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

FOR THE QUANTITATIVE DETERMINATION OF HUMAN TIMP-1 CONCENTRATIONS IN 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	TIMP-1 (HUMAN) ELISA KIT
Catalog No.	SK00039-08
Lot No.	
Formulation	96 T
Standard range	62.5-8000 pg/mL
Sensitivity	20 pg/mL
Sample Volume	100 µl
Sample Type	Serum, EDTA Plasma
Dilution Factor	100 (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 2 months. See page 2 for more information.
This kit contains sufficient materials to run approximately 40 samples duplicated provided that assay is run according to protocol.	

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INTRODUCTION

The TIMP-1 (Human) ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human TIMP-1 from serum or Plasma in a sandwich ELISA format.

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

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human TIMP-1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human TIMP-1 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for human 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

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_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 an antibody against TIMP-1.	**039-08-01**	**1 plate**
TIMP-1 Standard – 8000 pg/vial of recombinant human TIMP-1 in a buffered protein base with preservatives; lyophilized.	**039-08-02**	**1 vial**
Detection Antibody Concentrate – 1.05 mL/vial, 10-fold concentrate of biotinylated antibody against TIMP-1 with preservatives; lyophilized.	**039-08-03**	**1 vial**
Positive Control - one vial of recombinant human TIMP-1, lyophilized	**039-08-04**	**1 vial**
Streptavidin-HRP Conjugate – 120 µL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP	**SAHRP**	**1 vial**
Dilution Buffer - 45mL of buffered protein based solution with preservatives	**DB06**	**1 bottle**
Antibody & HRP Diluent Solution – 25 mL of buffered protein based solution with preservative.	**DB08B**	**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**
Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 – 8° C for up to 2 months. For longer storage up to 12 months, unopened

Standard, Positive Control, Detection Antibody Concentrate and Dilution Buffer 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.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- Microplate shaker (350-400rpm).
- 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

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. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Serum or EDTA plasma samples may require a 100 - fold dilution. A suggested 100-fold dilution is 5 µL sample + 495 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.

Dilution Buffer (DB06) - **Dilution Buffer (DB06) is highly viscous, prior to use warm it in 30 - 37° C water bath until liquid flows more freely.**

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 8000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 200 µL of Dilution Buffer into tube #1. Pipette 200 µL of Dilution Buffer into tubes #2 to #4. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **8000 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	8000 pg/ml
# 1	200 µl of stock	200 µl	4000 pg/ml
# 2	100 µl of 1	300 µl	1000 pg/ml
# 3	100 µl of 2	300 µl	250 pg/ml
# 4	100 µl of 3	300 µl	62.5 pg/ml

Detection Antibody - Reconstitute the Detection Antibody Concentrate with 1.05 mL of **Antibody & HRP Diluent Solution (DB08B)** to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of **Antibody & HRP Diluent Solution (DB08B)** 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 10.89 mL of **Antibody & HRP Diluent Solution (DB08B)** into a 15 mL centrifuge tube and transfer 110 µL of 100-fold concentrated stock solution to prepare working solution. **Note:** 1X working solution of Streptavidin-HRP Conjugate should be used within a few hours. **Protect from light.**

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

blank, standards, positive control and be assayed in duplicate.

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 wells (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 plate sealer. Incubate for 90 minutes 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 45 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 10-15 minutes on a micro-plate 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 micro-plate 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 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 by 50, the concentration read from the standard curve must be multiplied by the dilution factor 50.

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human TIMP-1 derived from HEK293 cells animal free cultures.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of TIMP-1 was 20 pg/mL.

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 OD450nm (Corrected)
Blank	0 (0.061)
62.5	0.049
250	0.209
1000	0.679
4000	2.229
8000	3.512

LINEARITY

To assess the linearity of the assay, pooled research human **EDTA plasma** samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (NG/ML)	RECOVERY (%)
50X	1609.173	80.459	100
100X	791.082	79.108	98

To assess the linearity of the assay, pooled research human **serum** samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (NG/ML)	RECOVERY (%)
100X	3927.504	392.75	100
200X	1906.103	381.22	97

SPECIFICITY

Protein	Cross-reactivity (%)
Human TIMP-1	100
Mouse TIMP-1	0
Human MMP-1	0
Human MMP-3	0

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 90 min on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Streptavidin-HRP conjugate working solution to each well. Incubate 45 minutes on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 10-15 min on the plate shaker. **Protect from light**.
↓
Add 100 µl Stop Solution to each well. Read 450nm within 3min