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# ab100606 – MMP2 Human ELISA Kit

For the quantitative measurement of Human MMP2 in serum, plasma, and cell culture supernates. (Collect plasma using heparin as an anticoagulant. EDTA and Citrate are not recommended as anticoagulants for use in this assay due to their chelating properties).

This product is for research use only and is not intended for diagnostic use.

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# 1. Overview

Abcam's MMP2 Human ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of Human MMP2 in serum, plasma, and cell culture supernates. (Collect plasma using heparin as an anticoagulant. EDTA and Citrate are not recommended as anticoagulants for use in this assay due to their chelating properties).

This assay employs an antibody specific for Human MMP2 coated on a 96-well plate. Standards and samples are pipetted into the wells and MMP2 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-Human MMP2 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of MMP2 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

## 2. Protocol Summary

Prepare all reagents, samples, and standards as instructed.



Add standard or sample each well used. Incubate at room temperature.



Add prepared biotin antibody to each well. Incubate at room temperature.



Add prepared Streptavidin solution. Incubate at room temperature.



Add TMB One-Step Development Solution to each well. Incubate at room temperature. Add Stop Solution to each well. Read at 450 nm immediately.

### 3. Precautions

**Please read these instructions carefully prior to beginning the assay.**

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

### 4. Storage and Stability

**Store kit at -20°C immediately upon receipt.**

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

## 5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

## 6. Materials Supplied

| Item                              | Amount   | Storage Condition (Before Preparation ) |
|-----------------------------------|----------|---|
| MMP2 Microplate (12 x 8 wells)    | 96 wells | -20°C                                   |
| 20X Wash Buffer Concentrate       | 25 mL    | -20°C                                   |
| Recombinant Human MMP2 Standard   | 2 vials  | -20°C                                   |
| 5X Assay Diluent                  | 15 mL    | -20°C                                   |
| Biotinylated anti-Human MMP2      | 2 vials  | -20°C                                   |
| 440X HRP-Streptavidin Concentrate | 200 µL   | -20°C                                   |
| TMB One-Step Substrate Reagent    | 12 mL    | -20°C                                   |
| Stop Solution                     | 8 mL     | -20°C                                   |

## 7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 2  $\mu$ L to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- Tubes to prepare standard or sample dilutions.

## 8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.



- When preparing your standards, it is very critical to briefly spin down the vial first. The powder may drop off from the cap when opening it if you do not spin down. Be sure to dissolve the powder thoroughly when reconstituting. After adding Assay Diluent to the vial, we recommend inverting the tube a few times, then flick the tube a few times, and then spin it down; repeat this procedure 3-4 times. This is a technique we find very effective for thoroughly mixing the standard without too much mechanical force.
- Do not vortex the standard during reconstitution, as this will destabilize the protein.
- Once your standard has been reconstituted, it should be used right away or else frozen for later use.
- Keep the standard dilutions on ice while during preparation, but the ELISA procedure should be done at room temperature.
- Be sure to discard the working standard dilutions after use – they do not store well.

## 9. Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use.

### 9.1 1X Assay Diluent

5X Assay Diluent should be diluted 5-fold with deionized or distilled water before use.

### 9.2 1X Wash Solution

If the 20X Wash Concentrate contains visible crystals, equilibrate to room temperature and mix gently until dissolved. Dilute 20 mL of 20X Wash Solution Concentrate into deionized or distilled water to yield 400 mL of 1X Wash Solution.

### 9.3 1X Biotinylated MMP2 Detection Antibody

Briefly spin the Biotinylated anti-Human MMP2 vial before use. Add 100 µL of 1X Assay Diluent into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can either be stored at 4°C for 5 days or aliquoted and frozen at -20°C for 2 months). The detection

antibody concentrate must be diluted 80-fold with 1X Assay Diluent prior to use in the Assay Procedure.

#### **9.4 1X HRP-Streptavidin Solution**

Briefly spin the 440X HRP-Streptavidin concentrate vial and pipette up and down to mix gently before use. HRP-Streptavidin concentrate must be diluted 440-fold with 1X Assay Diluent prior to use in the Assay Procedure.

For example: Briefly spin the vial and pipette up and down to mix gently. Add 25  $\mu$ L of 440X HRP-Streptavidin concentrate into a tube with 11 mL 1X Assay Diluent to prepare a 440-fold diluted 1X HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

## 10. Standard Preparation

- Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.
  - Standard (recombinant protein) should be stored at -20°C or -80°C (recommended at -80°C) after reconstitution.
- 10.1 Briefly spin the vial of MMP2 Standard. Prepare the 2 µg/mL MMP2 **Stock Standard** by adding 400 µL 1X Assay Diluent into the vial.
- 10.2 Dissolve the powder thoroughly by gentle mixing.
- 10.3 Label tubes #1-7.
- 10.4 Prepare **Standard #1** by adding 180 µL 2 µg/mL **Stock Standard** to 270 µL Assay Diluent into tube 1#. Mix thoroughly and gently.
- 10.5 Pipette 300 µL 1X Assay Diluent into remaining tubes.
- 10.6 Prepare **Standard #2** by transferring 200 µL from tube #1 to #2, mix thoroughly.
- 10.7 Prepare **Standard #3** by transferring 200 µL from tube #2 to #3, mix thoroughly.
- 10.8 Using the table below as a guide, prepare further serial dilutions.
- 10.9 1X Assay Diluent serves as the zero standard, (0 pg/mL).

| Standard # | Volume to dilute (µL) | Volume Diluent (µL) | Total Volume (µL) | Starting Conc. | Final Conc. |
|------------|-----------------------|---------------------|-------------------|----------------|-------------|
|------------|-----------------------|---------------------|-------------------|----------------|-------------|

|   |     |     |     | (ng/mL) | (ng/mL) |
|---|-----|-----|-----|---------|---------|
| 1 | 180 | 270 | 450 | 2,000   | 800     |
| 2 | 200 | 300 | 500 | 800     | 320     |
| 3 | 200 | 300 | 500 | 320     | 128     |
| 4 | 200 | 300 | 500 | 128     | 51.2    |
| 5 | 200 | 300 | 500 | 51.2    | 20.5    |
| 6 | 200 | 300 | 500 | 20.5    | 8.2     |
| 7 | 200 | 300 | 500 | 8.2     | 3.3     |
| 8 | 0   | 300 | 300 | 0       | 0       |

## 11. Sample Preparation

### General Sample Information:

- We recommend preparing serum-free or low-serum medium samples, as serum tends to contain cytokines which may produce significant background signals. If it is necessary to test serum containing medium, we recommend also running an uncultured media blank to assess baseline signals. This baseline can then be subtracted from the cultured media sample data.
- If your samples need to be diluted, 1X Assay Diluent should be used for dilution of serum, plasma and culture supernatants.
- Suggested dilution for normal serum/plasma: 5-50 fold.
- Please note that levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator.

### Cell culture supernatant preparation:

- On day 0, seed ~1 million cells in 100 mm tissue culture plate with complete medium.\*
- On day 3, remove medium and replace medium with 6-8 mL of serum-free or low serum containing medium (e.g. medium containing 0.2% calf serum).
- On day 5, collect medium into 15 mL tube. Centrifuge at 2,000 rpm in centrifuge at 4°C for 10 minutes. Save the supernatant. Transfer the supernatant into 1.5 mL Eppendorf tubes. Store supernatant at -80°C until experiment. Most samples can be stored this way for at least a year.

*\*The optimal number of seeded cells varies from one cell type to another and may need to be empirically determined.*

## 12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section.

### 13. Assay Procedure

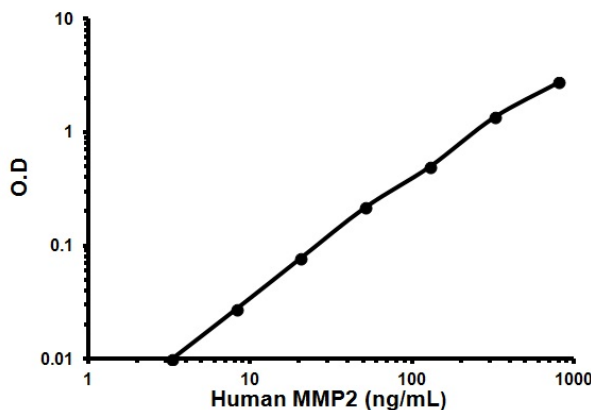
- Equilibrate all materials and prepared reagents to room temperature (18 - 25°C) prior to use.
  - It is recommended to assay all standards, controls and samples in duplicate.
- 
- 13.1** Add 100 µL of each standard (see Standard Preparation section 10) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4°C with gentle shaking.
  - 13.2** Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with 1X Wash Solution (300 µL) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining 1X Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
  - 13.3** Add 100 µL of 1X Biotinylated MMP2 Detection Antibody (Standard Preparation section 10) to each well. Incubate for 1 hour at room temperature with gentle shaking.
  - 13.4** Discard the solution. Repeat the wash as in step 13.2.
  - 13.5** Add 100 µL of 1X HRP-Streptavidin solution (see Reagent Preparation section 9) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
  - 13.6** Discard the solution. Repeat the wash as in step 13.2.
  - 13.7** Add 100 µL of TMB One-Step Substrate Reagent to each well.
  - 13.8** Incubate for 30 minutes at room temperature in the dark with gentle shaking.
  - 13.9** Add 50 µL of Stop Solution to each well. Read at 450 nm immediately.

## 14. Calculations

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

# 15. Typical Data

Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.



| Conc.<br>(ng/mL) | O.D.             |
|------------------|------------------|
|                  | Assay<br>Diluent |
| 3.3              | 0.010            |
| 8.2              | 0.028            |
| 20.5             | 0.078            |
| 51.2             | 0.217            |
| 128              | 0.497            |
| 320              | 1.355            |
| 800              | 2.755            |



## 16. Typical Sample Values

### SENSITIVITY –

The minimum detectable dose of MMP2 is typically less than 3.5 ng/mL.

### RECOVERY –

Recovery was determined by spiking Human MMP2 into normal Human serum, plasma and cell culture media. Mean recoveries are as follows:

| Sample Type        | Average % Recovery | Range (%) |
|--------------------|--------------------|-----------|
| Serum              | 81.27              | 70-93     |
| Plasma             | 84.53              | 72-95     |
| Cell Culture Media | 77.25              | 69-92     |

### LINEARITY OF DILUTION –

| Serum Dilution | Average % Expected Value | Range (%) |
|----------------|--------------------------|-----------|
| 1:2            | 93                       | 81-103    |
| 1:4            | 95                       | 82-104    |

| Plasma Dilution | Average % Expected Value | Range (%) |
|-----------------|--------------------------|-----------|
| 1:2             | 94                       | 82-105    |
| 1:4             | 92                       | 80-103    |

| Cell Culture Media Dilution | Average % Expected Value | Range (%) |
|-----------------------------|--------------------------|-----------|
| 1:2                         | 92                       | 82-103    |
| 1:4                         | 94                       | 84-104    |

**PRECISION –**

|        | Intra-assay Precision | Inter-Assay Precision |
|--------|-----------------------|-----------------------|
| CV (%) | <10%                  | <12%                  |

**17. Assay Specificity**

Cross Reactivity: This ELISA kit shows no cross-reactivity with the following cytokines tested: Human Angiogenin, BDNF, BLC, ENA-78, FGF-4, IL-1 $\alpha$ , IL-1 $\beta$ , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12 p70, IL-12 p40, IL-13, IL-15, IL-309, IP-10, G-CSF, GM-CSF, IFN- $\gamma$ , Leptin (OB), MCP-1, MCP-3, MDC, MIP-1 $\alpha$ , MIP-1  $\beta$ , MIP-1 $\delta$ , MMP-1, -3, -7, -8, -10, -12,-13, PARC, RANTES, SCF, TARC, TGF- $\beta$ , TIMP-1, TIMP-2, TNF- $\alpha$ , TNF- $\beta$ , TPO, VEGF.

## 18. Troubleshooting

| Problem                       | Reason   | Solution  |
|-------------------------------|--|---|
| <b>Low Precision</b>          | Use of expired components  | Check the expiration date listed before use. Do not interchange components from different lots  |
|                               | Splashing of reagents while loading wells                          | Pipette properly in a controlled and careful manner   |
|                               | Inconsistent volumes loaded into wells                             | Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance  |
|                               | Insufficient mixing of reagent dilutions                           | Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions  |
|                               | Improperly sealed microplate                                       | Check the microplate pouch for proper sealing. Check that the microplate pouch has no punctures. Check that three desiccants are inside the microplate pouch prior to sealing |
| <b>Precipitate in Diluent</b> | Precipitation and/or coagulation of components within the Diluent. | Precipitate can be removed by gently warming the Diluent to 37°C.   |

| Problem                    | Cause   | Solution   |
|----------------------------|---|--|
| <b>Poor standard curve</b> | Inaccurate Pipetting                            | Check pipettes   |
|                            | Improper standard dilution                      | Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing                     |
| <b>Low Signal</b>          | Incubation times too brief                      | Ensure sufficient incubation times; change to overnight standard/sample incubation   |
|                            | Inadequate reagent volumes or improper dilution | Check pipettes and ensure correct preparation  |
| <b>Large CV</b>            | Plate is insufficiently washed                  | Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.                            |
|                            | Contaminated wash buffer                        | Prepare fresh wash buffer  |
| <b>Low sensitivity</b>     | Improper storage of the ELISA kit               | Store your reconstituted standards at -80°C, all other assay components 4°C. Keep TMB substrate solution protected from light. |

## 19. Notes





# Technical Support

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