TIMP2 (Human) ELISA Kit

Catalog Number KA0549

96 assays

Version: 07

Intended for research use only

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Introduction

Intended Use

For quantitative detection of human TIMP-2 in cell culture supernatants, serum, plasma (heparin, EDTA) and saliva.

Background

TIMP2 gene is encoded by 5 exons spanning 83 kb of genomic DNA. TIMP2 is 83 kilobase pairs (kb) long with exon-intron splicing sites located in preserved positions among the three members of the TIMP family. The gene for tissue inhibitor of metalloproteinases-2 is localized on human chromosome arm 17q25. TIMP-2 abrogates angiogenic factor-induced endothelial cell proliferation in vitro and angiogenesis in vivo independent of MMP inhibition. The standard product used in this kit is recombinant human TIMP-2 with the molecular mass of 22Kda and 194 amino acid.

Principle of the Assay

TIMP2 (Human) ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. A monoclonal antibody from mouse specific for TIMP-2 has been precoated onto 96-well plates. Standards (CHO, C27-P220) and test samples are added to the wells, a biotinylated detection polyclonal antibody from goat specific for TIMP-2 is added subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the human TIMP-2 amount of sample captured in plate.

General Information

Materials Supplied

List of component

Component	Amount
One 96-well plate precoated with anti- human TIMP-2 antibody	96 (8 x 12) wells
Lyophilized recombinant human TIMP-2 standard	10 ng/tube x 2
Biotinylated anti- human TIMP-2 antibody, dilution 1:100	130 µL
Avidin-Biotin-Peroxidase Complex (ABC), dilution 1:100	130 µL
Sample diluent buffer	30 mL
Antibody diluent buffer	12 mL
ABC diluent buffer	12 mL
TMB color developing agent	10 mL
TMB stop solution	10 mL

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 in standard size.
- ✓ Automated plate washer.
- ✓ Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.
- ✓ Clean tubes and Eppendorf tubes.
- ✓ Washing buffer (neutral PBS or TBS).
- Preparation of 0.01M TBS:
  Add 1.2 g Tris, 8.5 g NaCl; 450 µL of purified acetic acid or 700 µL of concentrated hydrochloric acid to 1000 mL H_2O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1 L.
- Preparation of 0.01 M PBS:
  Add 8.5 g sodium chloride, 1.4 g Na_2HPO_4 and 0.2 g NaH_2PO_4 to 1000 mL distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1 L.

Precautions for Use

- ✓ 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.
- ✓ The TMB Color Developing agent is colorless and transparent before using, contact us freely if it is not the case.
- ✓ Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
- ✓ Duplicate well assay is recommended for both standard and sample testing.
- ✓ Don't let 96-well plate dry, for dry plate will inactivate active components on plate.
- ✓ Don't reuse tips and tubes to avoid cross contamination.
- ✓ To avoid to use the reagents from different batches together.
- ✓ In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution will be pre-warmed in 37°C for 30 min before using.

Assay Protocol

Reagent Preparation

- ✓ Reconstitution of the human TIMP-2 standard: TIMP-2 standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of TIMP-2 standard (10 ng per tube) are included in each kit. Use one tube for each experiment.
- 10,000 pg/mL of human TIMP-2 standard solution: Add 1 mL sample diluent buffer into one tube, keep the tube at room temperature for 10 min and mix thoroughly.
- 5000 pg/mL → 156 pg/mL of human TIMP-2 standard solutions: Label 6 Eppendorf tubes with 5000 pg/mL, 2500 pg/mL, 1250 pg/mL, 625 pg/mL, 312 pg/mL, 156 pg/mL, respectively. Aliquot 0.3 mL of the sample diluent buffer into each tube. Add 0.3 mL of the above 10,000 pg/mL TIMP-2 standard solution into 1st tube and mix. Transfer 0.3 mL from 1st tube to 2nd tube and mix. Transfer 0.3 mL from 2nd tube to 3rd tube and mix, and so on.
  Note: The standard solutions are best used within 2 hours. The 10 ng/mL standard solution may be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

- ✓ Preparation of biotinylated anti-human TIMP-2 antibody working solution: The solution should be prepared no more than 2 hours prior to the experiment.
- The total volume should be: 0.1 mL/well x (the number of wells). (Allowing 0.1-0.2 mL more than total volume)
- Biotinylated anti- human TIMP-2 antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly. (i.e. Add 1 µL Biotinylated anti-human TIMP-2 antibody to 99 µL antibody diluent buffer.)

- ✓ Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution: The solution should be prepared no more than 1 hour prior to the experiment.
- The total volume should be: 0.1 mL/well x (the number of wells). (Allowing 0.1-0.2 mL more than total volume)
- Avidin- Biotin-Peroxidase Complex (ABC) should be diluted in 1:100 with the ABC dilution buffer and mixed thoroughly. (i.e. Add 1 µL ABC to 99 µL ABC diluent buffer.)

Sample Preparation

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

- Cell culture supernate: Remove particulates by centrifugation, analyze immediately or aliquot and store at -20°C.
- Serum: Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1000 X g for 15 min. Analyze the serum immediately or aliquot and store

frozen at -20°C.

- Plasma: Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 min at 1000 x g within 30 min of collection. Analyze immediately or aliquot and store frozen at -20°C. Citrate is not recommended as the anticoagulant.
- Saliva: Collect saliva using a collection device without any protein binding or filtering capabilities such as a Salivette or aliquot and store samples at -20°C.

✓ Sample Dilution Guideline

The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve. Dilute the sample using the provided diluent buffer. The following is a guideline for sample dilution. Several trials may be necessary in practice. The sample must be well mixed with the diluents buffer.

- High target protein concentration (100-1000 ng/mL). The working dilution is 1:100. i.e. Add 1 µL sample into 99 µL sample diluent buffer.
- Medium target protein concentration (10-100 ng/mL). The working dilution is 1:10. i.e. Add 10 µL sample into 90 µL sample diluent buffer.
- Low target protein concentration (156-10,000 pg/mL). The working dilution is 1:2. i.e. Add 50 µL sample to 50 µL sample diluent buffer.
- Very Low target protein concentration (≤156 pg/mL). No dilution necessary, or the working dilution is 1:2.

Assay Procedure

The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 min before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard TIMP-2 detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of TIMP-2 amount in samples.

1. Aliquot 0.1 mL per well of the 10,000 pg/mL, 5000 pg/mL, 2500 pg/mL, 1250 pg/mL, 625 pg/mL, 312 pg/mL, 156 pg/mL human TIMP-2 standard solutions into the pre-coated 96-well plate. Add 0.1 mL of the sample diluent buffer into the control well (Zero well). Add 0.1 mL of each properly diluted sample of human cell culture supernates, serum, plasma (heparin, EDTA) or saliva to each empty well. See “Sample Dilution Guideline” above for details. It is recommend that each human TIMP-2 standard solution and each sample is measured in duplicate.
2. Seal the plate with the cover and incubate at 37°C for 90 min.
3. Remove the cover, discard plate content, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.
4. Add 0.1 mL of biotinylated anti-human TIMP-2 antibody working solution into each well and incubate the plate at 37°C for 60 min.
5. Wash the plate 3 times with 0.01 M TBS or 0.01 M PBS, and each time let washing buffer stay in the wells for 1 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent

material. (Plate Washing Method: Discard the solution in the plate without touching the side walls. Blot the plat onto paper towels or other absorbent material. Soak each well with at least 0.3 mL PBS or TBS buffer for 1~2 minutes. Repeat this process two additional times for a total of THREE washes. *Note: For automated washing, aspirate all wells and wash THREE times with PBS or TBS buffer, overfilling wells with PBS or TBS buffer. Blot the plate onto paper towels or other absorbent material.)*

6. Add 0.1 mL of prepared ABC working solution into each well and incubate the plate at 37°C for 30 min.
7. Wash plate 5 times with 0.01 M TBS or 0.01 M PBS, and each time let washing buffer stay in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (See Step 5 for plate washing method).
8. Add 90 µL of prepared TMB color developing agent into each well and incubate plate at 37°C in dark for 20-25 min *(Note: For reference only, the optimal incubation time should be determined by end user. And the shades of blue can be seen in the wells with the four most concentrated human TIMP-2 standard solutions; the other wells show no obvious color).*
9. Add 0.1 mL of prepared TMB stop solution into each well. The color changes into yellow immediately.
10. Read the O.D. absorbance at 450nm in a microplate reader within 30 min after adding the stop solution.

Summary

1. Add samples and standards and incubate the plate at 37°C for 90 min. Do not wash.
2. Add biotinylated antibodies and incubate the plate at 37°C for 60 min. Wash plate 3 times with 0.01 M TBS.
3. Add ABC working solution and incubate the plate at 37°C for 30 min. Wash plate 5 times with 0.01 M TBS.
4. Add TMB color developing agent and incubate the plate at 37°C in dark for 20-25 min.
5. Add TMB stop solution and read.

Data Analysis

Calculation of Results

For calculation, (the relative $O.D._{450}$) = (the $O.D._{450}$ of each well) – (the $O.D._{450}$ of Zero well). The standard curve can be plotted as the relative $O.D._{450}$ of each standard solution (Y) vs. the respective concentration of the standard solution (X). The human TIMP-2 concentration of the samples can be interpolated from the standard curve.

Note: if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

✓ Typical result

Typical Data Obtained from human TIMP-2

Concentration (pg/mL)	0	156	312	625	1250	2500	5000	10000
O.D.	0.005	0.074	0.183	0.309	0.618	1.201	1.794	2.133

(TMB reaction incubate at 37°C for 20 min)

Performance Characteristics

✓ Range

156 pg/mL – 10,000 pg/mL

✓ Sensitivity

< 2 pg/mL

✓ Specificity

Natural and recombinant human TIMP-2

✓ Cross-reactivity

No detectable cross-reactivity with other relative proteins.

Resources

References

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2. Hammani, K.; Blakis, A.; Morsette, D.; Bowcock, A. M.; Schmutte, C.; Henriet, P.; DeClerck, Y. A. : Structure and characterization of the human tissue inhibitor of metalloproteinases-2 gene. J. Biol. Chem. 271: 25498-25505, 1996.
3. Seo, D.-W.; Li, H.; Guedez, L.; Wingfield, P. T.; Diaz, T.; Salloum, R.; Wei, B.; Stetler-Stevenson, W. G. : TIMP-2 mediated inhibition of angiogenesis: an MMP-independent mechanism. Cell 114: 171-180, 2003

Plate Layout

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