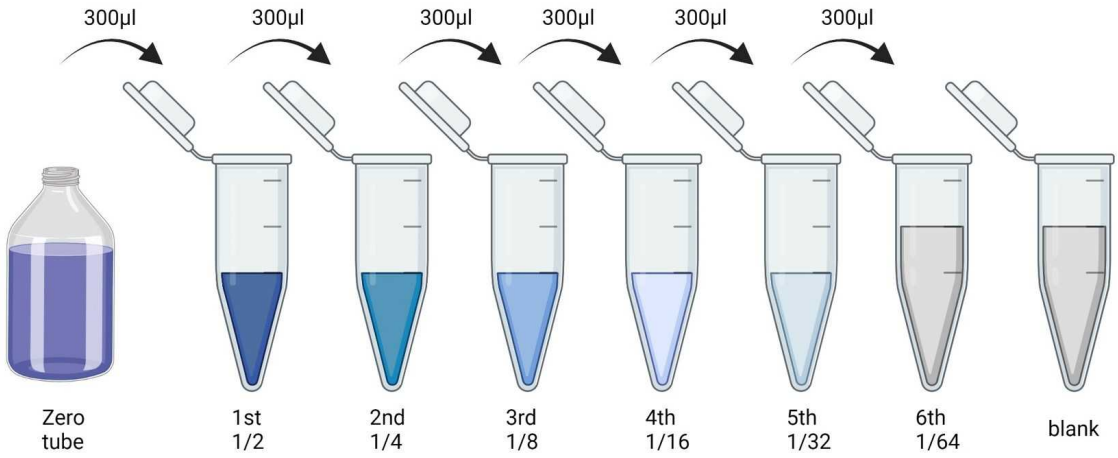
2. **Standards**

2. 1 Centrifuge standards tube for 1min at 10000 xg. Label it as Zero tube.

2. Add 1 ml sample dilution buffer into the standard tube. 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.)

2. 3 Centrifuge the tubes for 1min at 1000 xg, making the liquid towards the bottom of tube and removing possible bubbles.

2. 4 Standard dilution: Label 7 EP tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Add 0.3 ml 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 0.3 ml from 1/4 tube into 1/8 tube and mix them thoroughly, so on till 1/64 tube. Now blank tube only contain 0.3 ml sample dilution buffer. The standard concentration from zero tube to blank tube is 200 ng/ml, 100 ng/ml, 50 ng/ml, 25 ng/ml, 12.5 ng/ml, 6.25 ng/ml, 3.125 ng/ml, 0 ng/ml.



Notes: Store the zero tube with dissolved standards at 2-8°C and use it within 12h. Other diluted working solutions containing standards should be used in 2h.

3. **Preparation of Biotin-labeled Antibody Working**

The working solution should be prepared within 30min before the assay and can't be stored for a long time.

3. 1 Calculate required total volume of the working solution: 50 ul/well x quantity of wells. (It's better to prepare additional 100 ul-200 ul.)
3. 2 Centrifuge for 1min at 1000 xg in low speed and bring down the concentrated biotin-labeled antibody to the bottom of tube.
3. Dilute the biotinylated detection antibody with antibody dilution buffer at 1/100 and mix them thoroughly. (e.g. Add 10 µl concentrated biotin-labeled antibody into 990 µl antibody dilution buffer.)

4. **Preparation of HRP-Streptavidin Conjugate (SABC) Working Solution**

The working solution should be prepared within 30min before the assay and can't be stored for a long time.

4. 1 Calculate required total volume of the working solution: 100 ul/well x quantity of wells. (It's better to prepare additional 100ul-200ul.)
4. 2 Centrifuge for 1min at 1000 xg in low speed and bring down the concentrated SABC to the bottom of tube.
4. 3 Dilute the concentrated SABC with SABC dilution buffer at 1/100 and mix them thoroughly. (e.g. Add 10 µl concentrated SABC into 990 µl SABC dilution buffer.)

Assay Procedure

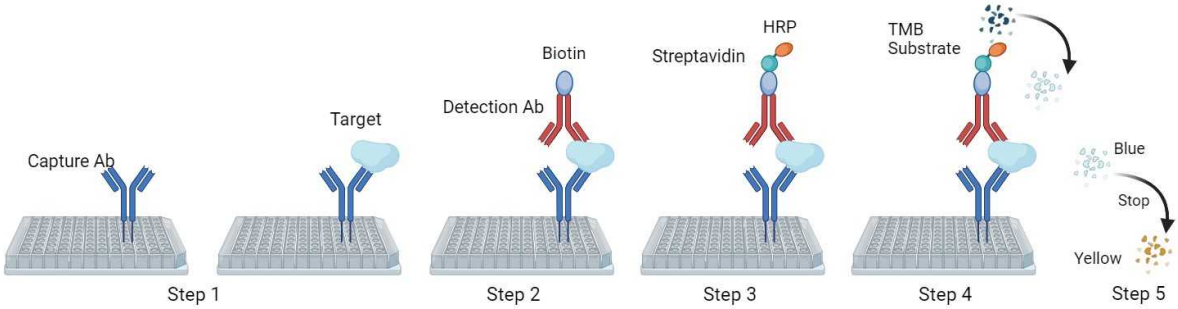
When diluting samples and reagents, they must be mixed completely and evenly. Before adding TMB into wells, equilibrate TMB Substrate for 30 minutes at 37°C. It is recommended to plot a standard curve for each test.

1. Set standard, test samples (diluted at least 1/2 with Sample Dilution Buffer), control (blank) wells on the pre-coated plate respectively, and then, records their positions. It is recommended to measure each standard and sample in duplicate.
2. Prepare Standards: Aliquot 100 µL of zero tube, 1sttube, 2ndtube, 3rdtube, 4thtube, 5thtube, 6thtube and Sample Dilution Buffer (blank) into the standard wells.
3. Add Samples: Add 100 µL of properly diluted sample into test sample wells.

4. Incubate: Seal the plate with a cover and incubate at 37°C for 90 minutes.
5. Wash: Remove the cover and discard the plate content, and wash plate 2 times with Wash Buffer. Do NOT let the wells dry completely at any time.
6. Biotin-labeled Antibody: Add 100 µL Biotin-labeled antibody working solution into above wells (standard, test sample and blank wells). Add the solution at the bottom of each well without touching the sidewall, cover the plate and incubate at 37°C for 60 minutes.
7. Wash: Remove the cover, and wash plate 3 times with Wash Buffer, and let the Wash Buffer stay in the wells for 1-2 minutes each time.
8. HRP-Streptavidin Conjugate (SABC): Add 100 µL of SABC Working Solution into each well, cover the plate and incubate at 37°C for 30 minutes.
9. Wash: Remove the cover and wash plate 5 times with Wash Buffer, and let the wash buffer stay in the wells for 1-2 minutes each time.
10. TMB Substrate: Add 90 µL TMB Substrate into each well, cover the plate and incubate at 37°C in dark within 10-20 minutes. (Note: The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. You can terminate the reaction when apparent gradient appeared in standard wells.)
11. Stop: Add 50µL Stop Solution into each well. The color will turn yellow immediately. The adding order of Stop Solution should be as the same as the TMB Substrate Solution.
12. OD Measurement: Read the O.D. absorbance at 450 nm in Microplate Reader immediately after adding the stop solution. Regarding calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of blank 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 target 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



Step1: Add 100 µL standard or sample to each well and incubate for 90 minutes at 37°C.

Wash step: Aspirate and wash plates 2 times.

Step2: Add 100 µL Biotin-labeled antibody working solution to each well and incubate for 60 minutes at 37°C.

Wash step: Aspirate and wash plates 3 times.

Step3: Add 100 µL SABC Working Solution into each well and incubate for 30 minutes at 37°C.

Wash step: Aspirate and wash plates 5 times.

Step4: Add 90 µL TMB Substrate Solution. Incubate 10-20 minutes at 37°C. (Accurate TMB visualization control is required.)

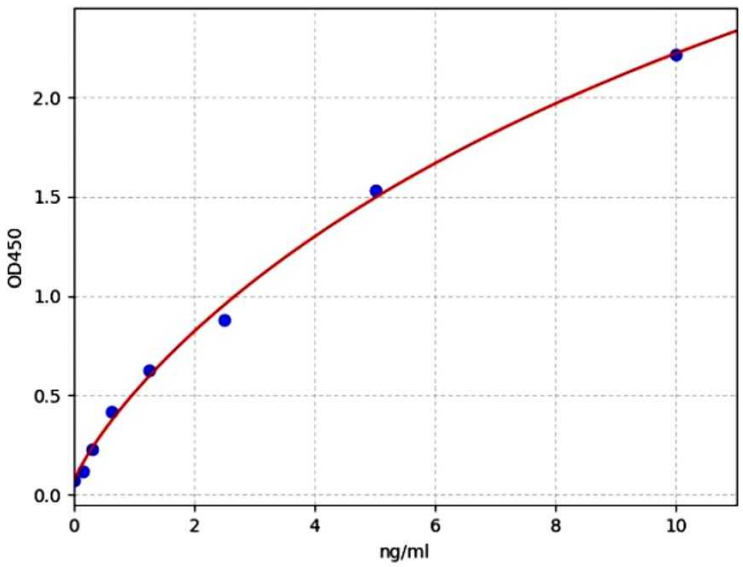
Step5: Add 50 µL Stop Solution. Read at 450 nm immediately and calculation.

Calculation

1. Calculate the mean OD450 value of the duplicate readings for each standard, control, and sample.
2. Create a four parameter logistic curve by plotting the mean absorbance for each standard on the y -axis against the concentration on the x-axis. Alternatively, you can use the curve fitting software offered by the microplate reader.
3. Calculate the sample concentration by substituting OD450 value into the standard curve. Diluted samples should be multiplied by the relevant dilution ratio.

Typical Standard Curve

STD.(ng/ml)	OD-1	OD-2	Average	Corrected
0	0.071	0.073	0.072	0
0.156	0.117	0.121	0.119	0.047
0.312	0.226	0.232	0.229	0.157
0.625	0.414	0.426	0.42	0.348
1.25	0.615	0.633	0.624	0.552
2.5	0.869	0.895	0.882	0.81
5	1.511	1.555	1.533	1.461
10	2.183	2.247	2.215	2.143



Precision

Item	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ng/ml)	0.3	1.2	5.25	0.32	1.18	5.21
Standard deviation	0.01	0.06	0.25	0.02	0.07	0.26
CV(%)	4.98	5.12	4.69	6.32	5.86	4.95

Detection Range

0.156-10 ng/ml

Detection Limit

0.094 ng/ml

Specificity

No obvious cross reaction with other analogues.

Linearity

Sample	1:2	1:4	1:8
Serum(n=5)	88-102%	85-104%	86-102%
EDTA Plasma(n=5)	86-98%	85-101%	82-96%
Heparin Plasma(n=5)	83-97%	85-100%	80-95%

Recovery

Matrix	Recovery Range (%)	Average (%)
Serum(n=5)	92-104	92
EDTA Plasma(n=5)	92-100	93
Heparin Plasma(n=5)	90-105	97