CELL APPLICATIONS, INC.

Human MMP-2 ELISA Kit

Cat. No. CL0459
96-wells

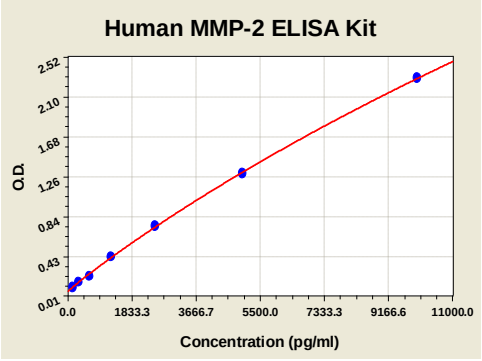
Target Protein Species:	Human
Range	156 pg/ml – 10,000 pg/ml
Specificity	No detectable cross-reactivity with other cytokines

KIT COMPONENTS

Component	Amount
96-well plate precoated with anti-human MMP-2 antibody	1 Plate
Protein Standard: Lyophilized recombinant human MMP-2	2 tubes, 50 ng/tube
Sample Diluent Buffer	30 ml
Biotinylated Antibody (Anti-human MMP-2)	130 µl (100x)
Antibody Diluent Buffer	12ml
Avidin-Biotin-Peroxidase Complex (ABC) Solution	130 µl (100x)
ABC Diluent Buffer	12 ml
Tetramethyllbenzidine (TMB) Color Developing Agent	10 ml
TMB Stop Solution	10 ml

Storage

Store at 4°C. Cell Applications, Inc. recommends using the kit within 6 months of order.



X	pg/ml	0.0	156	313	625	1250	2500	5000	10000
Y	O.D.450	0.065	0.105	0.157	0.219	0.424	0.751	1.298	2.298

Figure 1: MMP-2 Standard Curve. Using the Human MMP-2 ELISA Kit, O.D. data was graphed against MMP-2 protein concentration. The TMB reaction was incubated at 37°C for 27 min.

MMP-2 BACKGROUND

Matrix Metalloproteinase-2 (MMP2), also known as Type IV collagenase and gelatinase, 72-kD, plays an essential role in angiogenesis and arteriogenesis, two processes critical to restoration of tissue perfusion after ischemia. MMP-2 expression is increased in tissue ischemia, but the responsible mechanisms remain unknown.[1] MMPs catalyze extracellular matrix degradation. Control of their activity is a promising target for therapy of diseases characterized by abnormal connective tissue turnover. MMPs are expressed as latent proenzymes that are activated by proteolytic cleavage that triggers a conformational change in the propeptide (cysteine switch). The structure of proMMP-2 reveals how the propeptide shields the catalytic cleft and that the cysteine switch may operate through cleavage of loops essential for propeptide stability.[2] The gene was localized to 16q21 using somatic cell hybrids and in situ hybridization.[3] The standard protein used in this kit is recombinant human MMP-2, consisting of 631 amino acids with the molecular mass of 71KDa. The detected MMP-2 includes zymogen and active enzyme.

References

1. Lee, J. G, et al: Proc. Nat. Acad. Sci. 102: 16345-16350, 2005.
2. Morgunova, E, et al: Science 284: 1667-1670, 1999.
3. Huhtala, P, et al: Genomics 6: 554-559, 1990.

ELISA OVERVIEW

Cell Applications ELISA Kits are based on standard sandwich enzyme-linked immunosorbent assay technology. Freshly prepared standards, samples, and solutions are recommended for best results.

1. Prepare test samples.
2. Prepare a protein standard of the target protein.
3. Add test samples and standards to the pre-coated 96-well plate. Do not wash.
4. Add biotinylated detection antibodies. Wash.
5. Add Avidin-Biotin-Peroxidase Complex (ABC) Solution. Wash.
6. Add Tetramethyllbenzidine (TMB) Color Developing Agent, containing HRP substrate.
7. Add TMB Stop Solution
8. Subject the plate to analysis.

NOTES:

- Before using the kit, quick spin tubes to bring down all solution to the bottom of tube.
- Duplicate assay wells are recommended for both standard and sample testing.
- Do not let the 96-well plate dry, this will lead to inactivation of plate components.
- When diluting samples and reagents, ensure that they are mixed completely and evenly.
- Pre-warm diluted ABC and TMB solutions at 37°C for 30 min before use to avoid variable temperature effects.
- For washes, use TBS or PBS. Do not touch well walls.
- A protein standard is included in the kit. A protein standard detection curve should be generated with each experiment, no more than 2 hours prior to the experiment.
- The user will determine sample dilution fold by estimation of target protein amount in samples.

ELISA PROTOCOL

Preparation of Test Samples

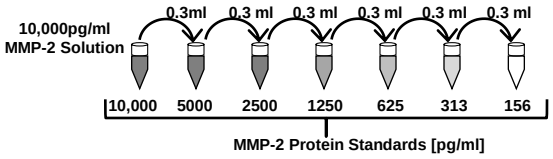
1. Process Test Samples in the following manner:
   - **Cell culture supernate, tissue lysate or body fluids**: Remove particulates by centrifugation.
   - **Serum**: Allow the serum to clot in a serum separator tube (about 30 min) at room temperature. Centrifuge at approximately 1000 x g for 15 min.
   - **Plasma**: Collect plasma using heparin as an anticoagulant. Centrifuge for 15 min at 1000 x g within 30 min of collection. EDTA and citrate are not recommended as anticoagulant.
2. Estimate the concentration of the target protein in the sample and select a proper dilution factor such that the diluted target protein concentration falls within the 156-10000 pg/ml standard curve range. Depending on the sample, several trial dilutions may be necessary. Dilute the sample using the provided diluent buffer, mixing well. Suggested working dilutions of samples are as follows:

Target Protein Concentration Range	Sample Working Dilution	Sample Vol.	Diluent Buffer Vol.
100-1000 ng/ml	1:100	1 µl	99 µl
10-100 ng/ml	1:10	10 µl	90 µl
156-10,000 pg/ml	1:2	50 µl	50 µl
≤156 pg/ml	n/a	100µl	n/a

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

Preparation of Standard Solutions (156-10000 pg/ml)

4. Reconstitute the Lypophilized Recombinant Protein to make a 50,000 pg/ml MMP-2 solution. Add 1 ml Sample Diluent Buffer to a tube of lypophilized protein, keep the tube at room temperature for 10 min. Mix thoroughly.
5. Aliquot 0.8 ml of the Sample Diluent Buffer to an eppendorf tube, and label as 10,000 pg/ml Protein Standard.
6. Add 0.2 ml of the mixed 50,000 pg/ml MMP-2 solution to the eppendorf tube containing 0.8 ml diluent buffer and mix to make a 10,000 pg/ml MMP-2 solution.
7. Label 6 eppendorf tubes with the MMP-2 protein concentrations to be prepared by serial dilution: 5000pg/ml, 2500pg/ml, 1250pg/ml, 625pg/ml, 313pg/ml, 156pg/ml.
8. Aliquot 0.3 ml of the Sample Diluent Buffer to the labeled tubes.
9. Serially dilute the protein standards into their respectively labeled tubes. Transfer 0.3 ml from the 10,000pg/ml MMP-2 Solution to the 5000pg/ml eppendorf tube and mix thoroughly. Transfer 0.3 ml of the 5000 pg/ml solution to the 2500 pg/ml tube and mix thoroughly, Transfer 0.3 ml of the 2500 pg/ml solution to the 1250 pg/ml tube and mix, and so on to make the 625, 313, 156 pg/ml solutions.
10. Store at 4°C until use.



Loading the 96-well Plate

11. Aliquot 0.1 ml of the sample diluent buffer into a control well to serve as the Blank. This will yield the O.D.450(Blank) reading.
12. Aliquot 0.1 ml of the standard solutions of the **Preparation of Standard Solutions** (156-10000 pg/ml) into empty wells of the precoated 96-well plate. Duplicate measurements of standards are recommended.
13. Aliquot 0.1 ml of each properly diluted test sample to empty wells prepared in **Step 2**. Duplicate measurements of each test sample are recommended.
14. Cover the 96-well plate and incubate at 37°C for 90 min.
15. During the **Step 14** incubation period, prepare a stock of Biotinylated 1:100 Antibody Working Solution. Count the number of reactions and multiply by 0.1 ml/well for the Working Solution total volume (preparation of 1-2 reactions in excess of the number of wells is recommended). Dilute the Biotinylated Antibody to 1:100 in Antibody Diluent Buffer and mix thoroughly. Use the working solution within 2 hours.
16. Upon completion of the 90 min incubation of **Step 14**, remove the cover of the 96 well plate and discard plate well contents. Blot the plate onto paper towels or other absorbent material. DO NOT let the wells completely dry at any time.
17. Add 0.1 ml of the Biotinylated 1:100 Antibody Working Solution (prepared in **Step 15**) to each well and incubate the plate at 37°C for 60 min.
18. During the incubation period of **Step 17**, prepare a stock of ABC Working Solution. Count the number of reactions and multiply by 0.1 ml/well for the Working Solution total volume (preparation of 1-2 reactions in excess of the number of wells is recommended). Dilute the ABC Stock Solution to 1:100 in ABC Diluent Buffer and mix thoroughly. Pre-warm the ABC working solution at 37°C for 30 min before use. Use the working solution within 1 hour.
19. Upon completion of the 60 min incubation of **Step 17**, wash the plate 3 times with 0.3 ml TBS or PBS. For each wash, leave washing buffer in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.
20. Add 0.1 ml of prepared ABC Working Solution (prepared in **Step 18**) to each well and incubate the plate at 37°C for 30 min.
21. During the incubation period of **Step 20**, pre-warm TMB Color Developing Agent at 37°C for 30 min before use.
22. Upon completion of the 30 min incubation of **Step 20**, wash the plate 5 times with 0.3 ml TBS or PBS. For each wash, leave the washing buffer in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.
23. Add 90 µl of the pre-warmed TMB Color Developing Agent into each well and incubate at 37°C for 25-30 min (shades of blue can be seen in the wells with the four most concentrated Protein Standard Solutions; the other control wells should show no obvious color).
24. Add 0.1 ml of the TMB Stop Solution to each well. The acidic stop solution will change the mixture color to yellow. The yellow intensity is proportional to the amount of target protein captured by the plate.
25. Read the O.D. absorbance at 450nm in a microplate reader within 30 min after adding the stop solution. These readings are the O.D.450(Reading).

Calculating Protein Concentration

- For all wells, determine O.D.450(Relative):
  O.D.450(Relative) = O.D.450(Reading) – O.D.450(Blank)

- Plot the standard curve: plot O.D.450(Relative) of each standard solution (Y) vs. the respective concentration of the standard solution (X). See **Figure 1** for a typical standard curve.
- The MMP-2 concentration of the samples can be interpolated from the standard curve. Multiply the interpolated concentration by the dilution factor to obtain the target protein concentration in the sample.