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MMP-2 (Mouse) ELISA Kit

(Catalog# E4725-100; 96 assays; Storage at 4ºC)

I. Introduction:

The mammalian Matrix metalloproteinases (MMPs) degrade extracellular matrix in physiological and pathological processes. After cleavage of a single peptide domain of about 20 amino acids, the MMPs are secreted in latent forms. Upon activation, the N-terminal propeptide domain is cleaved to generate the active forms of MMP. MMP-2 (72 kDa type IV collagenase, Gelatinase-A) contains the basic structure of propeptide, catalytic, and hemopexin domains. It is an important proteinase in tissue remodeling. BioVision's MMP-2 ELISA kit is based on the Sandwich-ELISA principle. The micro ELISA plate provided in the kit has been pre-coated with an antibody specific to mouse MMP-2. On addition of standards or samples to the micro ELISA plate, they react with Biotinylated detection antibody specific for mouse MMP-2 and Avidin-Horseradish Peroxidase (HRP) conjugate, which on the addition of substrate gives blue color. The enzyme-substrate reaction is terminated by the addition of stop solution to turn the reaction yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of mouse MMP-2.The concentration of Mouse MMP-2 in the samples was calculated by comparing the OD of the samples to the standard curve.

II. Applications:

- A sandwich ELISA kit for *in vitro* quantitative determination of mouse MMP-2
- Detection Range: 0.16 - 10 ng/ml
- Coefficient of variation is < 10%.
- Sensitivity: 0.10 ng/ml
- Specificity: This kit recognizes mouse MMP-2 in samples. No significant cross-reactivity or interference between mouse MMP-2 and analogues was observed.

III. Sample Type:

- Plasma, Serum, Other biological fluids

IV. Kit Contents:

Components	E4722-100	Part Number	Storage Temp
Micro ELISA Plate	8 wells x 12 strips	E4725-100-1	-20°C
Standard	2 vials	E4725-100-2	-20°C
Biotinylated Detection Ab (100x)	120 µl	E4725-100-3	-20°C
HRP Conjugate (100x)	120 µl	E4725-100-4	-20°C (In dark)
Standard & Sample Diluent	20 ml	E4725-100-5	4°C
Biotinylated Detection Antibody Diluent	14 ml	E4725-100-6	4°C
HRP Conjugate Diluent	14 m	E4725-100-7	4°C
Wash Buffer (25x)	30 ml	E4725-100-8	4°C
Substrate Reagent	10 ml	E4725-100-9	4°C (In dark)
Stop Solution	10 ml	E4725-100-10	4°C
Plate Sealer	4	E4725-100-11	RT

V. User Supplied Reagents and Equipment:

- Deionized or distilled water
- Microplate reader with 450 nm wavelength filter

VI. Storage Conditions and Reagent Preparation:

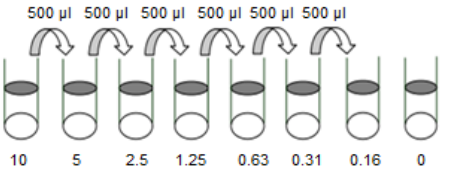
An unopened kit can be stored at 4ºC for 1 month**. If the kit is not used within 1 month, store the items separately according to the following conditions once the kit is received.**

1. HRP Conjugate: Calculate the required amount before the experiment (100 µl/well). Dilute the 100x Concentrated HRP Conjugate to 1x working solution with HRP Conjugate Diluent.

2. Biotinylated Detection Antibody: Calculate the required amount before the experiment (100µl/well). Centrifuge the stock tube before use; dilute the 100x Biotinylated Detection Antibody to 1x working solution with Biotinylated Detection Antibody Diluent.

3. Wash Buffer: Dilute 30 ml of Concentrated Wash Buffer with 720 ml of deionized or distilled water to prepare 750 ml of Wash Buffer. If crystals are present, warm it in a 40℃ water bath and mix gently until the crystals are completely dissolved.

4. Standard preparation: Centrifuge the standard at 10,000×g for 1 min. Add 1.0 ml of Standard and Sample Diluent, let it stand for 10 min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 10 ng/ml. Then make serial dilutions as needed. The recommended dilution gradient is as follows: 10, 10, 5, 2.5, 1.25, 0.63, 0.31, 0.16, 0 ng/ml. Prepare 7 tubes, add 500 µl of Standard & Sample Diluent to each tube. Pipette 500 µl of the 20 ng/ml stock solution to the first tube and mix up to produce a 10 ng/ml working solution. Transfer 500 µl of the solution into the other tube to form 2-fold serial dilutions of the highest standards to make the standard curve within the range of this assay.



5. Sample preparation:

Note: Samples should be assayed within 7 days when stored at 4°C, otherwise aliquot and stored at -20°C (≤1 month) or -80°C (≤3 months). Avoid repeated freeze-thaw cycles.

•**Serum:** Allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 15 min at 1000×g at 2~8ºC. Collect the supernatant to carry out the assay. Blood collection tubes should be disposable and be endotoxin free.

•**Plasma:** Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 min at 1000xg at 2~8°C within 30 min of collection. Collect the supernatant to carry out the assay. Hemolysed samples are not suitable for ELISA assay!

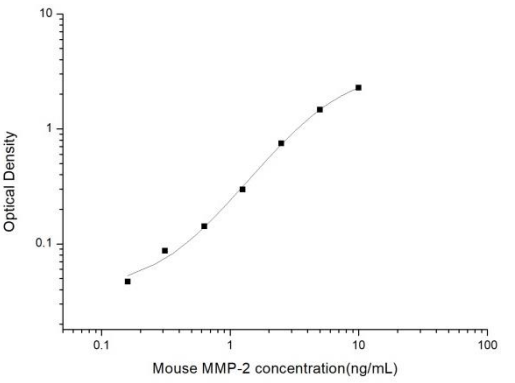
•**Cell lysates:** For adherent cells, gently wash the cells with moderate amount of pre-cooled PBS and dissociate the cells using trypsin. Collect the cell suspension into a centrifuge tube and centrifuge for 5 min at 1000xg. Discard the medium and wash the cells 3 times with pre-cooled PBS. For each $1x10^6$ cells, add 150-250 µl of pre-cooled PBS to keep the cells suspended. Repeat the freeze-thaw process several times until the cells are fully lysed. Centrifuge for 10min at 1500xg at 4°C. Remove the cell fragments; collect the supernatant to carry out the assay. Avoid repeated freeze-thaw cycles.

•**Tissue homogenates:** It is recommended to get detailed references from the literature before analyzing different tissue types. For general information, hemolysed blood may affect the results, so the tissues should be minced into small pieces and rinsed in ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then homogenized in PBS (tissue weight (g): PBS (mL) volume=1:9) with a glass homogenizer on ice. To further break down 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 5 min at 5000xg to get the supernatant.

•**Cell culture supernatant or other biological fluids:** Centrifuge samples for 20 min at 1000xg at 2~ 8°C. Collect the supernatant to carry out the assay.

VII. Assay Protocol:

1. Add 100 µl of each **standard or samples** into appropriate wells.
2. Cover the plate with the sealer provided in the kit. Incubate for 90 min at 37°C. Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and avoid bubble formation as much as possible.
3. Remove the liquid out of each well, do not wash. Immediately add 100 µl of **Biotinylated Detection Antibody** working solution to each well. Cover with the Plate sealer. Gently mix up. Incubate for 1 hour at 37°C.
4. Aspirate the solution from each well add 350 µl of 1x wash buffer to each well. Soak for 1~2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer can be used in this step and other wash steps.
5. Add 100 µl of **HRP Conjugate** working solution to each well. Cover with the Plate sealer. Incubate for 30 min at 37°C.
6. Aspirate or decant the solution from each well, repeat the wash process for five times as conducted in step 4
7. Add 90 µl of **Substrate Reagent** to each well. Cover with a new plate sealer. Incubate for about 15 min at 37°C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30 min.
8. Add 50 µl of **Stop Solution** to each well. Note: adding the stop solution should be done in the same order as the substrate solution.
9. Read the absorbance in micro plate reader set to 450 nm.
10. **Calculation:** Determine the average of the duplicate readings for each standard and samples, and then subtract the average blank standard readings. Plot a four-parameter logistic with standard concentration on the x-axis and OD values on the y-axis. If the samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample is above the upper limit of the standard curve, retest the samples with appropriate dilution. The actual concentration is the concentration obtained by calculated multiplied by the dilution factor. Typical standard curve and data is provided below for reference only. A standard curve must be run with each assay.



Conc. (ng/ml)	10	5	2.5	1.25	0.63	0.31	0.16	0
OD	2.336	1.523	0.808	0.356	0.201	0.146	0.106	0.059
OD corrected	2.277	1.464	0.749	0.297	0.142	0.087	0.047	-

VIII. Related Products:

MMP-2 (Rat) ELISA Kit (E4724)
MMP-2 (Human) ELISA Kit (E4723)

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