

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



ALWAYS REFER TO LOT SPECIFIC
PROTOCOL PROVIDED WITH EACH KIT FOR
INSTRUCTIONS. PROTOCOL MUST BE
READ BEFORE USING THIS PRODUCT.

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

PRODUCT INFORMATION:
THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	HUMAN TOTAL MMP-9 ELISA KIT
Catalog No.	SK00160-08
Lot No.	20114822
Formulation	96 T
Standard range	31.25 - 2000 pg/mL
Sensitivity	5 pg/mL
Sample require	10 - 20 µL
Dilution Factor	200 ~ 400 for EDTA Plasma (Optimal dilutions should be determined by each laboratory for each application)
Sample Type	Cell Culture Supernates, Serum, EDTA Plasma
Calibration	Human MMP-9 Recombinant (HEK293)
Specificity	Human MMP-9 (Pro and Active form)
Intra-assay Precision	4 - 6%
Inter-assay Precision	4 - 9%
Storage	2 - 8° C for 8 months. For longer storage up to 12 months, see page 2-3 for more information.
This kit contains sufficient materials to run 35 - 40 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Human Total MMP-9 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human total MMP-9 from cell culture supernates, serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human MMP-9 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural MMP-9 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a monoclonal antibody specific for human MMP-9. The capture antibody can bind to the human MMP-9 in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human MMP-9 is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution (TMB) is added to the wells and color develops in direct proportion to the amount of human MMP-9 bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

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

_Do not mix 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.

_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.

_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.

_Not all interfering factors have been tested in the immunoassay, therefore 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 IgG against MMP-9.	160-08-01	1 plate
MMP-9 Standard – 2000 pg/vial of recombinant human MMP-9 in a buffered protein base with preservative; lyophilized.	160-08-02	1 vial
Detection Antibody – 1.2 mL/vial, 10-fold concentrate of a biotinylated IgG against MMP-9 with preservative; lyophilized.	160-08-03	1 vial
Positive Control – one vial of recombinant MMP-9; lyophilized.	160-08-04	1 vial
Streptavidin-HRP Conjugate - 120 µL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	SAHRP	1 vial
Dilution Buffer – 45 mL of buffered protein based solution with preservative.	DB01	1 bottle
Antibody Diluent Solution – 12 mL of buffered protein based solution with preservative; lyophilized.	DB32	1 bottle
HRP Diluent Solution – 12 mL of buffered protein based solution with preservative; lyophilized.	DB08B	1 bottle
Wash Buffer 20X - 25 mL of 20-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 0.25M HCl.	S-STOP	1 bottle
Plate Sealer	EAPS	1
Plastic Pouch	P01	1

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 8 months. For longer storage for up to 12 months, unopened Standard, Positive Control, Detection Antibody Concentrated, Dilution Buffer, Antibody Diluent Solution and HRP Diluent Solution should be stored at -20 or -70°C. Do not use kit past expiration date. Streptavidin-HRP Conjugate 100-fold concentrated solution and TMB Substrate Solution may be stored at 2 - 8° C for up to 8 months.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

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.

SAMPLE PREPARATION

Human EDTA plasma samples may require a 200~400 fold dilution. A suggested 20-fold dilution is 10 µL sample +190 µL Dilution Buffer; then to make the final 200-fold dilution is 10 µL per well of 20-fold diluted sample per assay well + 90 µL of Dilution Buffer per assay well. To make the final 400-fold dilution is 5 µL per well of 20-fold diluted sample per assay well + 95 µL of Dilution Buffer per assay well.

Optimal dilutions should be determined by each laboratory for each application.

Use polypropylene test tubes.

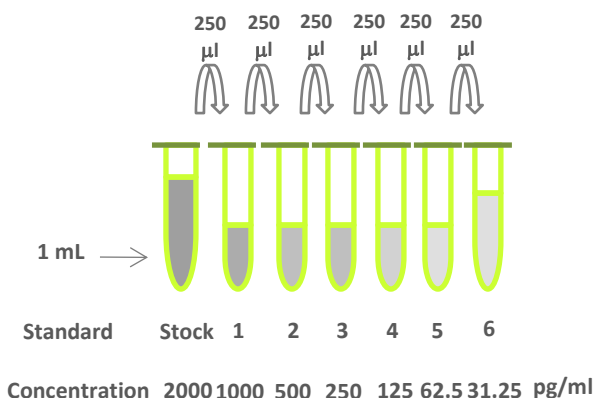
REAGENT PREPARATION

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 **25 mL of Wash Buffer Concentrate 20X** into deionized or distilled water (**475 mL**) to prepare 500 mL of 1x Wash Buffer.

MMP-9 Standard - 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 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). Store the stock solution at -70 °C for a few days.

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



Positive Control - Reconstitute the Positive Control with 1 mL of Dilution Buffer.

Detection Antibody - Reconstitute the Detection Antibody with 1.2 mL of **Antibody Diluent Solution (DB32)** to produce a 10-fold concentrated stock solution. For the 96 wells test, freshly Pipette 9.45 mL of **Antibody Diluent Solution (DB32)** into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution. Store the stock solution of 10-fold concentrated at -20°C for a few days.

Streptavidin-HRP Conjugate - Freshly Pipette 10.89 mL of HRP Diluent Solution (DB08B) into a 15 mL centrifuge tube and transfer 110 μL of 100-fold concentrated stock solution to prepare working solution of Streptavidin-HRP for 96 wells test. *Note: 1x working solution of Streptavidin-HRP Conjugate should be used within 20 min. **Protect from light.*** For the partial strip test, freshly prepare 900 μL of working solution per strip. Always store the stock solution at $2\sim 8^{\circ}\text{C}$ for 8 months.

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame and place into plastic pouch with desiccant pack.

3. Add 100 μL of Dilution Buffer to Blank wells.
4. Add 100 μL of standard dilutions, samples, or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature. **Prepare Detection Antibody working solution.**
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 90 minutes 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 45 minutes on microplate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 μL of TMB Substrate Solution to each well. Incubate for 15-20 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 3 minutes, using a microplate reader set to 450 nm.

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 or 4-parameter curve fit. 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.

SPECIFICITY

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

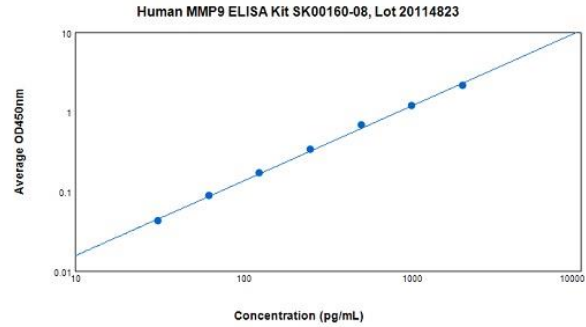
TYPICAL STANDARD CURVE

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

STANDARD (PG/ML)	AVERAGE OD405 NM (CORRECTED)
Blank	0 (0.089)
31.25	0.042
62.5	0.089
125	0.172
250	0.336
500	0.677
1000	1.190
2000	2.136

- Lot No.: 20114822
- Positive control: 150 ~ 400 pg/mL (log-log)

Standard Curve by log-log fit



SUMMARY OF ASSAY PROCEDURE

