

**COMPONENTS**

Kit Component	Amount
96-well plate precoated with anti-human MMP13 antibody	1 Plate
Protein Standard: Lyophilized recombinant human MMP13	2 tubes, 10 ng/tube
Sample Diluent Buffer	30 ml
Biotinylated Antibody (Anti-human MMP13)	130 µl (100x)
Antibody Diluent Buffer	12ml
Avidin-Biotin-Peroxidase Complex (ABC) Solution	130 µl (100x)
ABC Diluent Buffer	12 ml
Tetramethylbenzidine (TMB) Color Developing Agent	10 ml
TMB Stop Solution	10 ml

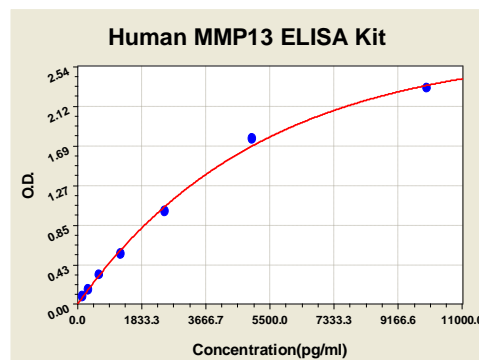
**Washing Buffer (not provided): TBS or PBS**

0.01M TBS: Add 1.2g Tris, 8.5g NaCl; 450µl of purified acetic acid or 700µl of concentrated hydrochloric acid to 900ml H<sub>2</sub>O and adjust pH to 7.2-7.6. Adjust the total volume to 1L.

0.01M PBS: Add 8.5g sodium chloride, 1.4g Na<sub>2</sub>HPO<sub>4</sub> and 0.2g NaH<sub>2</sub>PO<sub>4</sub> to 900ml distilled H<sub>2</sub>O and adjust pH to 7.2-7.6. Adjust the total volume to 1L.

**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	0.024	0.091	0.167	0.316	0.541	1.000	1.770	2.311

**Figure 1: MMP13 Standard Curve.** Using the Human MMP13 ELISA Kit, O.D. data was graphed against MMP13 protein concentration. The TMB reaction was incubated at 37°C for 27 min.

**BACKGROUND**

MMPs (matrix metalloproteinases) are zinc-dependent endopeptidases that are mainly involved in turnover and proteolytic degradation of the extracellular matrix and are believed to play a role in tissue remodeling in a number of normal and pathological conditions. MMPs may play a key role in physiologic and pathologic remodeling of tissues, including embryogenesis and tissue morphogenesis, wound repair, inflammatory diseases, cancerogenesis, etc. These multiple actions of MMPs are due to their ability to degrade extracellular matrices, basement membranes, and other proteins. So far, more than 25 members of the MMP family have been identified, and they can be divided into four groups on the basis of their domain structure, substrate specificity and cellular localization. These four groups are collagenases (MMP-1, -8 and -13), stromelysins (MMP-3, -7, -10, -11 and -12), gelatinases (MMP-2 and -9) and membrane-type MMPs.<sup>1</sup>

MMP-13 is a glycosylated protein of 60 kDa. It was reported that MMP-13 is tightly bound to tissues and utilizes heparan sulfate proteoglycans as extracellular docking molecules. MMP-13 is produced by many different cell types as an inactive proform that requires proteolytic activation to gain activity. Activation can involve MMP-2 and MMP-14. Plasmin is also an activating factor of the MMP-13 proform, but Plasmin can further deactivate active MMP-13. Trypsin-2 was found to directly activate MMP-13. MMP-13 has a wide substrate specificity, degrading collagen type 1, collagen type 3, collagen type 4. MMP-13 preferentially hydrolyzes soluble collagen type 2. Other substrates are aggrecan, gelatin, Galectin-3, the proform of MMP-9. It was also reported that MMP-13 can proteolytically process fibrinogen and also inactivates Factor XII (Hageman factor). MMP-13 was found to degrade fibrillin, the principal structural component of the 10-12 nm diameter elastic microfibrils of the extracellular matrix. MMP-13 is inhibited by the tissue inhibitors of metalloproteinases, TIMP-1, TIMP-2, and TIMP-3.<sup>2</sup> It has been shown that MMP-13 binds to, and is internalized by, a specific receptor, which has been identified as ENDO180 (CD280). MMP-13, together with other metalloproteinases, degrades major components of the extracellular matrix and thus can be assumed to play a role in skeletal development, tissue remodeling, and wound healing. MMP-13, as one of the collagenases, is responsible for collagen remodeling associated with angiogenesis.<sup>3</sup>

**References**

1. Mohamed, A. J. et al: Immunol. Rev. 228:58-73, 2009
2. Knäuper, V. et al: J. Biol. Chem. 271:17124-31, 1996
3. Hattori, N. et al: Am. J. Path. 175:533-46, 2009

**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 Tetramethylbenzidine (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.

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## PROTOCOL

### I. Plate Washing

Discard the solution in the plate without touching the side walls. Blot the plate onto paper towels or other absorbent material. Soak each well with at least 0.3 ml PBS or TBS buffer for 1–2 minutes. Repeat this process two additional times for a total of three washes.

### II. Preparation of Test Samples

#### Test Sample Processing

- **Cell culture supernate, tissue lysate or body fluids:** Remove particulates by centrifugation.
- **Serum:** Allow the serum to clot in a serum separator tube (about 2 hours) at room temperature. Centrifuge at approximately 1000 X g for 10 min.
- **Plasma:** Collect plasma using heparin, EDTA as an anticoagulant. Centrifuge for 30 min at 1000 x g within 30 min of collection. Analyze immediately or aliquot and store frozen at -20°C. Citrate is not recommended as the anticoagulant.

#### Sample Dilution Guideline

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 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.
100-100 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

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.

### III. Preparation of Reagents

#### Reconstitution of the Standard

The standard solutions should be prepared no more than 2 hours prior to the experiment. Two tubes of the standard are included in each kit. Use one tube for each experiment.

1. 10,000pg/ml of human MMP13 standard solution: Add 0.5 ml sample diluent buffer into one tube, keep the tube at room temperature for 10 min and mix thoroughly.
2. 5000pg/ml→156pg/ml of human MMP13 standard solutions: Label 6 Eppendorf tubes with 5000pg/ml, 2500pg/ml, 1250pg/ml, 625pg/ml, 313pg/ml, 156pg/ml, respectively. Aliquot 0.3 ml of the sample diluent buffer into each tube. Add 0.3 ml of the above 10, 000pg/ml MMP13 standard solution into 1st tube and mix. Transfer 0.3 ml from 1st tube to 2nd tube and mix. Transfer 0.3 ml from 2nd tube to 3rd tube and mix, and so on.

#### Preparation of Biotinylated Antibody Working Solution

The solution should be prepared no more than 2 hours prior to the experiment.

1. The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
2. Biotinylated antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly.

### Preparation of the Avidin-Biotin-Peroxidase Complex (ABC) Working Solution

The solution should be prepared no more than 1 hour prior to the experiment.

1. The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
2. Avidin- Biotin-Peroxidase Complex (ABC) should be diluted in 1:100 with the ABC dilution buffer and mixed thoroughly.

### IV. ELISA

The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 min before use. When diluting samples and reagents, they must be mixed completely and evenly. A standard detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of target protein amount in samples.

1. Aliquot 0.1ml per well of the 10,000pg/ml, 5000pg/ml, 2500pg/ml, 1250pg/ml, 625pg/ml, 313pg/ml, 156pg/ml human MMP13 standard solutions into the precoated 96-well plate. Add 0.1ml of the sample diluent buffer into the control well (Blank well). Add 0.1ml of each properly diluted sample of human sera, plasma, body fluids, tissue lysates or cell culture supernatants to each empty well. See "Sample Dilution Guideline" for details. We recommend that each human MMP13 standard solution and each sample is measured in duplicate.
2. Seal the plate with the cover and incubate at 37°C for 90 min.
3. Remove the cover, discard plate content, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.
4. Add 0.1ml of biotinylated anti-human MMP13 antibody working solution into each well and incubate the plate at 37°C for 60 min.
5. Wash plate 3 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (**Plate Washing Method:** Discard the solution in the plate without touching the side walls. Blot the plate onto paper towels or other absorbent material. Soak each well with at least 0.3 ml PBS or TBS buffer for 1–2 minutes. Repeat this process two additional times for a total of THREE washes. Note: For automated washing, aspirate all wells and wash THREE times with PBS or TBS buffer, overfilling wells with PBS or TBS buffer. Blot the plate onto paper towels or other absorbent material.)
6. Add 0.1ml of prepared ABC working solution into each well and incubate the plate at 37°C for 30 min.
7. Wash plate 5 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (See Step 5 for plate washing method).
8. Add 90 µl of prepared TMB color developing agent into each well and incubate plate at 25°C in dark for 25-30min (**Note:** For reference only, the optimal incubation time should be determined by end user. And the shades of blue can be seen in the wells with the four most concentrated human MMP13 standard solutions; the other wells show no obvious color).
9. Add 0.1ml of prepared TMB stop solution into each well. The color changes into yellow immediately.
10. Read the O.D. absorbance at 450nm in a microplate reader within 30 min after adding the stop solution.

### V. 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 target protein concentration in 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.

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