ELISA Kit
Catalog #KHC1491

Human
TIMP-1

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TABLE OF CONTENTS

INTRODUCTION

Matrix metalloproteinases (MMPs) are a family of metalloenzymes, that upon activation, cause the degradation of extracellular matrix components. The MMPs have the following similar properties: they share common amino acid sequences, they are translated as inactive pro-enzymes, and they require a zinc cofactor. There are four classes of MMPs: colleganases (MMP-1, 8, 13), gelatinases (MMP-2, 9), stromelysins (MMP-3, 7, 10, 11), and membrane type MMPs (MT-1). The activity of these enzymes is stringently controlled and inhibitors called tissue inhibitors of metalloproteinases (TIMPs) can block the destruction of the extracellular matrix. There are four known TIMPs (TIMPs 1-4) and all four are composed of two domains held in a fixed conformation by six disulfide bonds. One domain is primarily responsible for inhibition, while the other domain can bind to progelatinases, and also cause some cells to proliferate. All connective tissues contain TIMPs. TIMP-1 is a 21 kDa glycoprotein that inhibits MMPs by forming non-covalent complexes with the active enzymes. These complexes keep substrates from entering the MMP catalytic site. The crystal structure of the TIMP-1/MMP-3 complex was determined and it was found that Cys^{1} and Cys^{70} are involved in MMP inhibition. The N-terminal α-amino and carbonyl groups of Cys^{1} coordinate the catalytic Zn^{2+}. The major sites of TIMP-1 expression are in the ovary and bone. TIMPs inhibit tumorigenesis, metastasis, and angiogenesis. TIMP-1 stimulates fibroblasts to produce MMP-1.

PURPOSE

The Invitrogen Human Tissue Inhibitor of Metalloproteinases-1 (Hu TIMP-1) ELISA is to be used for the quantitative determination of Hu TIMP-1 in human serum, plasma, buffered solution, or cell culture medium. The assay recognizes natural Hu TIMP-1.

For Research Use Only. CAUTION: Not for human or animal therapeutic or diagnostic use.

Read entire protocol before use.

PRINCIPLE OF THE METHOD

The Invitrogen Hu TIMP-1 kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for Hu TIMP-1 has been coated onto the wells of the microtiter strips provided. Samples, including standards of known Hu TIMP-1 content, control specimens, and unknowns, are pipetted into these wells, followed by the addition of a biotinylated monoclonal second antibody.

During the first incubation, the Hu TIMP-1 antigen binds simultaneously to the immobilized (capture) antibody on one site, and to the solution phase biotinylated antibody on a second site.

After removal of excess second antibody, Streptavidin-Peroxidase (enzyme) is added. This binds to the biotinylated antibody to complete the four-member sandwich. After a second incubation and washing to remove all the unbound enzyme, a substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of Hu TIMP-1 present in the original specimen.

REAGENTS PROVIDED

Note: *Store all reagents at 2 to 8°C.*

Reagent	***96 Test Kit***
Hu TIMP-1 Standard, natural Hu TIMP-1. Contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume.	2 vials
Standard Diluent Buffer. Contains 0.1% sodium azide; 60 mL per bottle.	1 bottle
Antibody Coated Wells. 12 x 8 Well Strips.	1 plate
Hu TIMP-1 Biotin Conjugate (Biotin-labeled anti-*TIMP-1*). Contains 0.1% sodium azide; 6 mL per bottle.	1 bottle
Streptavidin-HRP (100X). Contains 3.3 mM thymol; 0.125 mL per vial.	1 vial
Streptavidin-HRP Diluent. Contains 3.3 mM thymol; 25 mL per bottle.	1 bottle
Wash Buffer Concentrate (25X); 100 mL per bottle.	1 bottle
Stabilized Chromogen, Tetramethylbenzidine (TMB); 25 mL per bottle.	1 bottle
Stop Solution; 25 mL per bottle.	1 bottle
Plate Covers, adhesive strips.	3

Disposal Note: This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin, and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

SUPPLIES REQUIRED BUT NOT PROVIDED

1. Microtiter plate reader capable of measurement at or near 450 nm.
2. Calibrated adjustable precision pipettes, preferably with disposable plastic tips. (A manifold multi-channel pipette is desirable for large assays.)
3. Distilled or deionized water.
4. Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.).
5. Data analysis and graphing software. Graph paper: linear (Cartesian), log-log, or semi-log, as desired.
6. Glass or plastic tubes for diluting and aliquoting standard.
7. Absorbent paper towels.
8. Calibrated beakers and graduated cylinders in various sizes.

PROCEDURAL NOTES/LAB QUALITY CONTROL

1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.
2. **Microtiter plates should be allowed to come to room temperature before opening the foil bags.** Once the desired number of strips has been removed, immediately reseal the bag and store at 2 to 8°C to maintain plate integrity.

3. Samples should be collected in pyrogen/endotoxin-free tubes.
4. Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
5. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
6. It is recommended that all standards, controls, and samples be run in duplicate.
7. Samples that are >25 ng/mL should be diluted with *Standard Diluent Buffer*.
8. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
9. Cover or cap all reagents when not in use.
10. **Do not mix or interchange different reagent lots from various kit lots**.
11. Do not use reagents after the kit expiration date.
12. Read absorbances within 2 hours of assay completion.
13. In-house controls should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
14. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. ***Never*** insert absorbent paper directly into the wells.
15. Because *Stabilized Chromogen* is light sensitive, avoid prolonged exposure to light. Also avoid contact between *Stabilized Chromogen* and metal, or color may develop.

SAFETY

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

DIRECTIONS FOR WASHING

Incomplete washing will adversely affect the test outcome. All washing must be performed with *Wash Buffer Concentrate (25X)* provided.

Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip (aspiration device) into the bottom of each well. Take care not to scratch the inside of the well.

After aspiration, fill the wells with at least 0.4 mL of diluted wash solution. Let soak for 15 to 30 seconds, then aspirate the liquid. Repeat as directed under **ASSAY METHOD**. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.

Alternatively, the wash solution may be put into a squirt bottle. If a squirt bottle is used, flood the plate with wash buffer, completely filling all wells. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.

If using an automated washer, the operating instructions for washing equipment should be carefully followed.

REAGENT PREPARATION AND STORAGE

A. Reconstitution and Dilution of Hu TIMP-1 Standard

This assay has been calibrated against natural Hu TIMP-1 from fetal lung fibroblast cell line (WI-38).

Note: Either glass or plastic tubes may be used for standard dilutions.

1. Reconstitute standard to 25.0 ng/mL with *Standard Diluent Buffer*. Refer to standard vial label for instructions. Swirl or mix gently and allow to sit for 10 minutes to ensure complete reconstitution. It is recommended that standard be used within 1 hour of reconstitution.
2. Add 0.150 mL of *Standard Diluent Buffer* to each of 4 tubes labeled 12.5, 6.25, 3.12, and 1.56 ng/mL Hu TIMP-1.
3. Make serial dilutions of the standard as described in the following dilution table. Mix thoroughly between steps.

B. Dilution of Hu TIMP-1 Standard

Standard:	Add:	Into:
25.0 ng/mL	Prepare as described in Step 1.	
12.5 ng/mL	0.150 mL of the 25.0 ng/mL std.	0.150 mL of the Diluent Buffer
6.25 ng/mL	0.150 mL of the 12.5 ng/mL std.	0.150 mL of the Diluent Buffer
3.12 ng/mL	0.150 mL of the 6.25 ng/mL std.	0.150 mL of the Diluent Buffer
1.56 ng/mL	0.150 mL of the 3.12 ng/mL std.	0.150 mL of the Diluent Buffer
0 ng/mL	0.150 mL of the Diluent Buffer	An empty tube

Discard all remaining reconstituted and diluted standards after completing assay. Return the *Standard Diluent Buffer* to the refrigerator.

C. Storage and Final Dilution of Streptavidin-HRP (100X)

Please Note: The *Streptavidin-HRP (100X)* is in 50% glycerol. This solution is viscous. To ensure accurate dilution, allow *Streptavidin-HRP (100X)* to reach room temperature. Gently mix. Pipette *Streptavidin-HRP (100X)* slowly. Remove excess concentrate solution from pipette tip by gently wiping with clean absorbent paper.

1. Dilute 10 µL of this 100X concentrated solution with 1 mL of *Streptavidin-HRP Diluent* for each 8-well strip used in the assay. Label as Streptavidin-HRP Working Solution.

For Example:

# of 8-Well Strips	Volume of Streptavidin-HRP (100X)	Volume of Diluent
2	20 µL solution	2 mL
4	40 µL solution	4 mL
6	60 µL solution	6 mL
8	80 µL solution	8 mL
10	100 µL solution	10 mL
12	120 µL solution	12 mL

2. Return the unused *Streptavidin-HRP (100X)* to the refrigerator.

D. Dilution of Wash Buffer

Allow the *Wash Buffer Concentrate (25X)* to reach room temperature and mix to ensure that any precipitated salts have redissolved. Dilute 1 volume of the *Wash Buffer Concentrate (25X)* with 24 volumes of deionized water (e.g., 50 mL may be diluted up to 1.25 liters, 100 mL may be diluted up to 2.5 liters). Label as Working Wash Buffer.

Store both the concentrate and the Working Wash Buffer in the refrigerator. The diluted buffer should be used within 14 days.

ASSAY METHOD: PROCEDURE AND CALCULATIONS

Be sure to read the *Procedural Notes/Lab Quality Control* section before carrying out the assay.

Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.

Note: A standard curve must be run with each assay.

1. Determine the number of 8-well strips needed for the assay. Insert these in the frame(s) for current use. (Re-bag extra strips and frame. Store these in the refrigerator for future use.)
2. Dilute serum and plasma samples 1:20 with *Standard Diluent Buffer* (e.g., add 10 µL of sample to 190 µL *Standard Diluent Buffer*). **NOTE:** Individual samples may require a greater or lesser dilution to fall within the range of the assay.
3. Add 50 µL of the *Standard Diluent Buffer* to zero wells. Well(s) reserved for chromogen blank should be left empty.
4. Add 50 µL of standards, samples or controls to the appropriate microtiter wells. (See **REAGENT PREPARATION AND STORAGE**, Section B.)

5. Pipette 50 µL of biotinylated *Hu TIMP-1* (*Biotin Conjugate*) solution into each well except the chromogen blank(s). Tap gently on the side of the plate to mix.
6. Cover plate with *plate cover* and incubate for **2 hours at room temperature**.
7. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**.
8. Add 100 µL Streptavidin-HRP Working Solution to each well except the chromogen blank(s). (Prepare the working dilution as described in **REAGENT PREPARATION AND STORAGE,** Section C.)
9. Cover plate with the *plate cover* and incubate for **30 minutes at room temperature**.
10. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**.
11. Add 100 µL of *Stabilized Chromogen* to each well. The liquid in the wells will begin to turn blue.
12. Incubate for **30 minutes at room temperature and in the dark**. ***Please Note*: Do not cover the plate with aluminum foil or metalized mylar**. The incubation time for chromogen substrate is often determined by the microtiter plate reader used. Many plate readers have the capacity to record a maximum optical density (O.D.) of 2.0. The O.D. values should be monitored and the substrate reaction stopped before the O.D. of the positive wells exceed the limits of the instrument. The O.D. values at 450 nm can only be read after the *Stop Solution* has been added to each well. If using a reader that records only to 2.0 O.D., stopping the assay after 20 to 25 minutes is suggested.
13. Add 100 µL of *Stop Solution* to each well. Tap side of plate gently

to mix. The solution in the wells should change from blue to yellow.

14. Read the absorbance of each well at 450 nm having blanked the plate reader against a chromogen blank composed of 100 µL each of *Stabilized Chromogen* and *Stop Solution*. Read the plate within 2 hours after adding the *Stop Solution*.
15. Plot on graph paper the absorbance of the standards against the standard concentration. (Optimally, the background absorbance may be subtracted from *all* data points, including standards, unknowns, and controls, prior to plotting.) Draw the best smooth curve through these points to construct the standard curve. If using curve fitting software, the four parameter algorithm provides the best curve fit.
16. Read the Hu TIMP-1 concentrations for unknown samples and controls from the standard curve plotted in step 15. **Multiply value(s) obtained for serum/plasma sample(s) by 20 to correct for the 1:20 dilution in step 2.** (Samples producing signals greater than that of the highest standard (25 ng/mL) should be further diluted in *Standard Diluent Buffer* and reanalyzed, multiplying the concentration found by the appropriate dilution factor.)

TYPICAL DATA

The following data were obtained for the various standards over the range of 0 to 25 ng/mL Hu TIMP-1.

Standard Hu TIMP-1 (ng/mL)	Optical Density (450 nm)
0	0.035
	0.038
1.56	0.079
	0.078
3.12	0.169
	0.165
6.25	0.511
	0.520
12.5	1.238
	1.355
25.0	2.242
	2.307

LIMITATIONS OF THE PROCEDURE

Do not extrapolate the standard curve beyond the 25 ng/mL standard point; the dose-response is non-linear in this region and accuracy is difficult to obtain. Dilute samples >25 ng/mL with *Standard Diluent Buffer*; reanalyze these and multiply results by the appropriate dilution factor.

The influence of various drugs, aberrant sera (hemolyzed, hyperlipidemic, jaundiced, etc.) and the use of biological fluids in place of serum samples have not been thoroughly investigated. The rate of degradation of native Hu TIMP-1 in various matrices has not been investigated. The immunoassay literature contains frequent references to aberrant signals seen with some sera, attributed to heterophilic antibodies. Though such samples have not been seen to date, the possibility of this occurrence cannot be excluded.

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PERFORMANCE CHARACTERISTICS

SENSITIVITY

The minimum detectable dose of Hu TIMP-1 is <1 ng/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times.

PRECISION

1. Intra-Assay Precision

Samples of known Hu TIMP-1 concentration were assayed in replicates of 16 to determine precision within an assay.

	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	6.3	13.6	22.9
SD	0.2	0.4	1.4
%CV	3.0	2.9	6.1

SD = Standard Deviation
CV = Coefficient of Variation

2. Inter-Assay Precision

Samples were assayed 48 times in multiple assays to determine precision between assays.

	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	6.4	13.2	21.5
SD	0.2	0.6	2.0
%CV	3.1	4.6	9.3

SD = Standard Deviation
CV = Coefficient of Variation

LINEARITY OF DILUTION

Human serum and tissue culture medium containing 1% fetal bovine serum were spiked with Hu TIMP-1 and serially diluted in *Standard Diluent Buffer* over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99 in both cases.

Dilution	Serum			Cell Culture		
	Measured (ng/mL)	**Expected (ng/mL)**	**% Expected**	**Measured (ng/mL)**	**Expected (ng/mL)**	**% Expected**
neat	23.9	-	-	26.6	-	-
1/2	11.8	12.0	98	11.2	13.3	84
1/4	5.8	6.0	97	5.6	6.7	84
1/8	2.7	3.0	90	2.7	3.3	82

RECOVERY

The recovery of Hu TIMP-1 added to human serum averaged 92%. The recovery of Hu TIMP-1 added to human plasma (EDTA) averaged 86%. The recovery of Hu TIMP-1 added to tissue culture medium containing 1% fetal bovine serum averaged 112%, while the recovery of Hu TIMP-1 added to tissue culture medium containing 10% fetal bovine serum averaged 107%.

SPECIFICITY

Buffered solutions of a panel of substances at 25 ng/mL were assayed with the Invitrogen Hu TIMP-1 kit. The following substances were tested and found to have no cross-reactivity: human IL-1β, IL-6, IL-8, IL-10, GM-CSF, IFN-γ, MCP-1, RANTES, TIMP-2, TNF-α, sVCAM-1; mouse IL-1β, IL-6, IL-10, IFN-γ, TNF-α; rat IL-1β, IL-6, IL-10, IFN-γ, TNF-α. The pro and active Hu MMPs 1, 2, and 3 showed no cross-reactivity, while the pro and active Hu MMP-9 showed 4% cross-reactivity. The pro Hu MMPs 1, 2, and 3 at concentrations of 100 ng/mL produced no interference when added to a 10 ng/mL TIMP-1 sample. Some interference was observed when the active Hu MMPs 1, 2, and 3 were added to a 10 ng/mL TIMP-1 sample:

HIGH DOSE HOOK EFFECT

Concentration MMP	**% Interference**		
	MMP-1	**MMP-2**	**MMP-3**
25 ng/mL	3	1	0
50 ng/mL	5	9	6
100 ng/mL	13	12	12

A sample spiked with Hu TIMP-1 up to 600 ng/mL gave a response higher than that obtained for the last standard point.

EXPECTED VALUES

Twenty sera and twenty plasma (EDTA) samples were evaluated in this assay. The values for sera ranged from 92 to 116 ng/mL (mean 101 ng/mL). The values for plasma ranged from 111 to 138 ng/mL (mean 123 ng/mL).

REFERENCES

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Explanation of symbols

Symbol	Description	Symbol	Description
	Catalogue Number		Batch code
	Research Use Only		*In vitro* diagnostic medical device
	Use by		Temperature limitation
	Manufacturer		European Community authorised representative
[-]	Without, does not contain	[+]	With, contains
	Protect from light		Consult accompanying documents
	Directs the user to consult instructions for use (IFU), accompanying the product.		

Human TIMP-1 Assay Summary

Add 50 µL of standards, controls & samples

Add 50 µL of Biotin Conjugate
Incubate for 2 hours at RT

↓ aspirate and wash 4x

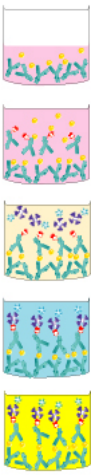
Incubate 100 µL of Streptavidin-HRP
Working Solution for 30 minutes at RT

↓ aspirate and wash 4x

Incubate 100 µL of Stabilized Chromogen
for 30 minutes at RT

↓

Add 100 µL of Stop Solution
and read at 450 nm



Total time: 3.0 hours