TIMP-1 (Mouse) ELISA Kit (For Lysates)

(Catalog # MBS843401, 100 assays; Store at -20°C) *rev 1/14*

Introduction:

Mouse TIMP-1 ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of mouse TIMP-1. This assay employs an antibody specific for mouse TIMP-1 coated on a 96-well plate. Standards and samples are pipetted into the wells and TIMP-1 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-mouse TIMP-1 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of TIMP-1 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm. The minimum detectable dose of TIMP-1 is typically less than 3 pg/ml. The intra-Assay reproducibility is CV<10% & inter-Assay is CV<12%. This ELISA kit shows no cross-reactivity with any of the cytokines tested e.g., Mouse CD30, L CD30, T CD40, CRG-2, CTACK, CXCL16, Eotaxin, Eotaxin-2, Fas Ligand, Fractalkine, GCSF, GM-CFS, IFN-γ, IGFBP-3, IGFBP-5, IGFBP-6, IL-1 α, IL-1β, IL-2, IL-3, IL-3 Rb, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12 p40/p70, IL-12 p70, IL-13, IL-17, KC, Leptin R, Leptin (OB), LIX, L-Selectin, Lymphotactin, MCP-1, MCP-5, M-CSF, MIG, MIP-1α, MIP-1γ, MIP-2, MIP-3β, MIP-3α, PF-4, P-Selectin, RANTES, SCF, SDF-1α, TARC, TCA-3, TECK, TNF-α, TNF RI, TNF RII, TPO, VCAM-1, VEGF).

II. Application:

Quantitative protein detection, establishing normal range, validation of antibody array results.

III. Specificity:

The antibody pair provided in this kit recognizes mouse TIMP-1.

IV. Sample Type:

- Cell lysates
- Tissue lysates

V. Kit Contents:

Components	MBS843401	Part No.
TIMP-1 Microplate (Item A) coated with anti- mouse TIMP-1, 96 wells	12 stripsx8 wells	MBS843401-1
Wash Buffer Concentrate (20x) (Item B)	25 ml	MBS843401-2
Standards (Item C) (recombinant mouse TIMP-1)	2 vials	MBS843401-3
Sample Diluent Buffer (Item D) for Standard/Sample (cell lysate/tissue lysate) diluent (5x concentrated)	10 ml	MBS843401-4
Assay Diluent (Item E) for Detection Antibody & HRP-Streptavidin concentrate diluent (5x concentrated)	15 ml	MBS843401-5
Detection Antibody TIMP-1 (Item F), biotinylated anti- mouse TIMP-1 (each vial enough for half plate)	2 vial	MBS843401-6
HRP-Streptavidin Concentrate (Item G), 120x concentrated	200 µl	MBS843401-7
TMB One-Step Substrate Reagent (Item H) 3,3',5,5'-tetramethylbenzidine (TMB) in buffer solution	12 ml	MBS843401-8
Stop Solution (Item I), 0.2 M sulfuric acid	8 ml	MBS843401-9
Cell Lysate buffer (Item J) (2x concentrated)	5 ml	MBS843401-10

VI. User Supplied Reagents and Equipment:

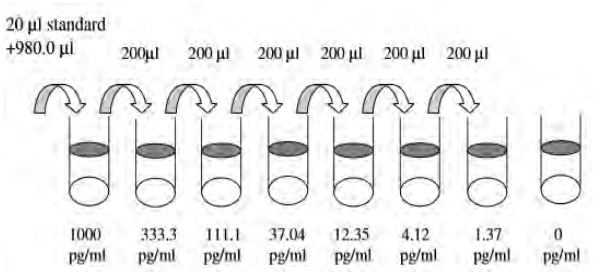
- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper.
- Distilled or deionized water.

VII. Storage and Handling:

May be stored for up to 6 months at 2° to 8°C from the date of shipment. Standard (recombinant protein) should be stored at -20°C or -80°C (recommended at –80°C) after reconstitution. Opened Microplate Wells or reagents may be stored for up to 1 month at 2° to 8°C. Return unused wells to the pouch containing desiccant pack, reseal along entire edge. **Note:** the kit can be used within one year if the whole kit is stored at -20°C. Avoid repeated freeze-thaw cycles.

VIII. Reagent Preparation:

- Bring all reagents and samples to room temperature (18 - 25°C) before use.
- Sample dilution: Tissue & cell lysate sample should be diluted at least 5-fold with 1X sample dilution buffer.
- Sample Diluent Buffer (Item D) and Assay Diluent (Item E) should be diluted 5-fold with deionized or distilled water.
- Preparation of standard: Briefly spin the vial of Item C then add 400 µl 1X Sample Diluent Buffer (Item D) into Item C vial to prepare a 50 ng/ml standard. Dissolve the powder thoroughly by a gentle mix. Add 20 µl TIMP-1 standard from the vial of Item C, into a tube with 980 µl Sample Diluent Buffer to prepare a 1,000 pg/ml stock standard solution. Pipette 400 µl 1X Sample Diluent Buffer into each tube. Use the stock standard solution to produce a dilution series (shown). Mix each tube thoroughly before the next transfer. 1x Sample Diluent Buffer serves as the zero standard (0 pg/ml).



- If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer.
- Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µl of 1x Assay Diluent into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Assay Diluent and used in step 4 of Assay Protocol.

MyBioSource.com

- Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 120-fold with 1x Assay Diluent. Mix well
- Cell lysate buffer should be diluted 2-fold with deionized or distilled water (for cell lysate and tissue lysate).

IX. Assay Protocol:

1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Add 100 µl of each standard and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or overnight at 4°C with gentle shaking.
   Note: We recommend using 50-500 µg/ml of total protein for lysate sample. The amount of sample used depends on the abundance of target protein. More of the sample can be used if signals are too weak. If signals are too strong, the sample can be diluted further.
3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel Pipette 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.
4. Add 100 µl of 1x prepared biotinylated antibody (see Reagent Preparation) to each well. Incubate for 1 hour at room temperature with gentle shaking. Discard the solution. Repeat the wash as in step 3.
5. Add 100 µl of prepared Streptavidin solution (see Reagent Preparation) to each well. Incubate for 45 minutes at room temperature with gentle shaking. Discard the solution. Repeat the wash as in step 3.
6. Add 100 µl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
7. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.
8. Calculation**:** Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

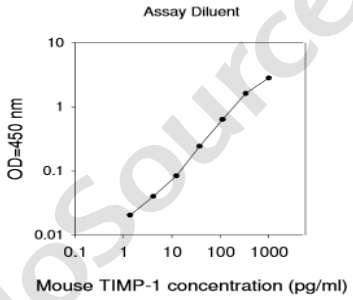


Figure 1: Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

Sample Type	Average % Recovery	Range (%)
Tissue lysate	89.53	79-102
Cell lysate	87.65	78-103

Table 1: Recovery: Recovery was determined by spiking various levels of mouse TIMP-1 into mouse tissue & cell lysate.

Sample Type		Tissue Lysate	Cell lysate
1:2	Average % of Expected Range (%)	92 82-101	89 83-102
1:4	Average % of Expected Range (%)	93 83-103	82 83-102

Table 2: Linearity

X. RELATED PRODUCTS:

TIMP-1 (Mouse) ELISA Kit
TIMP-1, human recombinant
MMP-1 Inhibitor Screening Kit
MMP-1, human recombinant
MMP-3 Antibody
MMP-3 Activity Fluorometric Assay Kit
MMP-11 Antibody
MMP-12 Antibody
TIMP-1, human recombinant
TIMP-2, human recombinant
MMP-12 Antibody
MMP-1 Antibody
MMP-3 Blocking Peptide
MMP-3 Inhibitor Screening Kit
MMP-11 Blocking Peptide
MMP-12 Blocking Peptide

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

MyBioSource.com