

HUMAN TOTAL MATRIX METALLOPROTEINASE 1 (MMP-1) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION
OF HUMAN TOTAL MMP-1
CONCENTRATIONS IN CELL CULTURE
SUPERNATES, SERUM AND EDTA PLASMA



FOR RESEARCH USE ONLY. NOT FOR USE IN
DIAGNOSTIC PROCEDURES.

PURCHASE INFORMATION:

ELISA NAME	HUMAN TOTAL MMP-1 ELISA
Catalog No.	SK00164-01
Lot No.	
Formulation	96 T
Standard range	156 – 20,000 pg/mL
Sensitivity	15 pg/mL
Sample Volume	100 µL
Sample Type	Cell Culture Supernates and Serum, EDTA Plasma
Dilution Factor	Optimal dilutions should be determined by each laboratory for each application
Specificity	Human Total MMP-1
Intra-assay Precision	4 - 8%
Inter-assay Precision	8 - 12%
Storage	2 – 8 °C

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INTRODUCTION

Human Total MMP-1 immunoassay is a solid phase ELISA designed to measure human MMP-1 in cell culture supernates, serum and EDTA plasma. It contains recombinant human MMP-1 and antibodies raised against this protein. It has been shown to accurately quantify recombinant human MMP-1. Results obtained with naturally occurring MMP-1 samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural human MMP-1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for MMP-1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any MMP-1 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for MMP-1 is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of MMP-1 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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_The kit should not be used beyond the expiration date on the kit label.

_Do not mix or substitute reagents with those from other lots or sources.

_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.

_If samples generate values higher than the highest standard, dilute the samples with Dilution Buffer and repeat the assay.

_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

_This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors

have been tested in the immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
MMP-1 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against MMP-1.	164-01-01	1 plate
MMP-1 Standard – 10,000 pg/vial of recombinant human MMP-1 in a buffered protein base with preservative; lyophilized.	164-01-02	2 vials
Detection Antibody Concentrate – 1.05 mL/vial, 10-fold concentrate of biotinylated antibody against MMP-1 with preservative; lyophilized.	164-01-03	1 vial
Positive Control - one vial of recombinant human MMP-1; lyophilized.	164-01-04	1 vial
Streptavidin-HRP Conjugate – 60 µL/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP.	SAHRP	1 vial
Dilution Buffer – 60 mL of buffered protein based solution with preservative.	DB01	1 bottle
Wash Buffer – 50 mL of 10-fold concentrated buffered surfactant, with preservative.	WB01	1 bottle
TMB Substrate Solution - 11 mL of TMB substrate solution.	TMB01	1 bottle
Stop Solution – 11 mL of 0.5M HCl.	S-STOP	1 bottle
Plate Sealer	EAPS	1 piece
Plastic Pouch	P01	1 piece

STORAGE

Unopened Kit: Store at 2 – 8 °C for up to 12 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20 °C or -70 °C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard (stock) solution and Detection Antibody concentrated solution could be stored for up to one

month at -20 °C or -70 °C. Streptavidin-HRP Conjugate 200-fold concentrated solution (protect from light) and other components may be stored at 2 – 8 °C for up to 12 months.

Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 – 8 °C after opening.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.

Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Plasma samples may not need to be diluted. Serum samples may need a 2-fold or greater dilution. A suggested 2-fold dilution is 125 µL sample + 125 µL dilution buffer. If samples values are greater than the highest standard of 20,000 pg/mL, then samples need to be diluted further. **Optimal dilutions should be determined by each laboratory for each application with a pretest.**

Use polypropylene test tubes.

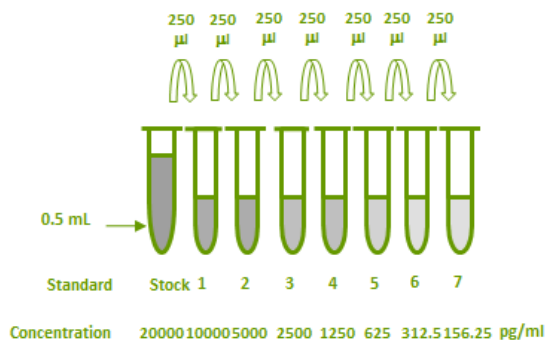
REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

MMP-1 Standard - Refer to vial label for reconstitution volume. Reconstitute the **MMP-1 Standard** with 0.5 mL of Dilution Buffer. This reconstitution produces a stock solution of 20,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Dilution Buffer into tubes #1 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 20,000 pg/mL standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	Powder	500 µl	20,000 pg/ml
# 1	250 µl of stock	250 µl	10,000 pg/ml
# 2	250 µl of 1	250 µl	5000 pg/ml
# 3	250 µl of 2	250 µl	2500 pg/ml
# 4	250 µl of 3	250 µl	1250 pg/ml
# 5	250 µl of 4	250 µl	625 pg/ml
# 6	250 µl of 5	250 µl	312.5 pg/ml
# 7	250 µl of 6	250 µl	156.25 pg/ml



Positive Control - Reconstitute the Positive Control with 0.5 mL of Dilution Buffer. **Note:** Positive Control could be reused within a few days if stored at -20 °C ~ -70 °C.

Detection Antibody - Reconstitute the Detection Antibody Concentrate with 1.05 mL of Dilution Buffer to produce a 10-fold concentrated stock

solution. Pipette 9.45 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

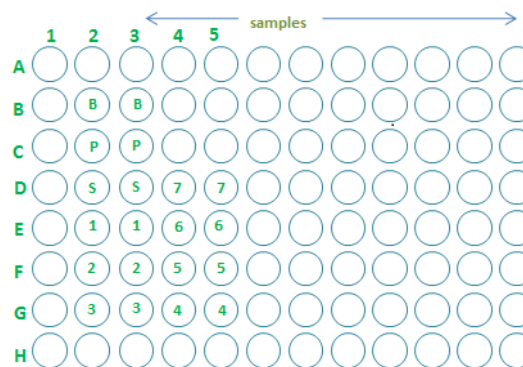
Streptavidin-HRP Conjugate - Pipette 11.94 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 60 μL of 200-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP Conjugate should be used within a few days (protect from light).

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100 μL of **Dilution Buffer** to Blank wells (B2, B3).
4. Add 100 μL of **Standard solutions** in reverse order of serial dilution (D4, D5 to G4, G5 and G2, G3 to D2, D3), **sample**, or **positive control** (C2, C3) per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with **1x Wash Buffer** (300 μL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 μL of **Detection Antibody working solution** to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 μL of **Streptavidin-HRP Conjugate working solution** to each well. Incubate for 60 minutes on microplate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.

10. Add 100 μL of **Substrate Solution** to each well. Incubate for 8-12 minutes on microplate shaker at room temperature. **Protect from light.**
11. Add 100 μL of **Stop Solution** to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a microplate reader set to 450 nm.



CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and samples, and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the MMP-1 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Calculation of samples with a concentration exceeding that of standard 20,000 pg/mL may result in inaccurate, low human MMP-1 levels. Such samples require further external predilution according to expected human MMP-1 values with Dilution Buffer in order to precisely quantify the actual human MMP-1 level.

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human MMP-1.

SENSITIVITY

The minimum detectable dose (MDD) of MMP-1 was 15 pg/mL.

TYPICAL DATA

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

MMP-1 Standard (pg/mL)	Average OD450nm (Corrected)
Blank	0 (0.105)
39.063 (optional)	0.017
78.125 (optional)	0.032
156.25	0.067
312.5	0.119
625	0.195
1250	0.274
2500	0.522
5000	0.803
10000	1.131
20000	1.416

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human MMP-1	100
Human MMP-2	0
Human MMP-3	0
Human MMP-7	0
Human TIMP-1	0
Human TIMP-2	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard, samples, positive control to the well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Streptavidin-HRP conjugate working solution to each well. Incubate 60 minutes on the plate shaker at RT. Protect from light.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 8-12 min on the plate shaker at RT. Protect from light.
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min.