RayBio®
Mouse TIMP-1 ELISA Kit

User Manual (for Cell Lysate and Tissue Lysate)
(Revised Mar 1, 2012)

RayBio® Mouse TIMP-1 ELISA Kit Protocol

(Cat#: ELM-TIMP1-001C)

RayBiotech, Inc.

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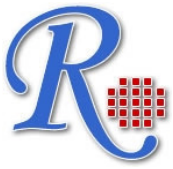

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I. INTRODUCTION

The RayBio® 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 cell lysate and tissue lysate. 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.

II. REAGENTS

1. TIMP-1 Microplate (Item A): 96 wells (12 strips x 8 wells) coated with anti-mouse TIMP-1.
2. Wash Buffer Concentrate (20x) (Item B): 25 ml of 20x concentrated solution.
3. Standards (Item C): 2 vials, recombinant mouse TIMP-1.
4. Sample Diluent Buffer (Item D): 10 ml of 5x concentrated buffer. For Standard/Sample (cell lysate/tissue lysate) diluent.
5. Assay Diluent (Item E): 15 ml of 5x concentrated buffer. For Detection Antibody (Item F) and HRP-Streptavidin concentrate (Item G) diluent.
6. Detection Antibody TIMP-1 (Item F): 2 vial of biotinylated anti-mouse TIMP-1 (each vial is enough to assay half microplate).
7. HRP-Streptavidin concentrate (Item G): 200 µl of 120x concentrated HRP-conjugated streptavidin.
8. TMB One-Step Substrate Reagent (Item H): 12 ml of 3,3',5,5'-tetramethylbenzidine (TMB) in buffered solution.

9. Stop Solution (Item I): 8 ml of 0.2 M sulfuric acid.
10. Cell lysate buffer (Item J): 5 ml 2x cell lysate buffer.

III. STORAGE

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 store 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.

IV. ADDITIONAL MATERIALS REQUIRED

1 Microplate reader capable of measuring absorbance at 450 nm.
2 Precision pipettes to deliver 2 µl to 1 ml volumes.
3 Adjustable 1-25 ml pipettes for reagent preparation.
4 100 ml and 1 liter graduated cylinders.
5 Absorbent paper.
6 Distilled or deionized water.
7 Log-log graph paper or computer and software for ELISA data analysis.
8 Tubes to prepare standard or sample dilutions.

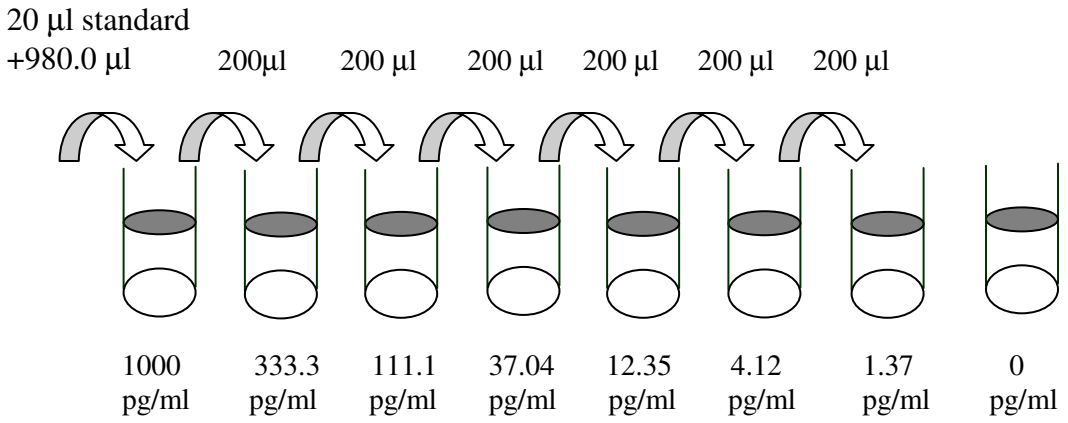
V. REAGENT PREPARATION

1. Bring all reagents and samples to room temperature (18 - 25°C) before use.

2. Sample dilution: Tissue lysate and cell lysate sample should be diluted at least 5-fold with 1x Sample Diluent Buffer.

3. Sample Diluent Buffer (Item D) and Assay Diluent (Item E) should be diluted 5-fold with deionized or distilled water before use.

4. Preparation of standard: **Briefly spin the vial of Item C**. 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 below). Mix each tube thoroughly before the next transfer. 1x Sample Diluent Buffer serves as the zero standard (0 pg/ml).



5. 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.

6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µl of 1x Assay Diuent 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 Diuent and used in step 4 of Part VI Assay Procedure.

7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) before use. HRP-Streptavidin Concentrate should be diluted 120-fold with 1x Assay Diuent.

   For example: Briefly spin the vial (Item G) and pipette up and down to mix gently . Add 100 µl of HRP-Streptavidin concentrate into a tube with 12 ml 1x Assay Diluent to prepare a final 120 fold diluted HRP-Streptavidin solution. Mix well.

8. Cell lysate buffer should be diluted 2-fold with deionized or distilled water (for cell lysate and tissue lysate).

VI. ASSAY PROCEDURE:

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 (see Reagent Preparation step 2) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4°C with gentle shaking.

   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 (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.

5. Discard the solution. Repeat the wash as in step 3.

6. Add 100 µl of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.

7. Discard the solution. Repeat the wash as in step 3.

8. 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.

9. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.

VII. ASSAY PROCEDURE SUMMARY

1. Prepare all reagents, samples and standards as instructed.

⇩

2. Add 100 µl standard or sample to each well.
   Incubate 2.5 hours at room temperature or over night at 4°C.

⇩

3. Add 100 µl prepared biotin antibody to each well.
   Incubate 1 hour at room temperature.

⇩

4. Add 100 µl prepared Streptavidin solution.
   Incubate 45 minutes at room temperature.

⇩

5. Add 100 µl TMB One-Step Substrate Reagent to each well.
   Incubate 30 minutes at room temperature.

⇩

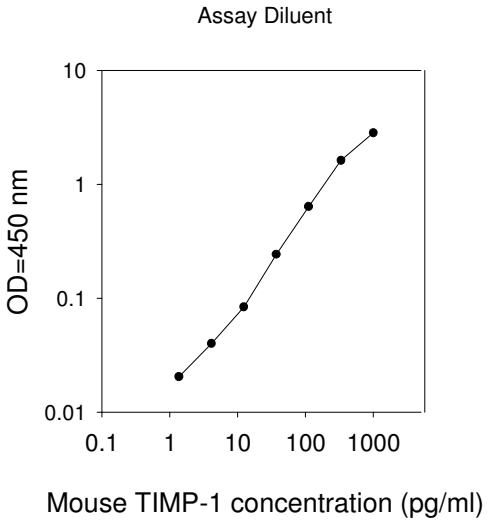
6. Add 50 µl Stop Solution to each well.
   Read at 450 nm immediately.

VIII. CALCULATION OF RESULTS

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.

A. TYPICAL DATA

These standard curves are for demonstration only. A standard curve must be run with each assay.



B. SENSITIVITY

The minimum detectable dose of TIMP-1 is typically less than 3 pg/ml.

C. RECOVERY

Recovery was determined by spiking various levels of mouse TIMP-1 into mouse tissue lysate and cell lysate. Mean recoveries are as follows:

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

D. LINEARITY

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

E. REPRODUCIBILITY

Intra-Assay: CV<10%
Inter-Assay: CV<12%

IX. SPECIFICITY

Cross Reactivity: 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).

X. TROUBLESHOOTING GUIDE

Problem	Cause	Solution
1. Poor standard curve	1. Inaccurate pipetting	1. Check pipettes
	2. Improper standard dilution	2. Ensure briefly spin the vial of Item C and dissolve the powder thoroughly by a gentle mix.
2. Low signal	1.Too brief incubation times	1. Ensure sufficient incubation time; assay procedure step 2 change to over night
	2. Inadequate reagent volumes or improper dilution	2. Check pipettes and ensure correct preparation
3. Large CV	1. Inaccurate pipetting	1. Check pipettes
4. High background	1. Plate is insufficiently washed	1. Review the manual for proper wash. If using an a plate washer, check that all ports are unobstructed.
	2. Contaminated wash buffer	2. Make fresh wash buffer
5. Low sensitivity	1. Improper storage of the ELISA kit	1. Store your standard at<-20^{o}C after reconstitution, others at 4 ^{o}C. Keep substrate solution protected from light
	2. Stop solution	2. Stop solution should be added to each well before measure

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