

Human TIMP-1 ELISA Kit

Cat: RK00051

This ELISA kit used for quantitation of human TIMP metalloproteinase inhibitor 1 (TIMP-1) concentration in cell culture supernate, serum and plasma. For research use only, and it's highly recommended to read thoroughly of this manual before using the product.

Manufactured by

Global Headquarters

86 Cummings Park

Woburn, MA 01801

Tel: +8887545670

China Branch

388# Gaoxin Road (No.2)

Tel: 400-999-6126

East Lake Development Zone

E-mail: market@abclonal.com

Wuhan P. R. China

[http: www.abclonal.com.cn](http://www.abclonal.com.cn)

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Introduction

Matrix Metalloproteinases (MMPs) are zinc-dependent endopeptidases that catalyze degradation of extracellular matrix proteins, thereby controlling such processes as development, tissue remodeling, wound healing and tumor metastasis (1-3). The activity of MMPs is controlled by regulation of expression and secretion, by proteolytic activation of pro-enzymes and by the Tissue Inhibitors of Metalloproteinases (TIMPs) (4, 5). TIMPs form 1:1, non-covalent complexes with MMPs, blocking access of substrates to the MMP catalytic site. TIMPs are highly specific for MMPs in general but not for any particular MMP. Functional specificity is conferred by other characteristics. TIMP-1 is an inducible protein and TIMP-2 is a constitutive protein and both are soluble and widely distributed. TIMP-3 is restricted to the extracellular matrix and TIMP-4 is largely restricted to cardiac tissue. For reviews on MMPs and TIMPs, see references 1-5. TIMP-1 is a 184 amino acid residue glycosylated protein, though glycosylation is not necessary for activity (6). It has 12 cysteines (conserved among all TIMPs) that form disulfide bonds in a pattern that gives distinct N- and C-terminal domains (7). The N-terminal domain contains sites that bind to the MMP substrate-binding site (8). Binding of TIMP-1 does not leave a peptide bond in position for proteolysis and is not cleaved (5). The TIMP/MMP complex can dissociate to yield enzyme and active TIMP-1 (9). The C-terminal domain binds to an external site on MMPs, increasing overall affinity (5). TIMP-1 binds with high affinity to the inactive pro-MMP-9, forming a complex in which TIMP-1 retains its ability to inhibit the activity of another active MMP via its N-terminal domain (10).

TIMP-1 is widely synthesized by many cells and tissues (4). Transcription of the TIMP-1 gene is induced by pro-inflammatory cytokines (IL-1, IL-6, OSM, LIF and

TNF- α), TGF- β 1 and phorbol esters (4, 11). Many physiological functions of TIMP-1 are closely tied to the functions of MMPs, and an improper balance of MMP and TIMP production correlates with pathological conditions such as arthritis, tumor growth and metastasis (4). On the other hand, TIMP-1 was independently discovered as an erythroid potentiating activity (12, 13), an activity that appears to be functionally distinct from MMP inhibitory activity (14). TIMP-1 binds to certain cell lines and is translocated to the nucleus (15). It inhibits apoptosis in B cells (16), further suggesting that it independently functions in multiple ways to support survival and growth of cells in contrast to its function of inhibition of MMPs.

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. Following incubation unbound samples are removed during a wash step, and then a detection antibody specific for TIMP-1 is added to the wells and binds to the combination of capture antibody-TIMP-1 in sample. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Following incubation and wash steps, a substrate is added. A colored product TMB is formed in proportion to the amount of TIMP-1 present in the sample. The reaction is terminated by addition of acid and absorbance is measured. A standard curve is prepared from seven TIMP-1 standard dilutions and TIMP-1 sample concentration determined.

Materials Provided

Description	Size (192T)	Size (96T)	Size (48T)	Storage	Cat NO.
Human TIMP-1 antibody coated plate	(8×12) ×2	8×12	8×6	4°C	RM00232
Human TIMP-1 Standard lyophilized	4 vials	2 vials	1 vial	4°C	RM00229
Standard/sample Diluent (R1)	2 bottles ×20 mL	1 bottle ×20 mL	1 bottle ×6 mL	4°C	RM00023
Human TIMP-1 concentrated biotin conjugate antibody (100X)	2 vials ×120 µL	1vial ×120 µL	1 vial ×60 µL	4°C	RM00230
Biotin-Conjugate antibody Diluent (R2)	1 bottle ×32 mL	1 bottle × 16 mL	1 bottle × 10 mL	4°C	RM00024
Streptavidin-HRP concentrated (100×)	2 vials ×120 µL	1 vial ×120 µL	1 vial ×60 µL	4°C	RM00231
Streptavidin-HRP Diluent	1 bottle ×32 mL	1 bottle ×16 mL	1 bottle ×16 mL	4°C	RM00025

Wash Buffer (20x)	2 bottles ×30 mL	1 bottle × 30 mL	1 bottle ×30 mL	4°C	RM00026
Substrate Solution (Dark)	2 bottles ×12 mL	1 bottle ×12 mL	1 bottle ×6 mL	4°C	RM00027
Stop Solution	1 bottle ×24 mL	1 bottle ×12 mL	1 bottle ×12 mL	4°C	RM00028
Plate Sealers	8 strips	4 strips	2 strips		
Specification	1				

Sample Collection And Storage

1. Cell Culture Supernates:

Centrifuge 1000x g for 10 min and detect; or aliquot and store samples at -20°C to -70°C (Stored at 2-8°C if tested within 24 hours). Avoid freeze/thaw cycles.

2. Serum:

Use a serum separator tube and allow samples to clot for 30 minutes before centrifugation for 10 minutes at 1000x g, and detect; or aliquot and store samples at -20°C to -70°C (Stored at 2-8°C if tested within 24 hours). Avoid freeze/thaw cycles.

3. Plasma

Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000x g within 30 minutes of collection, and detect; or aliquot and store samples at -20°C to -70°C (Stored at 2-8°C if tested within 24 hours). Avoid freeze / thaw cycles.

4. **Avoid hemolytic and hyperlipidemia sample for Serum and Plasma.**
5. **Dilution:**
Dilute samples at the appropriate multiple (recommend to do pre-test to determine the dilution factor).

Precautions For Use

1. **Reagents may be harmful, if ingested, rinse it with an excess amount of tap water.**
2. **Stop Solution contains strong acid. Wear eye, hand, and face protection.**
3. **Store the kits at 2 to 8°C before use, throw away the unspent kits.**
4. **Apart from the standard of kits, other components should not be refrigerated.**
5. **Please perform simple centrifugation to collect the liquid before use.**
6. **Apart from Stop Buffer and Concentrated Wash Buffer can be commonly used, the other components in the kits are specified. Do not mix or substitute reagents with those from other lots or other sources.**
7. **Adequate mixing is very important for good result. Use a mini-vortexer at the lowest frequency.**
8. **Mix the sample and all components in the kits adequately, and use clean plastainer to prepare wash buffer.**
9. **Both the sample and standard should be assayed in duplicate, and the sequence of the reagents should be added consistently.**
10. **The kit should not be used beyond the expiration date.**
11. **The kit should be away from light when it is stored or incubated.**
12. **To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma and other biological fluids in accordance with**

NCCLS regulations.

13. To avoid cross contamination, please use disposable pipette tips.
14. Please prepare all the kit components according to the requirement. If the kits will be used several times, please seal the rest strips and preserve with desiccants. Do use up within 2 months.

Experiment Materials

1. ELIASA (measuring absorbance at 450 nm, with the correction wavelength set at 570 nm or 630 nm)
2. Pipettes and pipette tips: 0.5-10, 2-20, 20-200, 200-1000 μ L
3. Microplate washer, Squirt bottle
4. Micro-oscillator
5. Deionized or double distilled water, graduated cylinder
6. Polypropylene Test tubes for dilution

Reagent Preparation

1. Bring all reagents to room temperature before use. If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.
2. Wash buffer: 1:20 diluted with double distilled or deionized water before use.
3. Biotin-Conjugate antibody: 1:100 diluted with the Biotin-Conjugate antibody Diluent (R2) before use, and the diluted solution should be used up within 30 min.

Dilution Method

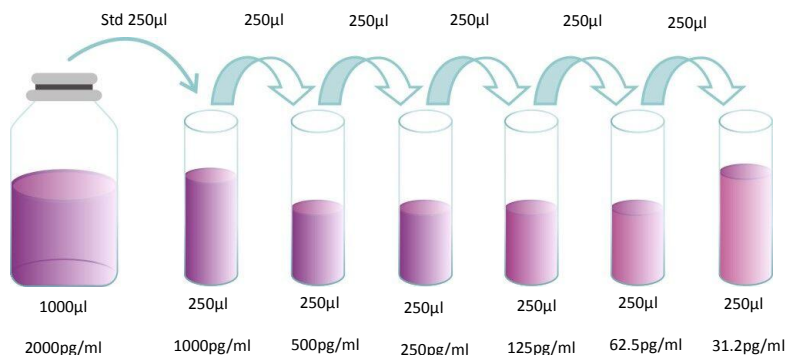
Strip	Concentrated Biotin- Conjugate antibody (1:100)	Testing dilution buffer (R2)
2	20	1980
4	40	3960
6	60	5940
8	80	7920
10	100	9900
12	120	11880

4. Streptavidin-HRP: 1:100 diluted with the Streptavidin-HRP Diluent (R3) before use, and the diluted solution should be used up within 30 min.

Dilution Method

Strip	Concentrated Streptavidin-HRP (1:100)	Testing dilution buffer (R3)
2	20	1980
4	40	3960
6	60	5940
8	80	7920
10	100	9900
12	120	11880

5. **Standard:** Add standard/sample dilution (R1) 1mL into freeze-dried standard, sit for a minimum of 15 minutes with gentle agitation prior to making dilutions (2000 pg/mL), then dilute according to the requirement (recommended concentration for standard curve: 2000,1000, 500, 250, 125, 62.5, 31.25, 0 pg/mL). Redissolved standard solution (2000 pg/mL), aliquot and store at -20°C— -70°C.



Wash Method

Automatic washer: Add wash buffer 300 µl/well, soak for about 10-20 seconds, and wash 5 times.

Washer: Throw all the solutions in the plate well, clean with absorbent paper, and then dispense wash buffer 300 µL/well, throw all the solutions in the plate well after holding 30 seconds, repeat 4 times.

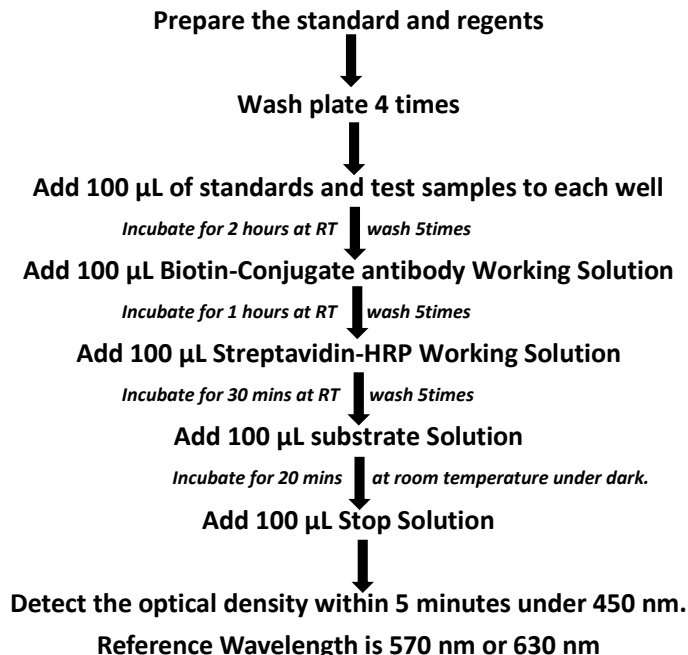
Assay Procedure

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
2. Add wash buffer 300 μL /well, aspirate each well after holding 30 seconds, repeating the process three times for a total of four washes. Then use enzyme-marked plate in a short time, do not let it dry.
3. Add 100 μL Standard /Sample Diluent (R1) in blank well.
4. Apart from blank well, add 100 μL different concentration of standard and sample in other wells, cover with the adhesive strip provided. Incubate for 2 hours at room temperature (20 to 25°C)
5. Wash the plate 5 times as in step 2.
6. Prepare the Biotin-Conjugate antibody Working Solution 20 minutes early.
7. Add Biotin-Conjugate antibody diluent (R2) in blank well and Biotin-Conjugate antibody Working Solution in other wells (100 μL /well), cover with new adhesive strip provided, shake with Micro-oscillator (100 r/min). Incubate for 1 hours at room temperature (20 to 25°C)
8. Prepare the Streptavidin-HRP Working Solution 20 minutes early, place away from light at room temperature.
9. Wash the plate 5 times as in step 2.
10. Aspirate Streptavidin-HRP diluent (R3) in blank well and aspirate Streptavidin-HRP Working Solution in other wells (100 μL /well), cover with new adhesive strip provided, shake with Micro-oscillator (100 r/min). Incubate for 30 minutes at room temperature (20 to 25°C)
11. Warm-up the ELIASA.
12. Wash the plate 5 times.
13. Aspirate substrate Solution (100 μL /well). Incubate for 20 minutes at room

temperature under dark.

14. Aspirate Stop Solution (100 μ L/well), mix, determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 570 nm or 630 nm. If wavelength correction is not available, subtract readings at 570 nm or 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Assay Procedure Summary

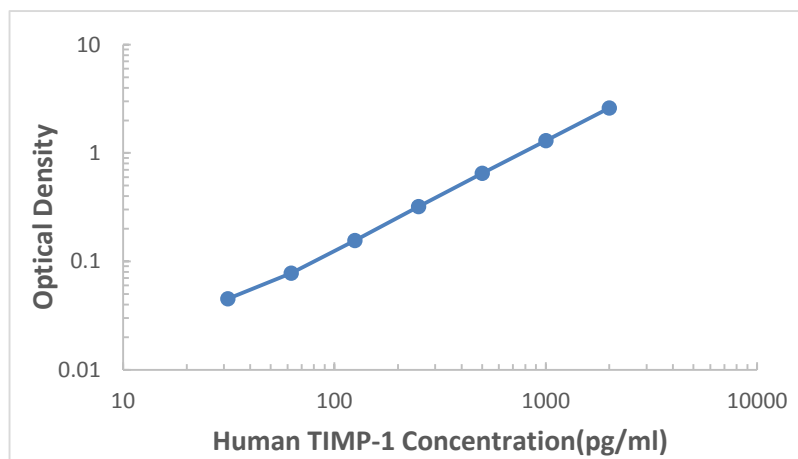


Calculation Of Results

1. Average the duplicate readings for each standard, control and sample, and subtract the average zero standard optical density (O.D.).
2. 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 a log/log graph. The data may be linearized by plotting the log of the TIMP-1 concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.
3. If the detect result is higher than the standard curve's upper limit, then dilute samples, and the concentration read from the standard curve must be multiplied.

Typical Data

Standard (pg/mL)	OD value		Average value	Correct value
0	0.048	0.049	0.0485	---
31.25	0.126	0.104	0.115	0.0665
62.5	0.236	0.259	0.2475	0.199
125	0.398	0.364	0.381	0.3325
250	0.625	0.654	0.6395	0.591
500	1.156	1.137	1.1465	1.098
1000	1.832	1.879	1.8555	1.807
2000	2.689	2.616	2.6525	2.604



The standard curves are provided for demonstration only. A standard curve should be generated for each set of TIMP-1 assayed.

Sensitivity

The minimum detectable dose (MDD) of TIMP-1 ranged from 15 pg/mL. The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

Specificity

This assay recognizes both recombinant and natural human TIMP-1. Use 50 ng/mL to do specificity assay. No significant cross-reactivity was observed with the following:

Recombinant human

MMP-1

MMP-2

MMP-3

TIMP-2

TIMP-3

TIMP-4

Precision

Intra-plate Precision

Repeat 20 times detection of 3 known concentration sample enzyme plate to evaluate the Intra-plate precision.

Sample	1	2	3
Repeat Times	20	20	20
Average Value (pg/mL)	65	482	1002
Standard Deviation (SD)	4.2	18.0	24.0
Variable Coefficient CV (%)	3.6	2.9	3.8

Inter-plate Precision

Repeat 20 times detection of 3 known concentration sample enzyme plate to evaluate the Inter-plate precision.

Sample	1	2	3
Repeat Times	20	20	20
Average Value (pg/mL)	60	491	1008
Standard Deviation (SD)	3.9	35.8	68.6
Variable Coefficient CV (%)	6.5	7.3	6.8

Recovery

Aspirate 3 different concentration of human TIMP-1 into healthy human serum and plasma, calculate the recovery.

Sample Form	Average Recover (%)	Range (%)
Serum	90	88-106
Plasma	94	90-112

Linearity Dilute

Aspirate high concentration of human TIMP-1 into 4 healthy human serum, dilute in the range of standard curve kinetics and evaluate the linearity.

Dilution	Average Value (%)	Range (%)
1:2	91	90-110
1:4	92	90-108
1:8	92	85-100
1:16	91	89-102

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