

Human CPB2(Carboxypeptidase B2) ELISA Kit

Catalog No.: MBS762600

Size: 48T/96T

Reactivity: Human

Range: 3.12-200ng/ml

Sensitivity: < 1.875ng/ml

Application: For quantitative detection of CPB2 in plasma, tissue homogenates and other biological fluids.

Storage: 4°C for 6 months

NOTE: FOR RESEARCH USE ONLY.

Kit Components

Item	Specifications(48T/96T)	Storage
Micro ELISA Plate(Dismountable)	8 × 6 or 8 × 12	4°C/-20°C
Lyophilized Standard	1 vial or 2 vial	4°C/-20°C
Sample / Standard dilution buffer	10ml/20ml	4°C
Biotin- detection antibody (Concentrated)	60ul/120ul	4°C
Antibody dilution buffer	5ml/10ml	4°C
HRP-Streptavidin Conjugate(SABC)	60ul/120ul	4°C(shading light)
SABC dilution buffer	5ml/10ml	4°C
TMB substrate	5ml/10ml	4°C(shading light)
Stop solution	5ml/10ml	4°C
Wash buffer (25X)	15ml/30ml	4°C
Plate Sealer	3/5pieces	
Product Description	1 copy	

Principle of the Assay

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Anti- CPB2 antibody was pre-coated onto 96-well plates. And the biotin conjugated anti- CPB2 antibody was used as detection antibodies. The standards, test samples and biotin conjugated detection

antibody were added to the wells subsequently, and wash with wash buffer. HRP-Streptavidin was added and unbound conjugates were washed away with wash buffer. TMB substrates were 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 CPB2 amount of sample captured in plate. Read the O.D. absorbance at 450nm in a microplate reader, and then the concentration of CPB2 can be calculated.

Precautions for Use

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. After opening and before using, keep plate dry.
3. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
4. Storage TMB reagents avoid light.
5. Washing process is very important, not fully wash easily cause a false positive.
6. Duplicate well assay is recommended for both standard and sample testing.
7. Don't let Micro plate dry at the assay, for dry plate will inactivate active components on plate.
8. Don't reuse tips and tubes to avoid cross contamination.
9. Avoid using the reagents from different batches together.

Material Required but Not Supplied

1. Microplate reader (wavelength: 450nm)
2. 37°C incubator
3. Automated plate washer
4. Precision single and multi-channel pipette and disposable tips
5. Clean tubes and Eppendorf tubes
6. Deionized or distilled water

Manual Washing

Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with 350ul wash buffer and soak for 1 to 2 minutes, then aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material. Repeat this procedure two more times for a total of THREE washes.

Automated Washing

Aspirate all wells, then wash plate THREE times with 350ul wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 minute.

Sample Collection and Storage

Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20°C for long term. Avoid multiple freeze-thaw cycles.

- Plasma: Collect plasma using EDTA-Na₂ 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. Avoid hemolysis, high cholesterol samples.

Note: 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.

Sample Dilution Guideline

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 falls the optimal detection range of the kit. Dilute the sample with the provided dilution buffer, and several trials may be necessary in practice. The test sample must be well mixed with the dilution buffer. And also standard curves and sample should be make in pre-experiment.

- High target protein concentration (2000-20000ng/ml): Dilution: 1:100. (i.e. Add 1μl of sample into 99 μl of Sample / Standard dilution buffer.)
- Medium target protein concentration (200-2000ng/ml): Dilution: 1:10.(i.e. Add 10 μl of sample into 90 μl of Sample / Standard dilution buffer.)
- Low target protein concentration (3.12-200ng/ml): Dilution: 1:2.(i.e. Add 50 μl of sample into 50 μl of Sample / Standard dilution buffer.)
- Very low target protein concentration (≤3.12ng/ml): Unnecessary to dilute, or dilute at 1:2.

Reagent Preparation and Storage

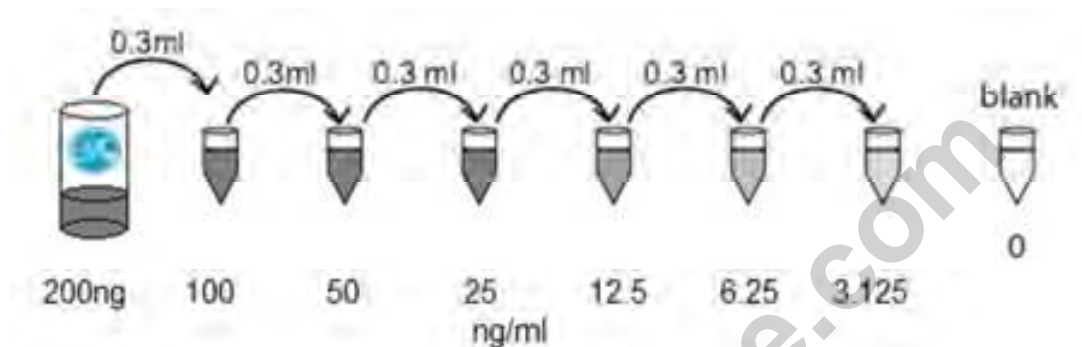
Bring all reagents to room temperature before use.

1, Wash Buffer:

Dilute 30mL 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, you can warm it with 40°C water bath (Heating temperature should not exceed 50°C) and mix it gently until the crystals have completely dissolved. The solution should be cooled to room temperature before use.

2, Standard:

- 1). 200ng/ml of standard solution: Add 1 ml of Sample / Standard dilution buffer into one Standard tube, keep the tube at room temperature for 10 min and mix thoroughly.
- 2). 100ng/ml→3.12ng/ml of standard solutions: Label 6 Eppendorf tubes with 100ng/ml, 50ng/ml, 25ng/ml, 12.5ng/ml, 6.25ng/ml, 3.12ng/ml, respectively. Aliquot 0.3 ml of the Sample / Standard dilution buffer into each tube. Add 0.3 ml of the above 200ng/ml standard solution into 1st tube and mix thoroughly. Transfer 0.3 ml from 1st tube to 2nd tube and mix thoroughly. Transfer 0.3 ml from 2nd tube to 3rd tube and mix thoroughly, and so on.



Note: The standard solutions are best used within 2 hours. The standard solution should be at 4°C for up to 12 hours. Or store at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

3, Preparation of Biotin- detection Antibody working solution

prepare within 1 hour before the experiment.

- 1) Calculate the total volume of the working solution: $0.1 \text{ ml / well} \times \text{quantity of wells}$. (Allow 0.1-0.2 ml more than the total volume)
- 2) Dilute the Biotin- detection antibody with Antibody dilution buffer at 1:100 and mix thoroughly. (i.e. Add 1 μl of Biotin- detection antibody into 99 μl of Antibody dilution buffer.)

4, Preparation of HRP-Streptavidin Conjugate (SABC) working solution:

prepare within 30min before the experiment.

- 1) Calculate the total volume of the working solution: $0.1 \text{ ml / well} \times \text{quantity of wells}$. (Allow 0.1-0.2 ml more than the total volume)
- 2) Dilute the SABC with SABC dilution buffer at 1:100 and mix thoroughly. (i.e. Add 1 μl of SABC into 99 μl of SABC dilution buffer.)

Assay Procedure

Before adding to wells, equilibrate the SABC working solution and TMB substrate for at least 30 min at 37°C. When diluting samples and reagents, they must be mixed completely and evenly. It is recommend to plot a standard curve for each test.

1. Set standard, test sample and control (zero) wells on the pre-coated plate respectively, and then, record their positions. It is recommend to measure each standard and sample in duplicate. **Wash plate 2 times before adding standard, sample and control (zero) wells!**
2. Aliquot 0.1ml of 200ng/ml, 100ng/ml, 50ng/ml, 25ng/ml, 12.5ng/ml, 6.25ng/ml, 3.12ng/ml, standard solutions into the standard wells.
3. Add 0.1 ml of Sample / Standard dilution buffer into the control (zero) well.
4. Add 0.1 ml of properly diluted sample (Human plasma, tissue homogenates and other biological fluids.) into test sample wells.
5. Seal the plate with a cover and incubate at 37°C for 90 min.
6. Remove the cover and discard the plate content, clap the plate on the absorbent filter papers or other absorbent material. Do NOT let the wells completely dry at any time. Do Not Wash Plate!
7. Add 0.1 ml of Biotin- detection antibody working solution into the above wells (standard, test sample & zero wells). Add the solution at the bottom of each well without touching the side wall.
8. Seal the plate with a cover and incubate at 37°C for 60 min.
9. Remove the cover, and wash plate 3 times with Wash buffer.
10. Add 0.1 ml of SABC working solution into each well, cover the plate and incubate at 37°C for 30 min.
11. Remove the cover and wash plate 5 times with Wash buffer, and each time let the wash buffer stay in the wells for 1-2 min.
12. Add 90 µl of TMB substrate into each well, cover the plate and incubate at 37°C in dark within 15-20min. (Note: This incubation time is for reference use only, the optimal time should be determined by end user.) And the shades of blue can be seen in the first 3-4 wells (with most concentrated CPB2 standard solutions), the other wells show no obvious color.
13. Add 50 µl of Stop solution into each well and mix thoroughly. The color changes into yellow immediately.
14. Read the OD. absorbance at 450 nm in a microplate reader immediately after adding the stop solution.

For calculation, (the relative OD.450) = (the OD.450 of each well) – (the OD.450 of Zero well). The standard curve can be plotted as the relative OD.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The CPB2 concentration of the samples can be interpolated from the standard curve. Recommended to use professional software curve expert to 1.3.

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

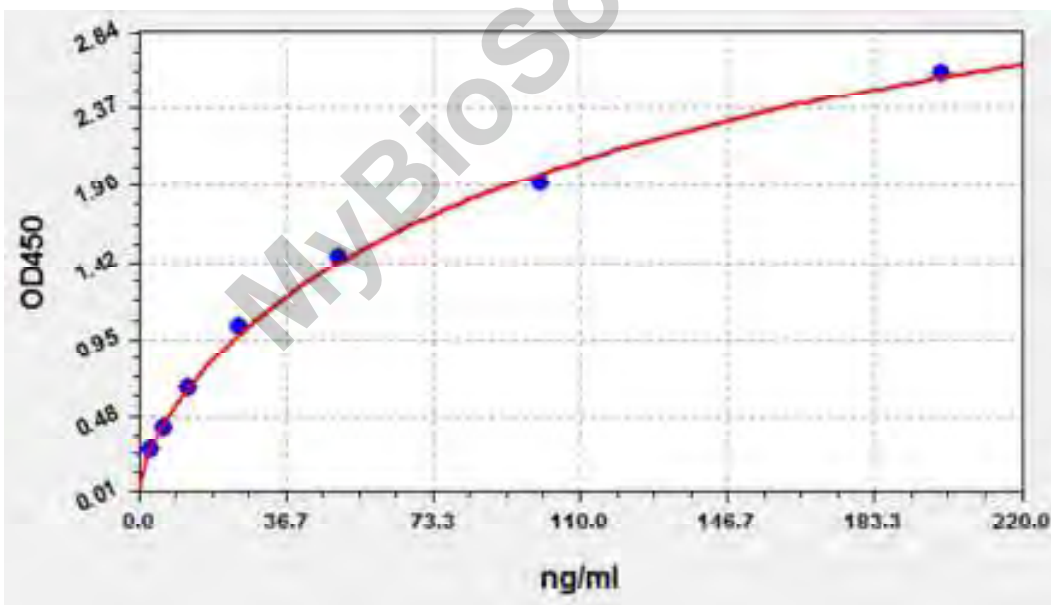
Summary

1. Wash plate 2 times before adding standard, sample and control (zero) wells!
2. Add 100 μ L standard or sample to each well for 90 minutes at 37°C
3. add 100 μ L Biotin- detection antibody working solution to each well for 60 minutes at 37°C
4. Aspirate and wash 3 times
5. Add 100 μ L SABC working solution to each well. Incubate for 30 minutes at 37°C
6. Aspirate and wash 5 times
7. Add 90 μ L TMB substrate. Incubate 15-20 minutes at 37°C
8. Add 50 μ L Stop Solution. Read at 450nm immediately
9. Calculation of results

Typical Data & Standard Curve

Results of a typical standard run of a CPB2 ELISA Kit are shown below. This standard curve was generated at our lab for demonstration purpose only. Each user should obtain their own standard curve as per experiment. (N/A=not applicable)

X	ng/ml	0	3.125	6.25	12.5	25	50	100	200
Y	OD450	0.068	0.269	0.407	0.648	1.035	1.453	1.907	2.589



Specificity

This assay has high sensitivity and excellent specificity for detection of CPB2 . No significant cross-reactivity or interference between CPB2 and analogues was observed.

Note: Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between CPB2 and all the analogues, therefore, cross reaction may still exist.

Recovery

Matrices listed below were spiked with certain level of CPB2 and the recovery rates were calculated by comparing the measured value to the expected amount of CPB2 in samples.

Matrix	Recovery range (%)	Average(%)
EDTA plasma(n=5)	88-102	95
heparin plasma(n=5)	87-104	97

Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of CPB2 and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

Sample	1:2	1:4	1:8	1:16
EDTA plasma(n=5)	82-100%	84-97%	89-99%	88-101%
heparin plasma(n=5)	85-99%	81-87%	82-96%	81-92%

Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level CPB2 were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level CPB2 were tested on 3 different plates, 8 replicates in each plate.

CV (%) = SD/meanX100

Intra-Assay: CV<8%

Inter-Assay: CV<10%

Stability

The stability of ELISA kit is determined by the loss rate of activity. The loss rate of this kit is less than 10% within the expiration date under appropriate storage condition.

Standard(n=5)	37°C for 1 month	4°C for 6 months
Average(%)	80	95-100

To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.

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