HUMAN TISSUE INHIBITOR OF METALLOPROTEINASES 1 (TIMP-1) ELISA KIT

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

PURCHASE INFORMATION:

ELISA NAME	HUMAN TIMP-1 ELISA
Catalog No.	SK00039-01
Lot No.	
Formulation	96 T
Standard range	3.12-200 pg/mL
Sensitivity	1 pg/mL
Sample Volume	100 µl
Sample Type	Cell Culture Supernates, Serum, EDTA Plasma
Dilution Factor	1000 to 2000 **(Optimal dilutions should be determined by each laboratory for each application)**
Specificity	Human TIMP-1
Intra-assay Precision	4-6%
Inter-assay Precision	8-12%
Storage	2°C - 8°C

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

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INTRODUCTION

Human TIMP-1 immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human TIMP-1 in cell culture supernates, serum, and EDTA plasma. It contains recombinant human TIMP-1 and antibodies raised against this protein. It has been shown to accurately quantify recombinant human TIMP-1. Results obtained with naturally occurring TIMP-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 TIMP-1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for TIMP-1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any TIMP-1 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated polyclonal antibody specific for TIMP-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 TIMP-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
TIMP-1 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against TIMP-1.	**039-01-01**	**1 plate**
TIMP-1 Standard – 200 pg/vial of recombinant human TIMP-1 in a buffered protein base with preservatives; lyophilized.	**039-01-02**	**2 vials**
Detection Antibody Concentrate – 105 µL/vial, 100-fold concentrated of Biotinylated polyclonal antibody against TIMP-1 with preservatives; lyophilized.	**039-01-03**	**1 vial**
Positive Control - one vial of recombinant human TIMP-1, lyophilized	**039-01-04**	**1 vial**
Streptavidin-HRP Conjugate – 75 uL/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP	**SAHRP**	**1 vial**
Dilution Buffer - 60mL of buffered protein based solution with preservatives	**DB01**	**1 bottle**
Wash Buffer - 50mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11mL of TMB substrate solution	**TMB01**	**1 bottle**
Stop Solution - 11mL of 0.5M HCl	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 6 months. For longer storage, unopened Standard, Positive Control and Antibody Concentrate should be stored at -20 or -70 °C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted standard and detection antibody concentrate could be stored for up to a month at -70°C. Diluted

standard working solution and positive control should be prepared and used immediately. Streptavidin-HRP Conjugate 200-fold concentrate and other components may be stored at 2 - 8°C for up to 6 months.
Microplate Wells: Return unused wells to the plastic pouch containing the desiccant pack, reseal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8° C.

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.
Note: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation.

SAMPLE PREPARATION

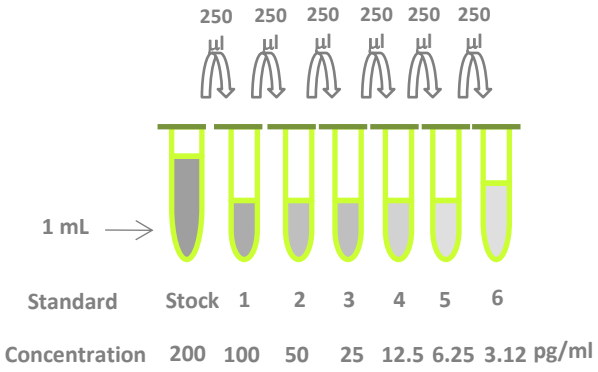
Serum or EDTA plasma samples may require a 1000 to 2000 fold dilution. A suggested 1000-fold dilution is 10 µL sample + 190 µL Dilution Buffer, follow 10 µL of 20-fold diluted sample + 490 µL Dilution Buffer. A suggested 2000-fold dilution is 10µL sample + 490 µL Dilution Buffer, follow 10 µL of 50-fold diluted sample + 390 µL 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.

TIMP-1 Standard - Refer to vial label for reconstitution volume. Reconstitute the **TIMP-1 Standard** with 1 mL of Dilution Buffer. This reconstitution produces a stock solution of 200 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 200 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	1000 µl	200 pg/ml
# 1	250 µl of stock	250 µl	100 pg/ml
# 2	250 µl of 1	250 µl	50 pg/ml
# 3	250 µl of 2	250 µl	25 pg/ml
# 4	250 µl of 3	250 µl	12.5 pg/ml
# 5	250 µl of 4	250 µl	6.25 pg/ml
# 6	250 µl of 5	250 µl	3.125 pg/ml



Detection Antibody - Reconstitute the **Detection Antibody Concentrate** with 105 µL of Dilution Buffer to produce a 100-fold concentrated stock solution. Pipette 10.395 mL of Dilution Buffer into a 15 ml centrifuge tube and transfer 105 µL of 100-fold concentrated stock solution to prepare working solution.

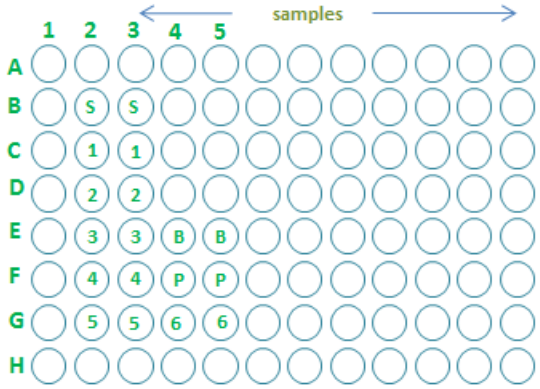
Streptavidin-HRP Conjugate - Pipette 11.94 mL of Dilution Buffer into a 15 ml centrifuge tube and transfer 60 µL of 200-fold concentrated stock solution to prepare working solution. **Note:** 1X working solution of Streptavidin- HRP Conjugate should be used within a few days.

Positive Control - Reconstitute the **Positive Control** with 1.0 mL of Dilution Buffer. **Note:** Positive Control should be prepared and used immediately.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that standards and positive control 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 containing the desiccant pack, reseal.
3. Add 100 µL of **Dilution Buffer** to Blank well (E4, E5).
4. Add 100 µL of **Standard** (from B2, B3 to G2, G3 and G4, G5), **sample, or positive control** (F4, F5) 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 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 **Substrate Solution** to each well. Incubate for 20-30 minutes 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 micro-plate reader set to 450 nm. If wavelength correction is needed, set to 540 nm or 570 nm. This will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.



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 TIMP-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.

CALIBRATION

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

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of TIMP-1 was 1pg/mL.

TYPICAL DATA

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

sAXL Standard (pg/mL)	Average OD450nm (Corrected)
Blank	0 (0.067)
3.12	0.011
6.25	0.022
12.5	0.052
25	0.088
50	0.284
100	0.760
200	1.742

SPECIFICITY

PROTEINS	CROSSREACTIVITY (%)
Human TIMP-1	100
Human MMP-1	0
Human MMP9	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100µl 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 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 20-30 min on the bench top. **Protect from light**.
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min