Product Manual

TIMP-1 (human) ELISA kit

Catalog #: ENZ-KIT147-0001

96 Well Kit

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Product Manual

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Please read entire booklet before proceeding with the assay.

Carefully note the handling and storage conditions of each kit component.

Please contact Enzo Life Sciences Technical Support if necessary.

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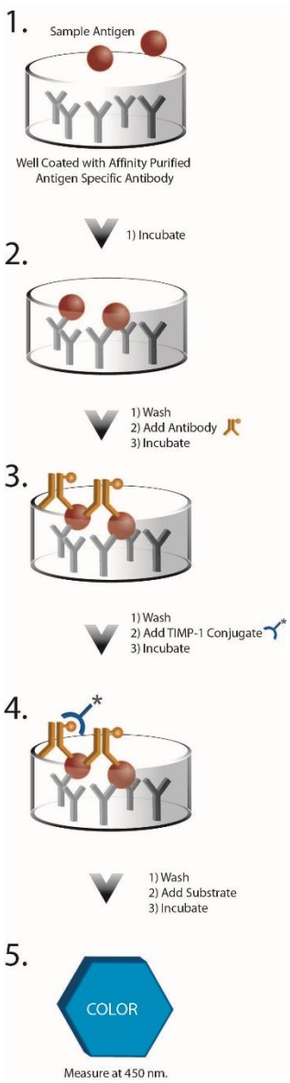
INTRODUCTION

The TIMP-1 (human) ELISA kit is a complete kit for the quantitative determination of TIMP-1 in human serum, human plasma, human saliva, human urine and tissue culture media. Please read the complete kit insert before performing this assay.

Tissue inhibitor of metalloproteinases-1 (TIMP-1) is one of the four members of the TIMP family which inhibit the activity of the matrix metalloproteinases (MMPs), a large group of peptidases involved in degradation of the extracellular matrix[1]. TIMP-1 displays multiple biological functions. TIMP-1 is capable of inhibiting the activities of most MMPs except membrane-type MMP subfamily[1]. Associated with its inhibitory activity on MMPs, TIMP-1 is able to promote cell proliferation and survival in a wide range of cell types[2]. Recent findings have separated the MMP inhibitory activity of TIMP-1 from its growth promoting effect. TIMP-1 exhibits growth factor-like activity and acts as a cell survival factor which may be mediated through ligand-receptor interactions[3].

The balance of TIMPs/MMPs plays a crucial role in extracellular matrix homeostasis and remodeling, the imbalance of TIMPs/MMPs is involved in various kinds of diseases in multiple organs. Breaking the balance between MMPs and TIMPs occurs in various pathologic processes and involves all the members of these two families[4]. Elevation of TIMP-1 has been reported in diseases such as systemic sclerosis[5], amyotrophic lateral sclerosis (ALS)[6], renal disease[7] and invasive tumors[8-9]. Down regulation of TIMP-1 was found to be associated with ischemic heart failure[10]and chronic inflammation[11]. The contribution of TIMP-1 in the pathologic processes is not fully understood. Our TIMP-1 ELISA kit provides a useful tool in investigating the TIMP-1 levels in multiple human matrices and will help advance the studies on TIMP-1 function.



PRINCIPLE

1. A monoclonal antibody to TIMP-1 is immobilized on a microtiter plate. Standards and samples containing TIMP-1 are added to the plate and incubated.
2. During this incubation the TIMP-1 antibody immobilized on the plate binds TIMP-1 in the standards or samples.
3. The plate is washed, removing excess unbound sample or standard, and a solution of detector antibody to TIMP-1 is added. This antibody binds to the TIMP-1 captured on the plate.
4. After a short incubation the plate is washed to remove unbound detector antibody. Streptavidin conjugated to Horseradish Peroxidase (SA-HRP) is added to bind the biotinylated TIMP-1 detector antibody. The plate is then incubated.
5. The plate is washed to remove excess conjugate. TMB substrate solution is added to all wells and incubated. An HRP-catalyzed reaction generates a blue color in the solution.
6. Stop solution is added to stop the substrate reaction. The resulting yellow color is read at 450nm. The amount of signal is directly proportional to the level of TIMP-1 in the sample or standard.

MATERIALS SUPPLIED

Do not mix components from different kit lots or use beyond the expiration date of the kit.

1. **TIMP-1 Microtiter Plate, One plate of 96 wells. Catalog No. 80-2716**

   Plate with break-apart strips coated with a monoclonal antibody specific to TIMP-1.

2. **TIMP-1 Standard, 1 vial, 60 ng, Catalog No. 80-2715**

   Vial containing 60 ng lyophilized TIMP-1 protein, purified from human origin.

3. **TIMP-1 Detector Antibody, 1 vial, Catalog No. 80-2717**

   Vial containing lyophilized TIMP-1 detector antibody.

4. **TIMP-1 Conjugate, 10 mL, Catalog No. 80-2724**

   A blue solution of streptavidin conjugated to horseradish peroxidase.

5. **Antibody Diluent, 14 mL, Catalog No. 80-2722**

   A yellow solution of Tris buffered saline containing BSA and detergents.

6. **Assay Buffer 13, 50 mL, Catalog No. 80-1500**

   Tris buffered saline containing BSA and detergents.

Protect substrate from prolonged exposure to light.

7. **TMB Substrate, 10 mL, Catalog No. 80-0350**

   Solution of 3,3',5,5' tetramethylbenzidine (TMB) and hydrogen peroxide.

8. **Stop Solution 2, 10 mL, Catalog No. 80-0377**

   A 1N solution of hydrochloric acid in water. Keep tightly capped. Caution: **Caustic**.

Stop solution is caustic. Keep tightly capped.

9. **Wash Buffer Concentrate, 100 mL, Catalog No. 80-1287**

   20x Tris buffered saline containing detergents.

10. **TIMP-1 Assay Layout Sheet, Catalog No. 30-0336**

11. **Plate Sealer, 3 each, Catalog No. 30-0012**

STORAGE

Reagents require separate storage conditions.

The standard and detector antibody should be stored at -20°C (before and after reconstitution) until the kit's expiration date. All other components of this kit should be stored at 4°C. Avoid repeated freeze-thaw cycles.

OTHER MATERIALS NEEDED

1. Deionized or distilled water.
2. Precision pipets for volumes between 100 µL and 1,000 µL.
3. Repeater pipet for dispensing 100 µL.
4. Disposable beakers for diluting buffer concentrates.
5. Graduated cylinders.
6. A microplate shaker.
7. Adsorbent paper for blotting.
8. Microplate reader capable of reading at 450 nm.
9. Software for extrapolating sample values from optical density readings utilizing a four parameter logistic curve fit.

SAMPLE HANDLING

If buffers other than those provided are used, the end-user must determine the appropriate dilution and assay variation.

This assay is suitable for the measurement of TIMP-1 in human serum, plasma (EDTA), saliva, urine in addition to tissue culture media. Prior to assay, frozen specimens should be brought to 4°C and centrifuged. If it is necessary, filter to remove residual debris.

Diluted samples have been validated for use in this assay (please refer to the Spike and Recovery section on page 7 for details). However, due to variation in samples, users must determine the optimal dilutions for their unique set of samples.

SAMPLE MATRIX PROPERTIES

Linearity

The minimum required dilution for human serum, plasma (EDTA), saliva, urine was determined by serially diluting specimens into the provided assay buffer and identifying the dilution at which linearity was observed. According to the below table, the 16-fold diluted human serum and plasma, 32-fold diluted saliva and 2-fold diluted urine are the minimum dilutions in the linear detection range.

Dilutional Linearity, %				
Dilution	**Human Serum**	**Human Plasma (EDTA)**	**Human Saliva**	**Human Urine**
Neat				
1:2				100
1:4				113
1:8				133
1:16	100	100		
1:32	97	97	100	
1:64	98	93	100	
1:128	95	91	107	
1:256	99	103	106	

Spike and Recovery

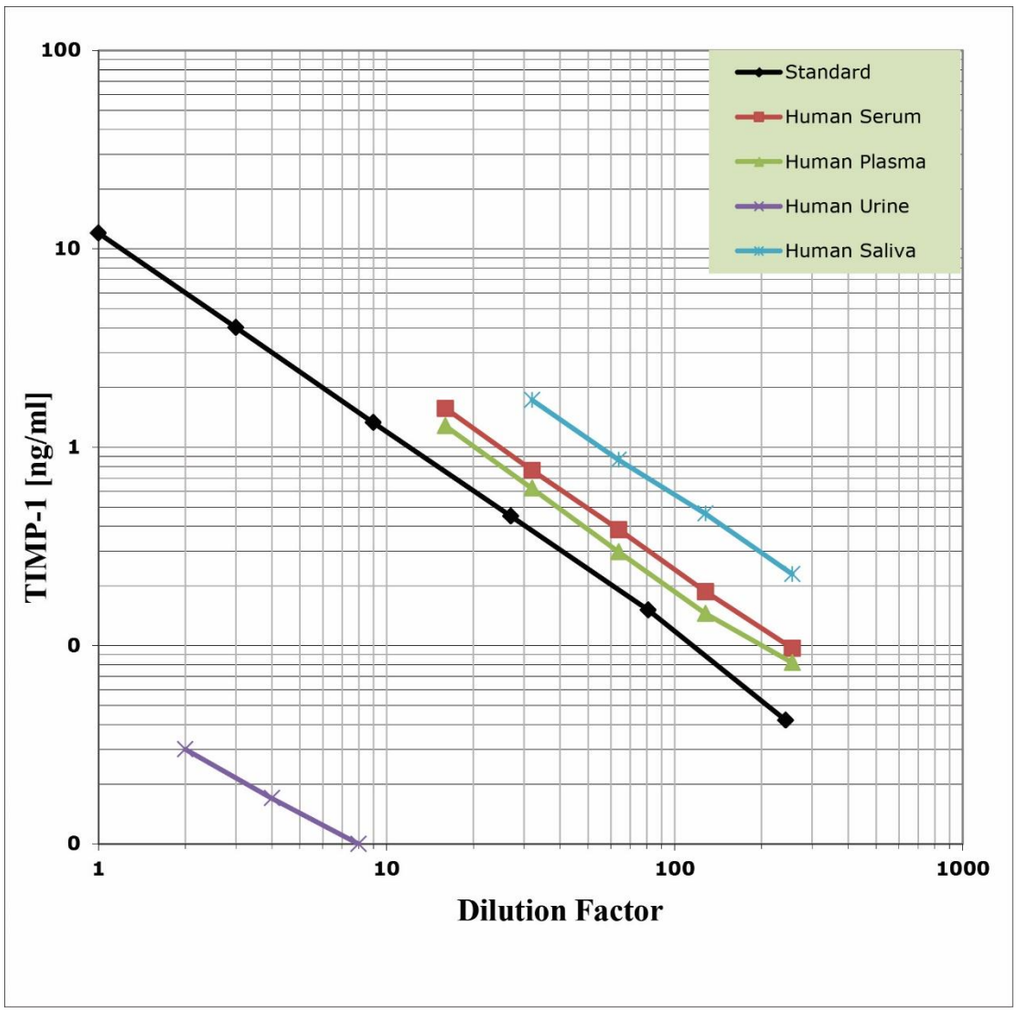
Purified TIMP-1 protein was spiked at three concentrations into minimum recommended dilution of human matrices or tissue culture media. Matrix background was subtracted from the spiked values and the average percent recovery for each matrix at minimum required dilution is presented below. These results showed the tested human matrices and tissue culture media at minimum recommended dilution have no obvious interference with the TIMP-1 ELISA assay.

Sample	Spike Concentration ng/ml	% Recovery	Minimum Recommended Dilution
Serum	6	98	1:16
	2	117	
	0.67	117	
Plasma	6	96	1:16
	2	112	
	0.67	86	
Saliva	6	98	1:32
	2	109	
	0.67	102	
Urine	6	102	1:2
	2	104	
	0.67	86	
***Tissue Culture Medium**	6	107	1:2
	2	118	
	0.67	105	

*Tissue culture medium used in this test was DMEM with phenol red and 10% FBS.

Parallelism

The parallelism of various matrices was determined by running serial dilutions in the assay, assigning concentrations to each dilution and plotting the dilution factor against the determined concentration of each matrix dilution. Parallelism of the curves demonstrates that the antigen binding characteristics are similar enough to allow the accurate determination of native analyte levels in diluted samples of human origin.



REAGENT PREPARATION

Sample handling procedures should be completed prior to reagent preparation.

1. Wash Buffer

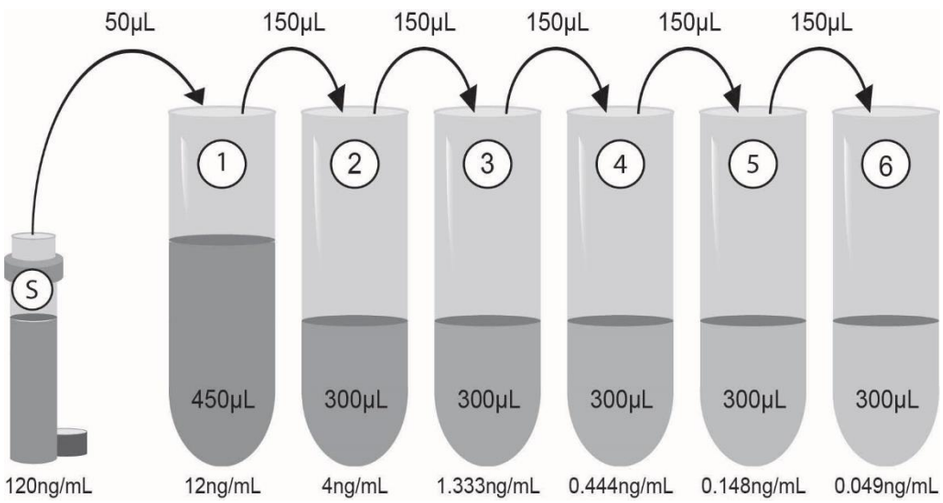
Prepare the Wash Buffer by diluting 50 mL of the supplied concentrate with 950 mL of deionized water. The diluted wash buffer can be stored at room temperature for up to 3 months.

Polypropylene tubes may be used for standard preparation. Avoid polystyrene.

2. TIMP-1 Standard Curve

Reconstitute the TIMP-1 standard in 0.5 mL of the provided Assay Buffer 13 to make 10x (120 ng/mL) stock.

Label six 12 x 75 mm (or similar) polypropylene tubes #1 through #6. Add 450 µL Assay Buffer 13 into tube #1 and 300 µL Assay Buffer 13 into tubes #2 through #6. Add 50 µL of 10x standard stock into tube #1 and vortex gently. Keep the 10x standard stock on ice while at the bench or store unused 10x stock at -20°C. Serially dilute 150 µL of tube #1 standard to tubes #2 through #6 by gently vortexing after each serially dilutional transfer. The diluted standards and samples should be kept on ice if a prolonged amount of bench set-up time is anticipated. Allow the TIMP-1 standards and samples to warm to room temperature before adding to the plate.



Diluted standards should be used within 30 minutes of preparation. Discard any unused standard dilutions. However, unused 10x stock may be stored at -20°C; avoid repeated freeze thaw cycles.

3. TIMP-1 Detector Antibody

Reconstitute the detector antibody in 1.1 mL of the Antibody Diluent to make a 10x stock. Dilute appropriate volume to 1x (i.e. 0.5 mL 10x stock into 4.5 mL Antibody Diluent) and **store unused 10x stock at -20°C. Avoid repeated freeze thaw cycles.**

ASSAY PROCEDURE

Bring all reagents to room temperature for at least 30 minutes prior to opening.

All standards and samples should be run in duplicate.

Add the reagents to the sides of the wells to avoid possible contamination.

Refer to the Assay Layout Sheet to determine the number of wells to be used. Remove unneeded wells and return them, with the desiccant, to the plate bag and seal. Store the unused wells at 4°C.

1. Add 100 µL of Assay Buffer 13 into NSB (non-specific binding) and standard 0 wells. Leave the Blank and TA (total activity) wells empty.
2. Add 100 µL of standards #1 through #6 into the appropriate wells.
3. Add 100 µL of the samples into the appropriate wells.
4. Seal the plate and incubate at room temperature (RT) on a plate shaker for 30 min at ~500 rpm*. **See note.**
5. Empty the contents of the wells and wash by adding a full well volume ~400 µL of 1X Wash Buffer to each well. Empty or aspirate the wells and repeat the wash 3 more times for a total of 4 washes. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
6. Add 100 µL of 1x reconstituted TIMP-1 Detector Antibody into all wells except for the NSB, TA and blank. Add 100 µL of Assay Buffer 13 to NSB wells and leave Blank and TA wells empty.
7. Seal the plate and incubate at RT on a plate shaker for 1 hour at ~500 rpm.
8. Wash as above (Step 5).
9. Add 100 µL of TIMP-1 Conjugate (blue) to each well, except the Blank and TA.
10. Seal the plate and incubate at RT on a plate shaker for 30 minutes at ~500 rpm.
11. Wash as above (Step 5).
12. Dilute TIMP-1 Conjugate 1:50 with Assay Buffer 13 and add 5 µL of this dilution to TA wells.
13. Add 100 µL TMB solution into all wells.
14. Seal the plate and incubate at RT on a plate shaker for 30 minutes at ~500rpm.
15. Add 100 µL of the Stop Solution 2 into each well.

16. After blanking the plate reader against the substrate, read optical density at 450 nm. If the plate reader is not capable of adjusting for the blank, manually subtract the mean OD of the substrate blank from all readings.

*** Note: The plate shaker speed was based on a BellCo Mini Orbital Shaker (mod no. 7744-08096). The actual speed of the plate shaker should be such that the liquid in the plate wells mixes thoroughly, but does not splash out of the well.**

Be sure to multiply sample concentrations by the dilution factor used during sample preparation.

CALCULATION OF RESULTS

Several options are available for the calculation of the concentration of TIMP-1 in the samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic (4PL) curve fitting program. The concentration of TIMP-1 can be calculated as follows:

1. Calculate the average net OD for each standard and sample by subtracting the average NSB OD from the average OD for each standard and sample.

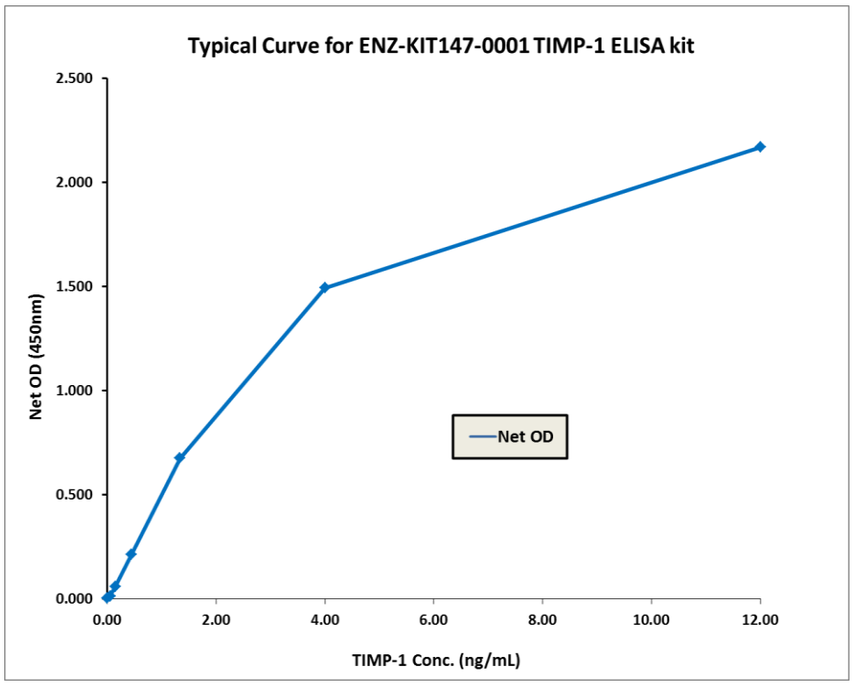
   Average Net OD = Average OD - Average NSB OD

2. Using data analysis software, plot the Average Net OD for each standard versus TIMP-1 concentration in each standard.
   Samples with concentrations outside of the standard curve range will need to be reanalyzed using alternative dilution.

TYPICAL RESULTS

The results shown below are for illustration only and should not be used to calculate results.

Sample	Mean OD	Net OD	TIMP-1 [ng/mL]
NSB	0.048	---	---
Std 0	0.052	0.004	0
Std 1	2.216	2.168	12
Std 2	1.541	1.493	4
Std 3	0.722	0.675	1.333
Std 4	0.261	0.213	0.444
Std 5	0.105	0.057	0.148
Std 6	0.061	0.013	0.049



PERFORMANCE CHARACTERISTICS

Specificity

The specificity of the assay was determined by running serial dilutions of the analytes, including the cross-reactants, in the assay, fitting the resulting dose response curve(s) to a 4PL curve-fit and determining the ED50. The ED50 of the standard curve was then divided by the determined ED50 of the cross-reactant and multiplied by 100.

Analyte	Cross reactivity
TIMP1	100%
TIMP2	Undetected
TIMP3	Undetected
TIMP4	Undetected
MMP1	Undetected
MMP2	≤0.019%
MMP3	Undetected
MMP7	Undetected
MMP8	Undetected
MMP9	Undetected
MMP13	Undetected

Sensitivity

The sensitivity or limit of detection of this assay is ≤ 30 pg/mL. The assay sensitivity was determined by interpolation at two standard deviations above the net OD of 20 zero standard replicates utilizing a four parameter logistic (4PL) curve fit.

Intra-assay precision was determined by analyzing 20 replicates of three matrix controls containing TIMP-1 in a single assay.

Intra-assay precision	
[ng/mL]	**%CV**
7.99	9.86
2.43	10.49
0.46	5.81

Inter-assay precision was determined by measuring matrix controls containing TIMP-1 in multiple assays over several days.

Inter-assay precision	
[ng/mL]	**%CV**
7.79	13.16
2.35	11.69
0.45	9.06

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NOTES

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