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

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.

PRODUCT INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	**HUMAN TOTAL MMP-1 ELISA**
Catalog No.	SK00164-01
Lot No.	
Formulation	96 T
Standard range	156 – 10,000 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
Calibration	Human MMP-1 Rec. (HEK293 cell derived)
Intra-assay Precision	4 - 8%
Inter-assay Precision	8 - 12%
Storage	2 – 8° C for 1 month. See page 2-3 for detail
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

Order Contact:
AVISCERA BIOSCIENCE, INC.
2348 WALSH AVE., SUITE C
SANTA CLARA, CA 95051
USA
Tel: (408) 982 0300
Fax: (408) 982 0301
Email: Info@AvisceraBioscience.com
Website: www.AvisceraBioscience.com
www.AvisceraBioscience.net

DESCRIPTION

This Human Total MMP-1 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human MMP-1 from cell culture supernates, serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human MMP-1 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural MMP-1 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human MMP-1. The capture antibody can bind to the human MMP-1 in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human MMP-1 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-1 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.

COMPONENTS 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 – refer to lot of recombinant human MMP-1 in a buffered protein base with preservative; lyophilized.	**164-01-02**	**1 vial**
Detection Antibody Concentrate – refer to lot, 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 – 120 µL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Dilution Buffer – 50 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 1 month. For longer storage for up to 10 months, unopened Standard, Positive Control, Detection Antibody Concentrate and Dilution Buffer (DB01) should be stored at -20° C. Streptavidin-HRP Conjugate and TMB Substrate Solution 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

Plasma samples DO 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 10,000 pg/mL, then samples need to be diluted further. **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 1x Wash Buffer.

MMP-1 Standard - Reconstitute the MMP-1 Standard with refer to lot of Dilution Buffer. 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 **10,000 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	Refer to lot	10,000 pg/ml
# 1	250 µl of stock	250 µl	5000 pg/ml
# 2	250 µl of 1	250 µl	2500 pg/ml
# 3	250 µl of 2	250 µl	1250 pg/ml
# 4	250 µl of 3	250 µl	625 pg/ml
# 5	250 µl of 4	250 µl	312.5 pg/ml
# 6	250 µl of 5	250 µl	156.25 pg/ml

Positive Control - Reconstitute the Positive Control with refer to lot of Dilution Buffer

Detection Antibody - Reconstitute the Detection Antibody Concentrate with refer to lot 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 11.88 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working **solution (protect from light)**.

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. Add 100 µL of **Dilution Buffer** to Blank wells.
3. Add 100 µL of **Standard dilutions** in reverse order of serial dilution, **samples,** or **positive control** per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
4. 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.
5. 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.
6. Repeat the aspiration/wash as in step 4.
7. 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.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of **Substrate Solution** to each well. Incubate for refer to lot on microplate shaker at room temperature. **Protect from light.**
10. 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.
11. Determine the optical density of each well 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 10,000 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.

TYPICAL STANDARD CURVE

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

MMP-1 Standard (pg/mL)	Average OD450nm (Corrected)
Blank	0 (refer to lot)
156.25	0.063
312.5	0.131
625	0.280
1250	0.481
2500	0.890
5000	1.521
10000	2.319

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human MMP-1 (HEK293 cell derived)	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 dilutions, samples or 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 refer to lot on the plate shaker at RT. **Protect from light.**

Add 100 µl Stop Solution to each well. Read at 450nm.