HUMAN TOTAL MATRIX METALLOPROTEINASE 9 (MMP-9) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN TOTAL MMP-9 CONCENTRATIONS IN CELL CULTURE SUPERNATES, SERUM AND EDTA PLASMA

PURCHASE INFORMATION:

ELISA NAME	HUMAN TOTAL MMP-9 ELISA
Catalog No.	SK00160-02
Lot No.	
Formulation	96 T
Standard range	31.25-2000 pg/mL
Sensitivity	15.6 pg/mL
Sample require	10-20 μL
Dilution Factor	100 (Optimal dilutions should be determined by each laboratory for each application)
Sample Type	Cell Culture Supernates, Serum, EDTA Plasma
Specificity	Human MMP-9 (Pro and Active form)
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 12%
Storage	2 - 4°C

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

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INTRODUCTION

Human Total MMP-9 immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human Total MMP-9 (Pro-MMP-9 92KD and Active Form 82KD) in cell culture supernates, serum and EDTA plasma. It contains recombinant Human MMP-9 and antibodies raised against this protein. It has been shown to accurately quantify recombinant human MMP-9. Results obtained with naturally occurring MMP-9 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-9.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for MMP-9 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any MMP-9 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated polyclonal antibody specific for MMP-9 is added to the wells. Following a wash to remove any unbound antibody reagent, a Streptavidin HRP conjugate 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-9 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-9 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a purified monoclonal IgG against MMP-9.	**160-02-01**	**1 plate**
MMP-9 Standard – 2000 pg/vial of recombinant Human MMP-9 in a buffered protein base with preservatives; lyophilized.	**160-02-02**	**1 vial**
Detection Antibody – 105 µL/vial, 100-fold concentrated of a purified polyclonal IgG against MMP-9 with preservatives; lyophilized.	**160-02-03**	**1 vial**
Positive Control – one vial of recombinant MMP-9, lyophilized	**160-02-04**	**1 vial**
Streptavidin-HRP Conjugate - 120 µL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP	**SAHRP**	**1 vial**
Dilution Buffer - 60mL of buffered protein based solution with preservatives	**DB01**	**1 bottle**
HRP Diluent Solution – 12mL of buffered protein based solution with preservatives.	**DB08**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
ABTS Substrate Solution - 12 mL of ABTS substrate solution	**ABTS01**	**1 bottle**
Stop Solution - 12 mL of 0.9% SDS solution	**SDS-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 bag**

STORAGE

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

Opened / Reconstituted Reagents: Reconstituted Standard, Antibody Solution Concentrate SHOULD BE STORED at -20 °C or -70°C for up to one month. Streptavidin-HRP Conjugate 100-fold concentrate 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, reseal along entire edge of zip-seal. 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 405nm or 650nm.
- 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.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted SDS solution. Appropriate care, therefore, should be taken while handling this solution. We therefore recommend that this product be handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

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.

Note: Use Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

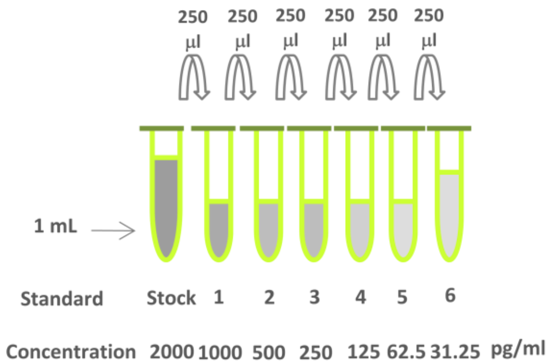
SAMPLE PREPARATION

Serum and plasma samples require a 100 -fold dilution. A suggested 100-fold dilution is 10 µL sample + 990µ L Dilution Buffer. **Optimal dilutions should be determined by each laboratory for each application.**
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 Wash Buffer.
MMP-9 Standard - Refer to vial label for reconstitution volume. Reconstitute the **MMP-9** standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 2000 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 #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 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	1.0 mL	2000 pg/mL
# 1	250µL of stock	250µL	1000 pg/mL
# 2	250µL of 1	250µL	500 pg/mL
# 3	250µL of 2	250µL	250 pg/mL
# 4	250µL of 3	250µL	125 pg/mL
# 5	250µL of 4	250µL	62.5 pg/mL
# 6	250µL of 5	250µL	31.25 pg/mL



Detection Antibody Concentrate - Reconstitute with 105 µL of Dilution Buffer to produce a 100-fold concentrated stock solution. Transfer 105 µL of 100-fold concentrated stock solution to 10.395 mL Dilution Buffer in a 15 mL centrifuge tube to prepare working solution of Detection Antibody.

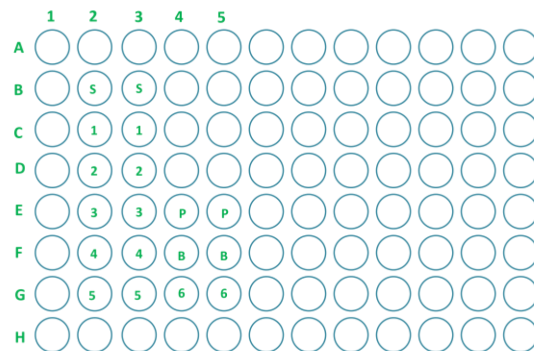
Streptavidin-HRP Conjugate - Pipette 11.88 mL of **HRP Diluent Solution (DB08)** into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution of Streptavidin-HRP. *Note: 1x working solution of Streptavidin-HRP Conjugate should be used within a few days.*

Positive Control - Reconstitute the Positive Control with 1.0 mL of Dilution Buffer. *Note: Positive Control should be prepared and used immediately*. Reconstituted Positive Control CAN NOT BE REUSED.

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 duplicates.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch with the desiccant pack and seal.
3. Add 100 µL of Dilution Buffer to Blank well (F4, F5).
4. Add 100 µL of Standard (from B2, B3 to G2, G3 and G4, G5), sample, or positive control (E4, E5) per well. Cover with plate sealer. Incubate for 2 hours on micro-plate 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 micro-plate 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 micro-plate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of ABTS Substrate Solution to each well. Incubate for 25-35 minutes at room temperature. **Protect from light.**
11. Add 100 µL of Stop Solution to each well. That yields a green end product upon reaction with peroxidase. The green product has two major absorbance peaks, 405 nm and 650 nm.
12. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 405 nm or 650nm.



CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control, and sample 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-9 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 2000 pg/mL may result in inaccurate, low human MMP-9 levels. Such samples require further external predilution according to expected human MMP-9 values with Dilution Buffer in order to precisely quantify the actual human MMP-9 level.

CALIBRATION

This immunoassay is calibrated against a highly purified CHO cell-expressed recombinant human MMP-9.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of MMP-9 was 15.6 pg/mL.

SPECIFICITY

This assay recognizes both natural and recombinant human MMP-9. The factors listed below were prepared at 50 ng/mL in Dilution Buffer, and assayed for cross reactivity.

PROTEIN NAME	CROSS-REACTIVITY
Human MMP-9	100%
Mouse MMP-9	0
Human MMP-1	0
Human MMP-2	0
Human MMP-3	0
Human MMP-7	0
Human MMP-8	0
Human MMP-10	0

TYPICAL DATA

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

STANDARD (PG/ML)	AVERAGE OD405 (CORRECTED)
Blank	0 (0.066)
31.25	0.013
62.5	0.026
125	0.053
250	0.102
500	0.208
1000	0.430
2000	0.930

- Lot No.:
- Positive control: 100-300 pg/mL

SUMMARY OF ASSAY PROCEDURE