# MMP2 (Human) ELISA Kit

Catalog Number KA0391

96 assays

Version: 29

Intended for research use only

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# Table of Contents

## Introduction

### Intended Use

For the quantitation of Human MMP2 concentrations in cell culture supernatants, serum and plasma (heparin).

### Background

Type IV collagenase, 72-kD, is officially designated matrix metalloproteinase-2 (MMP2). It is also known as gelatinase, 72-kD. MMP-2 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. Matrix metalloproteinases (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. The gene is localized to 16q21 using somatic cell hybrids and in situ hybridization. The standard product used in this kit is recombinant human MMP-2, consisting of 631 amino acids with the molecular mass of 71 KDa. The detected MMP-2 includes zymogen and active enzyme.

### Principle of the Assay

The MMP2 (human) ELISA Kit is a solid phase immunoassay specially designed to measure Human MMP2 with a 96-well strip plate that is pre-coated with antibody specific for MMP2. The detection antibody is a biotinylated antibody specific for MMP2. The capture antibody is monoclonal antibody from mouse, the detection antibody is polyclonal antibody from goat. The kit contains recombinant Human MMP2 with immunogen: Expression system for standard: NS0; Immunogen sequence: A30-C660. The kit is analytically validated with ready to use reagents.

To measure Human MMP2, add standards and samples to the wells, then add the biotinylated detection antibody. Wash the wells with PBS or TBS buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbounded ABC-HRP with PBS or TBS buffer and add TMB. TMB is substrate to HRP and will be catalyzed to produce a blue color product, which changes into yellow after adding acidic stop solution. The absorbance of the yellow product at 450nm is linearly proportional to Human MMP2 in the sample. Read the absorbance of the yellow product in each well using a plate reader, and benchmark the sample wells' readings against the standard curve to determine the concentration of Human MMP2 in the sample

Uniprot: P08253

## General Information

### Materials Supplied

List of component

| Component | Amount | Storage of opened/reconstituted material |
|---|---|---|
| Anti-Human MMP2 Pre-coated 96-well strip microplate | 96 (8x12) wells | Return unused wells to the foil pouch. Reseal along the entire edge of the zip-seal. May be stored for up to 1 month at 4°C provided this is within the expiration date of the kit. |
| Human MMP2 Standard | 40 ng/tube x 2 | Discard the MMP2 stock solution after 12 hours at 4°C. May be stored at -20°C for 48 hours. |
| Human MMP2 Biotinylated antibody (100x) | 100 µL | May be stored for up to 1 month at 4°C provided this is within the expiration date of the kit. |
| Avidin-Biotin-Peroxidase Complex (100x) | 100 µL | |
| Sample Diluent | 30 mL | |
| Antibody Diluent | 12 mL | |
| Avidin-Biotin-Peroxidase Diluent | 12 mL | |
| Color Developing Reagent (TMB) | 10 mL | |
| Stop Solution | 10 mL | |
| Wash Buffer (25x) | 20 mL | |
| Plate Sealers | 4 slides | |

### Storage Instruction

Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles.

### Materials Required but Not Supplied

- ✓ Microplate Reader capable of reading absorbance at 450 nm.
- ✓ Automated plate washer (optional).
- ✓ Pipettes and pipette tips capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.
- ✓ Multichannel pipettes are recommended for large amount of samples.
- ✓ Deionized or distilled water.
- ✓ 500 mL graduated cylinders.
- ✓ Test tubes for dilution.

## Precautions for Use

This protocol must be read in its entirety before using this product. For research use only. Not for use in diagnostic procedures.

✓ Preparations Before Assay

Please read the following instructions before starting the experiment.

1. Read this manual in its entirety in order to minimize the chance of error.
2. Confirm that you have the appropriate non-supplied equipment available.
3. Confirm that the species, target antigen, and sensitivity of this kit are appropriate for your intended application.
4. Confirm that your samples have been prepared appropriately based upon recommendations (see Sample Preparation) and that you have sufficient sample volume for use in the assay.
5. When first using a kit, appropriate validation steps should be taken before using valuable samples. Confirm that the kit adequately detects the target antigen in your intended sample type(s) by running control samples.
6. If the concentration of target antigen within your samples is unknown, a preliminary experiment should be run using a control sample to determine the optimal sample dilution (see Sample Preparation).
7. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, a pilot experiment using standards and a small number of samples is recommended.
8. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
9. Don’t let 96-well plate dry for dry plate will inactivate active components on plate.
10. Don’t reuse tips and tubes to avoid cross contamination.
11. Avoid using the reagents from different batches together.
12. The kit should not be used beyond the expiration date on the kit label. Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding. Variations in sample collection, processing, and storage may cause sample value differences.

## Assay Protocol

**Reagent Preparation**

✓ Bring all reagents to room temperature (18-25°C) prior to use. Please DO NOT equilibrate unused plate well strips to room temperature. They should be sealed and stored in the original packaging. We recommend doing it at 37°C for best consistency with our QC results. Also the TMB incubation time estimate (15-25 min) is based on 37°C.

- Wash buffer

  Prepare 500 mL of Working Wash Buffer by diluting the supplied 20 mL of Wash Buffer (25 x) with 480 mL of deionized or distilled water. If crystals have formed in the concentrate, warm to room temperature and mix it gently until crystals have completely dissolved.
- Biotinylated Anti-Human MMP2 antibody

  It is recommended to prepare this reagent immediately prior to use by diluting the Human MMP2 Biotinylated antibody (100x) 1:100 with Antibody Diluent. Prepare 100 µL by adding 1 µL of Biotinylated antibody (100x) to 99 µL of Antibody Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
- Avidin-Biotin-Peroxidase Complex

  It is recommended to prepare this reagent immediately prior to use by diluting the Avidin-Biotin-Peroxidase Complex (100x) 1:100 with Avidin-Biotin-Peroxidase Diluent. Prepare 100 µL by adding 1 µL of Avidin-Biotin-Peroxidase Complex (100x) to 99 µL of Avidin-Biotin-Peroxidase Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
- Human MMP2 Standard

  It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. Use one 40 ng of lyophilized Human MMP2 standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 40 ng/mL using 1 mL of sample diluent. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions.
- Microplate

  The included microplate is coated with capture antibodies and ready-to-use. It does not require additional washing or blocking. The unused well strips should be sealed and stored in the original packaging.

✓ Dilution of Human MMP2 Standard

1. Number tubes 1-8. Final Concentrations to be Tube # 1 –40000 pg/mL, #2 –20000 pg/mL, #3 – 10000 pg/mL, #4 – 5000 pg/mL, #5 – 2500 pg/mL, #6 – 1250 pg/mL, #7 – 625 pg/mL, #8 –Sample Diluent serves as the zero standard (0 pg/mL).
2. For standard #1, add 1000 µL of undiluted standard stock solution to tube #1.
3. Add 300 µL of sample diluent to tubes # 2-7.
4. To generate standard #2, add 300 µL of standard #1 from tube #1 to tube #2 for a final volume of 600

µL. Mix thoroughly.

5. To generate standard #3, add 300 µL of standard #2 from tube #2 to tube #3 for a final volume of 600 µL. Mix thoroughly.
6. Continue the serial dilution for tube #4-7.

## Sample Preparation

✓ Sample Preparation and Storage

These sample collection instructions and storage conditions are intended as a general guideline and the sample stability has not been evaluated.

- Cell culture supernatants: Clear sample of particulates by centrifugation, assay immediately or store samples at -20°C.
- Serum: Use a serum separator tube (SST) and allow serum to clot at room temperature for about four hours. Then, centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C.
- Plasma: Collect plasma using heparin as an anticoagulant. Centrifuge for 15 min at approximately 1,000 x g. Assay immediately or store samples at -20°C.

  **Note: it is important to not use anticoagulants other than the ones described above to treat plasma for other anticoagulants could block the antibody binding site.*

✓ *Sample Collection Notes*

1. *We recommend that samples are used immediately upon preparation.*
2. *Avoid repeated freeze/thaw cycles for all samples.*
3. *In the event that a sample type not listed above is intended to be used with the kit, it is recommended that the customer conduct validation experiments in order to be confident in the results.*
4. *Due to chemical interference, the use of tissue or cell extraction samples prepared by chemical lysis buffers may result in inaccurate results.*
5. *Due to factors including cell viability, cell number, or sampling time, samples from cell culture supernatant may not be detected by the kit.*
6. *Samples should be brought to room temperature (18-25°C) before performing the assay without the use of extra heating.*
7. *Sample concentrations should be predicted before being used in the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.*
8. *Abnova is responsible for the quality and performance of the kit components but is NOT responsible for the performance of customer supplied samples used with the kit.*

✓ Sample Dilution

The user needs to estimate the concentration of the target protein in the sample and use an appropriate dilution factor so that the diluted target protein concentration falls in the range of O.D. values of the standard curve. Dilute the sample using provided diluent buffer. Pilot tests using a dilution series of each sample type are necessary. The sample must be mixed thoroughly with Sample Diluent.

Dilute the sample so that the expected range of concentrations fall within the detection range of this kit.

If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples.

Some PubMed article(s) citing the expression level of this target are as follows: 17239302

Our internal QC testing used Dilution ratio of 1:10, concentration in serum is around 50 ng/mL.

## Assay Procedure

It is recommended that all reagents and materials be equilibrated to 37°C/room temperature prior to the experiment (see Reagent Preparation before the experiment if you have missed this information).

1. Prepare all reagents and working standards as directed previously.
2. Remove excess microplate strips from the plate frame and seal and store them in the original packaging.
3. Add 100 µL of the standard, samples, or control per well. Add 100 µL of the sample diluent buffer into the zero well. At least two replicates of each standard, sample, or control is recommended.
4. Cover with the plate sealer provided and incubate for 120 minutes at RT (or 90 minutes at 37°C).
5. Remove the cover and discard the liquid in the wells into an appropriate waste receptacle. Invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
6. Add 100 µL of the prepared 1x Biotinylated Anti-Human MMP2 antibody to each well.
7. Cover with plate sealer and incubate for 90 minutes at RT (or 60 minutes at 37°C).
8. Wash the plate 3 times with the 1x wash buffer.

   a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.

   b. Add 300 µL of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).

   c. Repeat steps a-b 2 additional times.

   d. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid.
9. Add 100 µL of the prepared 1x Avidin-Biotin-Peroxidase Complex into each well. Cover with the plate sealer provided and incubate for 40 minutes at RT (or 30 minutes at 37°C).
10. Wash the plate 5 times with the 1x wash buffer.

    a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that

the wells are not allowed to completely dry at any time.

b. Add 300 µL of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).

c. Repeat steps a-b 4 additional times.

d. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid.

11. Add 90 µL of Color Developing Reagent to each well. Cover with the plate sealer provided and incubate in the dark for 30 minutes at RT (or 15-25 minutes at 37°C). (The optimal incubation time must be empirically determined. A guideline to look for is blue shading the top four standard wells, while the remaining standards remain clear.)
12. Add 100 µL of Stop Solution to each well. The color should immediately change to yellow.
13. Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450 nm.

✓ *Assay Protocol Notes*

1. *Solutions: To avoid cross-contamination, change pipette tips between additions of each standard, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.*
2. *Applying Solutions: All solutions should be added to the bottom of the ELISA plate well. Avoid touching the inside wall of the well. Avoid foaming when possible.*
3. *Assay Timing: The interval between adding samples to the first and last wells should be minimized. Delays will increase the incubation time differential between wells, which will significantly affect the experimental accuracy and repeatability. For each step in the procedure, total dispensing time for addition of reagents or samples should not exceed 10 minutes.*
4. *Incubation: To prevent evaporation and ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods of time between incubation steps. Do not let wells dry out at any time during the assay. Strictly observe the recommended incubation times and temperatures.*
5. *Washing: Proper washing procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. Residual liquid in the reaction wells should be patted dry against absorbent paper during the washing process. Do not put absorbent paper directly into the reaction wells.*
6. *Controlling Substrate Reaction Time: After the addition of the TMB Substrate, periodically monitor the color development. Stop color development before the color becomes too deep by adding Stop Solution. The excessively strong color will result in inaccurate absorbance readings.*
7. *Reading: The microplate reader should be preheated and programmed prior to use. Prior to taking O.D. readings, remove any residual liquid or fingerprints from the underside of the plate and confirm that there are no bubbles in the wells.*
8. *Reaction Time Control: Control reaction time should be strictly followed as outlined.*
9. *Stop Solution: The Stop Solution contains an acid, therefore proper precautions should be taken during its use, such as protection of the eyes, hands, face, and clothing.*
10. *To minimize the external influence on the assay performance, operational procedures and lab conditions (such as room temperature, 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.*

## Data Analysis

### Calculation of Results

Average the duplicate readings for each standard, sample, and control. Subtract the average zero standard O.D. reading.

It is recommended that a standard curve be created using computer software to generate a four parameter logistic (4-PL) curve assay.

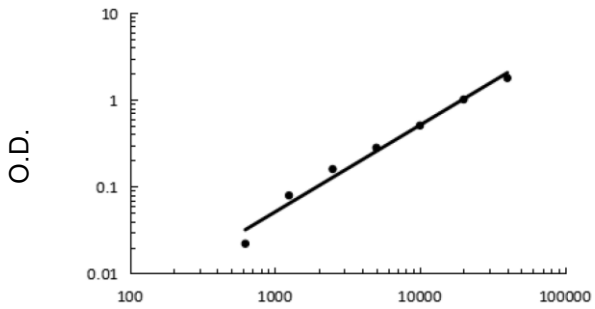
Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative OD against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data.

For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor.

✓ MMP2 (Human) ELISA Kit Standard Curve Sample

The highest O.D. value might be higher or lower than in the example. The experiment result is statistically significant if the highest O.D. value is no less than 1.0.

| Concentration (pg/mL) | 0 | 625 | 1250 | 2500 | 5000 | 10000 | 20000 | 40000 |
|---|---|---|---|---|---|---|---|---|
| O.D | 0.033 | 0.055 | 0.112 | 0.192 | 0.313 | 0.538 | 1.041 | 1.812 |




A standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

### Performance Characteristics

✓ Detection Range: 625 pg/mL - 40000 pg/mL

✓ Sensitivity: < 10 pg/mL

* The sensitivity or the minimum detectable dose (MDD) is the lower limit of target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration.

✓ Specificity: Natural and recombinant human MMP2

✓ Cross-reactivity: There is no detectable cross-reactivity with other relevant proteins

✓ Intra/Inter Assay Variability

- Intra-Assay Precision (Precision within an assay)

  Three samples of known concentration were tested on one plate to assess intra-assay precision.

- Inter-Assay Precision (Precision across assays)

  Three samples of known concentration were tested in separate assays to assess inter-assay precision.

| | Intra-Assay Precision | | | Inter-Assay Precision | | |
|---|---|---|---|---|---|---|
| Sample | 1 | 2 | 3 | 1 | 2 | 3 |
| n | 16 | 16 | 16 | 24 | 24 | 24 |
| Mean (pg/mL) | 1326 | 3925 | 14099 | 1253 | 3970 | 14093 |
| Standard deviation | 80.88 | 282.6 | 690.85 | 76.43 | 349.36 | 690.55 |
| CV (%) | 6.1% | 7.2% | 4.9% | 6.1% | 8.8% | 4.9% |

✓ Reproducibility

To assay reproducibility, three samples with differing target protein concentrations were assayed using four different lots.

| Lots | Lot1 (pg/mL) | Lot2 (pg/mL) | Lot3 (pg/mL) | Lot4 (pg/mL) | Mean (pg/mL) | Standard Deviation | CV (%) |
|---|---|---|---|---|---|---|---|
| Sample 1 | 1326 | 1365 | 1368 | 1233 | 1323 | 54.53 | 4.1% |
| Sample 2 | 3925 | 3610 | 3426 | 3883 | 3711 | 204.2 | 5.5% |
| Sample 3 | 14099 | 16330 | 14083 | 16849 | 15340 | 1262.66 | 8.2% |

*number of samples for each test n=16.

## Resources

**Plate Layout**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 12 | | | | | | | | |
| 11 | | | | | | | | |
| 10 | | | | | | | | |
| 9 | | | | | | | | |
| 8 | | | | | | | | |
| 7 | | | | | | | | |
| 6 | | | | | | | | |
| 5 | | | | | | | | |
| 4 | | | | | | | | |
| 3 | | | | | | | | |
| 2 | | | | | | | | |
| 1 | | | | | | | | |
| | A | B | C | D | E | F | G | H |