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

PRODUCT INFORMATION:
THIS IS FOR ONE TIME USE ONLY.

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
Calibration	Human MMP-9 Recombinant
Specificity	Human MMP-9 (Pro and Active form)
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 12%
Storage	2 - 8° C for 1 month. See page 2-3 for more information.
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

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

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

ORDER CONTACT
AVISCERA BIOSCIENCE, INC.
2348 WALSH AVE., SUITE C
SANTA CLARA, CA 95051
USA
TEL: (408) 982 0300
FAX: (408) 982 0301
EMAIL: SALES@AVISCERABIOSCIENCE.COM
INFO@AVISCERABIOSCIENCE.COM
WWW.AVISCERABIOSCIENCE.COM

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 an 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

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_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.	**160-02-01**	**1 plate**
MMP-9 Standard – 2000 pg/vial of recombinant human MMP-9 in a buffered protein base with preservative; lyophilized.	**160-02-02**	**1 vial**
Detection Antibody – 1.05 mL/vial, 10-fold concentrate of a biotinylated IgG against MMP-9 with preservative; 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 – 40 mL of buffered protein based solution with preservative.	**DB01**	**2 bottles**
HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	**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 1 month. For longer storage up to 10 months, unopened Standard, Positive Control, Detection Antibody Concentrated, Dilution Buffer and HRP Diluent Solution should be stored at -20 C. **Streptavidin-HRP Conjugate** should be stored only at 2-8 °C. Do not use kit past expiration date.

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.

Optional: Use Aprotinin (enzyme inhibitor) 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 5 µL sample + 450µ L Dilution Buffer. **Optimal dilutions should be determined by each laboratory for each application.**

Use polypropylene test tubes.

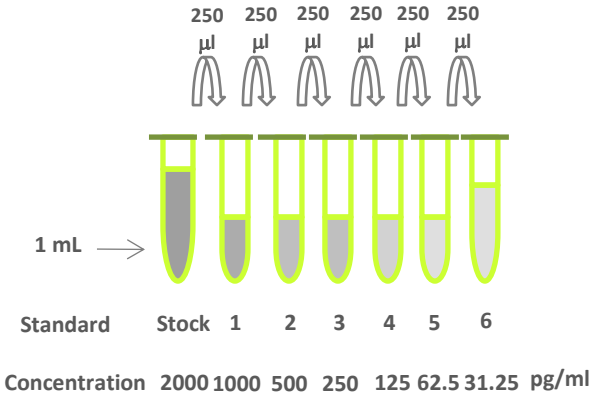
REAGENT PREPARATION

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 1.05 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Transfer 1.05 mL of 10-fold concentrated stock solution to 9.45 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.

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 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.
4. Add 100 µL of Standard, sample, or positive control per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
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 20-40 minutes on micro-plate shaker 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.

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 (CORRECTED)
Blank	0 (0.101)
31.25	0.059
62.5	0.119
125	0.247
250	0.517
500	1.019
1000	1.789
2000	2.918

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard, samples, positive control to each 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 working solution to each well. Incubate 60 min on the plate shaker at RT. Protect from light.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 20-40 min on plate shaker. Protect from light.
↓
Add 100 µl Stop Solution to each well. Read 405 nm or 650 nm within 5 min.