Timp1 (Rat) ELISA Kit

Catalog Number KA0437

96 assays

Version: 08

Intended for research use only

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Introduction

Intended Use

For the quantitation of rat Timp1 concentrations in cell culture supernates, cell lysates, serum and plasma (heparin, EDTA).

Background

The tissue inhibitor of metalloproteinases 1 (TIMP1) is also called erythroid-potentiating activity (EPA). The X-linked gene for human TIMP1 is expressed in some but not all inactive X-containing somatic-cell hybrids, suggesting that this gene is either prone to reactivation or variable in its inactivation. Purified EPA specifically stimulates human and murine cells of the erythroid lineage, unlike murine interleukin-3 (IL-3) which stimulates precursor cells from all haematopoietic lineages. TIMP1 is thought to play a regulatory role in connective tissues by forming inactive complexes with those metalloproteinases that are normally responsible for connective tissue turnover. The human gene encoding TIMP has been mapped to the X chromosome in the region Xp11.1-p11.4. The standard product used in this kit is recombinant rat TIMP-1, consisting of 194 amino acids with the molecular mass of 21.5 KDa. As a result of glycosylation, the molecular mass is 32-34 KDa.

Principle of the Assay

The Timp1 (Rat) ELISA Kit is a solid phase immunoassay specially designed to measure Rat Timp1 with a 96-well strip plate that is pre-coated with antibody specific for Timp1. The detection antibody is a biotinylated antibody specific for Timp1. The capture antibody is monoclonal antibody from mouse, the detection antibody is polyclonal antibody from goat. The kit contains recombinant Rat Timp1 with immunogen: Expression system for standard: NSO; Immunogen sequence: C24-A217. The kit is analytically validated with ready to use reagents.

To measure Rat Timp1, add standards and samples to the wells, then add the biotinylated detection antibody. Wash the wells with PBS or TBS buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbounded ABC-HRP with PBS or TBS buffer and add TMB. TMB is substrate to HRP and will be catalyzed to produce a blue color product, which changes into yellow after adding acidic stop solution. The density of the yellow product is linearly proportional to Rat Timp1 in the sample. Read the density of the yellow product in each well using a plate reader, and benchmark the sample wells' readings against the standard curve to determine the concentration of Rat Timp1 in the sample.

General Information

Materials Supplied

List of component

Component	Amount
Anti-Rat Timp1 Pre-coated 96-well strip microplate	96 (8x12) wells
Rat Timp1 Standard	10 ng/tube x 2
Rat Timp1 Biotinylated antibody (100x)	130 µL
Avidin-Biotin-Peroxidase Complex (100x)	130 µL
Sample Diluent	30 mL
Antibody Diluent	12 mL
Avidin-Biotin-Peroxidase Diluent	12 mL
Color Developing Reagent (TMB)	10 mL
Stop Solution	10 mL
Wash Buffer Powder	1 pack
Plate Sealers	4 slides

Storage Instruction

Store at 4 °C for 6 months, at -20 °C for 12 months. Avoid multiple freeze-thaw cycles.

Materials Required but Not Supplied

- ✓ Microplate Reader capable of reading absorbance at 450 nm.
- ✓ Automated plate washer (optional).
- ✓ Pipettes and pipette tips capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.
- ✓ Multichannel pipettes are recommended for large amount of samples.
- ✓ Deionized or distilled water.
- ✓ 500 mL graduated cylinders.
- ✓ Test tubes for dilution.

Precautions for Use

This protocol must be read in its entirety before using this product. For research use only. Not for use in diagnostic procedures.

✓ Notice Before Application

Please read the following instructions before starting the experiment.

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
3. Don’t let 96-well plate dry, for dry plate will inactivate active components on plate.
4. Don’t reuse tips and tubes to avoid cross contamination.
5. Avoid using the reagents from different batches together.

Assay Protocol

Reagent Preparation

✓ Bring all reagents to 37°C prior to use. The assay can also be done at room temperature however we recommend doing it at 37°C for best consistency with our QC results. Also the TMB incubation time estimate (25-30 min) is based on 37°C.

- Wash buffer

  Dissolve the included powder in 1000 mL of deionized water. Excess wash buffer can be stored for up to one week at 4°C.

- Biotinylated Anti-Rat Timp1 antibody

  It is recommended to prepare this reagent immediately prior to use by diluting the Rat Timp1 Biotinylated antibody (100x) 1:100 with Antibody Diluent. Prepare 100 µL by adding 1 µL of Biotinylated antibody (100x) to 99 µL of Antibody Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.

- Avidin-Biotin-Peroxidase Complex

  It is recommended to prepare this reagent immediately prior to use by diluting the Avidin-Biotin-Peroxidase Complex (100x) 1:100 with Avidin-Biotin-Peroxidase Diluent. Prepare 100 µL by adding 1 µL of Avidin-Biotin-Peroxidase Complex (100x) to 99 µL of Avidin-Biotin-Peroxidase Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.

- Rat Timp1 Standard

  It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. Use one 10 ng of lyophilized Rat Timp1 standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 10 ng/mL using 1 mL of sample diluent. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions.

- Microplate

  The included microplate is coated with capture antibodies and ready-to-use. It does not require additional washing or blocking. The unused well strips should be sealed and stored in the original packaging.

✓ Dilution of Rat Timp1 Standard

1. Number tubes 1-8. Final Concentrations to be Tube # 1 –2000 pg/mL, #2 –1000 pg/mL, #3 – 500 pg/mL, #4 – 250 pg/mL, #5 – 125 pg/mL, #6 – 62.5 pg/mL, #7 – 31.25 pg/mL, #8 –0.0 (Blank).
2. To generate standard #1, add 200 µL of the reconstituted standard stock solution of 10 ng/mL and 800 µL of sample diluent to tube #1 for a final volume of 1000 µL. Mix thoroughly.
3. Add 300 µL of sample diluent to tubes # 2-7.
4. To generate standard #2, add 300 µL of standard #1 from tube #1 to tube #2 for a final volume of 600 µL. Mix thoroughly.
5. To generate standard #3, add 300 µL of standard #2 from tube #2 to tube #3 for a final volume of 600

µL. Mix thoroughly.

6. Continue the serial dilution for tube #4-7.
7. Tube #8 is a blank standard to be used with every experiment.

Sample Preparation

✓ Sample Preparation and Storage

These sample collection instructions and storage conditions are intended as a general guideline and the sample stability has not been evaluated.

- Cell culture supernatants: Clear sample of particulates by centrifugation, assay immediately or store samples at -20°C.
- Serum: Use a serum separator tube (SST) and allow serum to clot at room temperature for about four hours. Then, centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C.
- Plasma: Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 min at approximately 1,000 x g. Assay immediately or store samples at -20°C.

  **Note: it is important to not use anticoagulants other than the ones described above to treat plasma for other anticoagulants could block the antibody binding site.*
- Cell lysates: Lyse the cells, make sure there are no visible cell sediments. Centrifuge cell lysates at approximately 10000 X g for 5 min. Collect the supernatant..

✓ Sample Dilution Guideline

The target protein concentration should be estimated and appropriate sample dilutions should be selected such that the final protein concentration lies near the middle of the linear dynamic range of the assay.

It is recommended to prepare 150 µL of sample for each replicate to be assayed. The samples should be diluted with sample diluent and mixed gently.

Assay Procedure

It is recommended that all reagents and materials be equilibrated to 37°C/room temperature prior to the experiment (see Reagent Preparation before the experiment if you have missed this information).

1. Prepare all reagents and working standards as directed previously.
2. Remove excess microplate strips from the plate frame and seal and store them in the original packaging.
3. Add 100 µL of the standard, samples, or control per well. Add 100 µL of the sample diluent buffer into the control well (zero well). At least two replicates of each standard, sample, or control is recommended.
4. Cover with the plate sealer provided and incubate for 120 minutes at RT (or 90 minutes at 37°C).
5. Remove the cover and discard the liquid in the wells into an appropriate waste receptacle. Invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is

recommended that the wells are not allowed to completely dry at any time.

6. Add 100 µL of the prepared 1x Biotinylated Anti-Rat Timp1 antibody to each well.
7. Cover with plate sealer and incubate for 90 minutes at RT (or 60 minutes at 37°C).
8. Wash the plate 3 times with the 1x wash buffer.

   a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.

   b. Add 300 µL of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).

   c. Repeat steps a-b 2 additional times.
9. Add 100 µL of the prepared 1x Avidin-Biotin-Peroxidase Complex into each well. Cover with the plate sealer provided and incubate for 40 minutes at RT (or 30 minutes at 37°C).
10. Wash the plate 5 times with the 1x wash buffer.

    a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.

    b. Add 300 µL of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).

    c. Repeat steps a-b 4 additional times.
11. Add 90 µL of Color Developing Reagent to each well and incubate in the dark for 30 minutes at RT (or 25-30 minutes at 37°C). (The optimal incubation time must be empirically determined. A guideline to look for is blue shading the top four standard wells, while the remaining standards remain clear.)
12. Add 100 µL of Stop Solution to each well. The color should immediately change to yellow.
13. Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450 nm

Data Analysis

Calculation of Results

Average the duplicate readings for each standard, sample, and control. Subtract the average zero standard O.D. reading.

It is recommended that a standard curve be created using computer software to generate a four parameter logistic (4-PL) curve-fit.

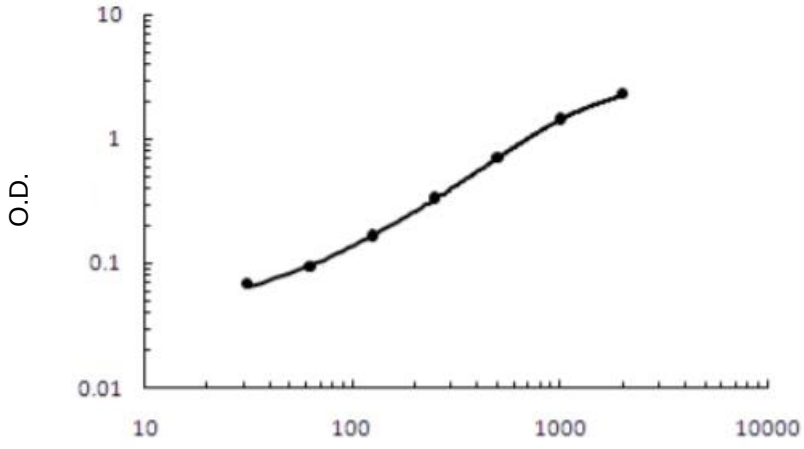
Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative OD against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data.

Note: For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor.

✓ The Timp1 (Rat) ELISA Kit Standard Curve example

Highest O.D. value might be higher or lower than in the example. The experiment result is statistically significant if the highest O.D. value is no less than 1.0.

Concentration(pg/mL)	0	31.2	62.5	125	250	500	1000	2000
O.D	0.045	0.068	0.094	0.165	0.333	0.706	1.437	2.283



Concentration (pg/mL)

A standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Performance Characteristics

✓ Detection Range: 31.2 pg/mL-2000 pg/mL

✓ Sensitivity: < 3 pg/mL

* The sensitivity or the minimum detectable dose (MDD) is the lower limit of target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration.

✓ Specificity: Natural and recombinant Rat Timp1

✓ Cross-reactivity: There is no detectable cross-reactivity with other relevant proteins.

✓ Intra/Inter Assay Variability

- Intra-Assay Precision (Precision within an assay)

  Three samples of known concentration were tested on one plate to assess intra-assay precision.

- Inter-Assay Precision (Precision across assays)

  Three samples of known concentration were tested in separate assays to assess inter-assay precision.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	16	16	16	24	24	24
Mean (pg/mL)	59	252	889	61	252	895
Standard Deviation	4.24	13.1	45.33	5	13.35	46.54
CV (%)	7.2%	5.2%	5.2%	8.2%	5.3%	5.2%

✓ Reproducibility

To assay reproducibility, three samples with differing target protein concentrations were assayed using four different lots.

Lots	Lot1 (pg/mL)	Lot2 (pg/mL)	Lot3 (pg/mL)	Lot4 (pg/mL)	Mean (pg/mL)	Standard Deviation	CV (%)
Sample 1	59	60	56	65	60	3.24	5.4%
Sample 2	252	245	247	261	251	6.17	2.4%
Sample 3	889	980	917	861	911	44.09	4.8%

*number of samples for each test n=16.

Resources

Plate Layout

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H