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METAP2 (Human) ELISA Kit

02/20

(Catalog # E4807-100, 96 assays, Store at 4°C)

I. Introduction:

Methionine aminopeptidase (MetAP) is a bifunctional protein that plays a critical role in the regulation of post-translational processing and protein synthesis. MetAP function is essential for cell proliferation and viability. MetAP2 plays an important role in the development of different types of cancer. BioVision's METAP2 (Human) ELISA Kit is based on the Competitive ELISA principle for the quantitative measurement of human METAP2 in serum, plasma and other biological fluids. The microtiter plate provided in this kit has been pre-coated with target. During the reaction, target in the sample or standard competes with a fixed amount of target on the solid phase supporter for sites on the Biotinylated Detection Antibody specific to target. Excess conjugate and unbound sample or standard are washed from the plate, and HRP-Streptavidin (SABC) is added to each microplate well and incubated. Then TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of target in the samples is then determined by comparing the OD of the samples to the standard curve.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Human METAP2.
Detection Range: 0.78-50 ng/ml
Sensitivity: 0.469 ng/ml
Precision: Intra-Assay: CV<8%, Inter-Assay: CV<10%

III. Sample Type:

Human serum, plasma, tissue homogenates and other biological fluids

IV. Kit Contents:

Components	E4807-100	Part No.	Storage Temp.
Micro ELISA Plate	8 X 12 strips	E4807-100-1	-20°C
Lyophilized Standard (50 ng)	2 vials	E4807-100-2	-20°C
Sample / Standard dilution buffer	20 ml	E4807-100-3	4°C
Biotin- labeled antibody (Concentrated)	60 µl	E4807-100-4	4°C
Antibody dilution buffer	10 ml	E4807-100-5	4°C
HRP-Streptavidin Conjugate (SABC)	120 µl	E4807-100-6	4°C (Avoid light)
SABC dilution buffer	10 ml	E4807-100-7	4°C
TMB substrate	10 ml	E4807-100-8	4°C (Avoid light)
Stop Solution	10 ml	E4807-100-9	4°C
Wash buffer (25X)	30 ml	E4807-100-10	4°C
Plate sealers	5	E4807-100-11	4°C

V. User Supplied Reagents and Equipment:

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

VI. Storage and Handling:

The entire kit may be stored at 4°C for up to 6 months from the date of shipment.

VII. Reagent and Sample Preparation:

Note: Prepare reagents within 30 minutes before the experiment.
Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Biotin- detection antibody working solution**: Calculate the total volume of the working solution: 0.05 ml / well × quantity of wells with additional 0.1 - 0.2 ml of the total volume. Dilute the Biotin- detection antibody with Antibody dilution buffer at 1:100 and mix thoroughly.
2. **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. Dilute the SABC with SABC dilution buffer at 1:100 and mix thoroughly.
3. **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.
4. **Standard Preparation:**
   - Add 1 ml Sample Dilution Buffer into one Standard tube (labeled as zero tube), keep the tube at room temperature for 10 minutes and mix them thoroughly.

- Label 7 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 of the above Standard solution (from zero tube) into 1st tube and mix them thoroughly.
- Transfer 0.3 ml from 1st tube to 2nd tube and mix them thoroughly. Transfer 0.3 ml from 2nd tube to 3rd tube and mix them thoroughly, and so on. Sample Dilution Buffer was used for the blank control. (Note: Please use Standard Solutions within 2 hours of preparation).

5. Sample Preparation:

Note: Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20°C (≤1 month) or -80°C(≤2 months). Avoid multiple freeze-thaw cycles. The hemolytic samples are not suitable for this assay.

- **Serum**: Place whole blood sample at room temperature for 2 hours or put it at 2-8°C overnight and centrifugation for 20 minutes at approximately 1000xg, Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and endotoxin free.
- **Plasma:** Collect plasma using EDTA-Na_2 or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000xg at 2 - 8°C within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.
- **Tissue homogenates**: As hemolysis blood has relation to assay result, it is necessary to remove residual blood by washing tissue with pre-cooling PBS buffer (0.01M, pH=7.4). Mince tissue after weighing it and get it homogenized in PBS (the volume depends on the weight of the tissue. Normal, 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitors are recommended to add into the PBS) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5minutes at 5000×g to get the supernatant. The total protein concentration was determined by BCA kit and the total protein concentration of each pore sample should not exceed 0.3mg.
- **Cell culture supernatant**: Centrifuge supernatant for 20 minutes at 1000×g at 2 - 8°C to remove insoluble impurity and cell debris. Collect the clear supernatant and carry out the assay immediately.
- **Cell Culture Lysate:** Commercial RIPA kits are recommended to follow the instructions provided. Generally, 0.5 ml RIPA lysis buffer would be appropriate to $2x10^6$ cells, DNA must to be removed. The total protein concentration was determined by BCA kit and the total protein concentration of each pore sample should not exceed 0.3 mg.
- **Other biological fluids:** Centrifuge samples for 20 min at 1000×g at 4°C. Collect the supernatant and carry out the assay immediately.

Note: 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.

VIII. Assay Protocol:

Note: 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 should be run for each assay.

1. Prepare all reagents, samples and standards as instructed in section VII.
2. Wash plate 2 times with **1X Wash buffer** before adding standard, sample and control wells.
3. Add 100 µl of each **standards** or **samples** into appropriate wells. Cover well and incubate for 1.5 hours at 37ºC.
4. Remove the cover and discard the plate content. Wash the plate 2 times with **1x wash buffer** without letting the wells completely dry.
5. Add 0.05 ml of **Biotin-detection antibody** work solution into the above wells. Seal the plate and incubate at 37ºC for 60 min.
6. Discard the solution and wash 3 times with **1X Wash buffer**. Wash by filling each well with Wash Buffer (350 µl) using a multi-channel pipette or autowasher. Let it soak for 1-2 minutes, and then remove all residual wash-liquid from the wells by aspiration. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Clap the plate on absorbent filter papers or other absorbent materials.
7. Add 0.1 ml of **SABC working solution** into each well, cover the plate and incubate at 37ºC for 30 min.
8. Discard the solution and wash 5 times with **1X Wash buffer** as step 6.
9. Add 90 µl of **TMB substrate** into each well, cover the plate and incubate at 37 ºC in dark within 10-20 min. (Note: The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. The reaction can be terminated when apparent gradient appeared in standard wells).
10. Add 50 µl of **Stop Solution** to each well. Read result at 450 nm within 20 minutes.

IX. 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 CD 97 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.

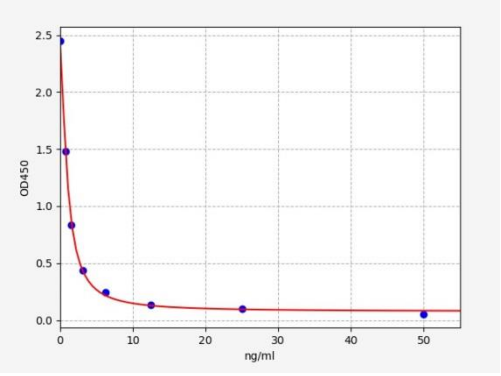

Figure: Typical Standard Curve and OD values: These standard curves are for demonstration only. A standard curve must be run with each assay.

FOR RESEARCH USE ONLY! Not to be used on humans.

STD.(ng/ml)	OD-1	OD-2	Average
0	2.412	2.482	2.447
0.781	1.461	1.503	1.482
1.562	0.809	0.833	0.821
3.125	0.429	0.441	0.435
6.25	0.242	0.248	0.245
12.5	0.132	0.136	0.134
25	0.099	0.101	0.1
50	0.05	0.052	0.051

X. Recovery:

Matrices listed below were spiked with certain level of METAP2 and the recovery rates were calculated by comparing the measured value to the expected amount of METAP2 in samples.

Matrix	Recovery Range (%)	Average (%)
Serum(n=5)	85-105	94
EDTA Plasma(n=5)	90-105	95
Heparin Plasma(n=5)	90-101	96

XI. Linearity:

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of METAP2 and their serial dilutions. The results were demonstrated by percentage of calculated concentration to the expectation.

Sample	1:2	1:4	1:8
Serum(n=5)	86-100%	87-102%	85-105%
EDTA Plasma(n=5)	82-98%	84-99%	82-96%
Heparin Plasma(n=5)	81-92%	81-95%	84-95%

XII. RELATED PRODUCTS:

- c-MET (Human) ELISA Kit (E4351)
- β-catenin (human) ELISA Kit (K3381)
- Glutathione Reductase (GR)(Human) ELISA Kit (E4623)
- 2-Methoxyestradiol (2-ME2) ELISA Kit (E4635)