



TIMP-4 (Human) ELISA Kit

06/20

(Catalog # E4843-100; 96 assays, Storage at 4°C)

I. Introduction:

TIMP-4 belongs to the TIMP gene family. It complexes with metalloproteinases (such as collagenases) and irreversibly inactivates them by binding to their catalytic zinc cofactor. TIMP-4 is known to act on MMP-1, MMP-2, MMP-3, MMP-7 and MMP-9. BioVision's TIMP-4 (Human) ELISA Kit is based on the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human TIMP-4. Standards or samples are added to the micro ELISA plate wells that bind to the specific antibody. Then a biotinylated detection antibody specific for Human TIMP-4 and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. The wells are washed, a TMB substrate solution is added to the wells and blue color develops in proportion to the amount of TIMP-4 bound. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of Human TIMP-4. The concentration of Human TIMP-4 in the samples can be calculated by comparing the OD of the samples to the standard curve.

II. Applications:

in vitro quantitative determination of human TIMP-4 concentrations in serum, plasma and other biological fluids.

Sensitivity: 46.88 pg/mL

Detection Range: 78.13-5000 pg/mL

Specificity: No significant crossreactivity or interference between Human TIMP-4 and analogues was observed.

Precision: Coefficient of variation is < 10%.

III. Sample Type:

Serum, plasma and other biological fluids

IV. Kit Contents:

Components	E4843-100	Part Number	Storage
Micro ELISA Plate	8 wells x12 strips	E4843-100-1	-20°C
Reference Standard	2 vials	E4843-100-2	-20°C
Biotinylated Detection Ab (100x)	120 µl	E4843-100-3	-20°C
HRP Conjugate (100x)	120 µl	E4843-100-4	-20°C (protect from light)
Reference Standard & Sample Diluent	20 ml	E4843-100-5	4°C
Biotinylated Detection Antibody Diluent	14 ml	E4843-100-6	4°C
HRP Conjugate Diluent	14 ml	E4843-100-7	4°C
Wash Buffer (25X)	30 ml	E4843-100-8	4°C
Substrate Reagent	10 ml	E4843-100-9	4°C (protect from light)
Stop Solution	10 ml	E4843-100-10	4°C
Plate Sealer	4	E4843-100-11	4°C

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- Clean Eppendorf tubes for preparing standards or sample dilutions

VI. Storage and Handling:

An unopened kit can be stored at 2-8°C for 1 month. If the kit is not used within 1 month, store the items separately according to the above mentioned conditions once the kit is received.

VII. Reagent and Sample Preparation:

Bring all reagents to room temperature before use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.

- **Wash Buffer (25X):** Dilute 30 ml of Concentrated Wash Buffer with 720 ml of deionized or distilled water to prepare 750 ml of Wash Buffer. (Note: if crystals have formed in the concentrate, warm it in a 40°C water bath and mix it gently until the crystals have completely dissolved)
- **Biotinylated Detection Antibody working solution:** Calculate the required amount (100 µL/well). Centrifuge the stock tube before use; dilute the 100x Concentrated Biotinylated Detection Antibody to 1x working solution with Biotinylated Detection Antibody Diluent.
- **HRP Conjugate working solution:** Calculate the required amount before the experiment (100 µL/well). In preparation, slightly more than calculated should be prepared. Dilute the 100x Concentrated HRP Conjugate to 1x working solution with Concentrated HRP Conjugate Diluent.



X. Calculation:

Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Plot a four-parameter logistic curve on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis.

If the samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample surpasses the upper limit of the standard curve, you should re-test it with an appropriate dilution. The actual concentration is the calculated concentration multiplied by the dilution factor.

Typical standard curve and data is provided below for reference only. A standard curve must be run with each assay.

Concentration(pg/mL)	5000	2500	1250	625	312.5	156.25	78.13	0
OD	2.551	1.678	0.979	0.49	0.274	0.194	0.14	0.085
Corrected OD	2.466	1.593	0.894	0.405	0.189	0.109	0.055	-

XI. Precision:

Intra-assay Precision (Precision within an assay): 3 samples with low, mid-range and high level Human TIMP-4 were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, mid-range and high level Human TIMP-4 were tested on 3 different plates, 20 replicates in each plate.

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	262.70	728.80	2324.30	269.70	684.90	2391.60
Standard deviation	14.70	32.10	102.30	15.10	29.50	90.90
C V (%)	5.60	4.40	4.40	5.60	4.31	3.80

XII. Recovery:

The recovery of Human TIMP-4 spiked at three different levels in samples throughout the range of the assay was evaluated in various matrices.

Sample Type	Range (%)	Average Recovery (%)
Serum (n=5)	85-96	91
EDTA plasma (n=5)	93-110	101
Cell culture media (n=5)	91-102	97

XIII. Linearity:

Samples were spiked with high concentrations of Human TIMP-4 and diluted with Reference Standard & Sample Diluent to produce samples with values within the range of the assay.

		Serum (n=5)	EDTA plasma (n=5)	Cell culture media (n=5)
1:2	Range (%)	89-102	97-114	84-97
	Average (%)	95	104	90
1:4	Range (%)	96-111	81-92	93-104
	Average (%)	104	87	99
1:8	Range (%)	98-113	84-94	96-111
	Average (%)	105	89	103
1:16	Range (%)	94-110	85-98	96-109
	Average (%)	101	91	102

XIV. RELATED PRODUCTS:

- TIMP-2 (Human) ELISA Kit (E4841)
- TIMP-3 (Human) ELISA Kit (E4842)
- TIMP-1 (Human) ELISA Kit (E4840)

FOR RESEARCH USE ONLY! Not to be used on humans.