Human TIMP-1 ELISA Kit

Catalog Number：CEA-B292

Assay Tests: 96 tests

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures

IMPORTANT: Please carefully read this user guide before performing your experiment.

Product information

This kit is specifically designed for the accurate quantitation of human TIMP-1 from cell culture supernates, serum and plasma.

The principle of this assay employs a quantitative sandwich enzyme immunoassay approach. Initially, a microplate is coated with a capture antibody. Then, samples and biotinylated capture antibody are added to the wells. After the removal of any unbound materials through washing, streptavidin-HRP (SA-HRP) conjugate is added to the wells. Streptavidin has a very high affinity for biotin, so it binds to the biotinylated capture antibody that is already bound to the target antigen. After washing, a substrate specific to HRP is added to the wells. HRP catalyzes a reaction that converts the substrate into a detectable signal, often a color change or luminescence, depending on the substrate used. This enzymatic reaction amplifies the signal, allowing for higher sensitivity in detecting the target analyte. The intensity of the signal is measured using a spectrophotometer.

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NOTE:

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. Please do not use the kit after the expiration date indicated on the kit label.
3. Do not mix or substitute reagents with those from other lots or sources.

Manufactured and distributed by
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ACROBiosystems Inc.
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Contents

The kit contains sufficient reagents for 96 wells.

Catalog	Contents	Amount
CEA292-C01	Pre-coated Anti-TIMP-1 Antibody Microplate	1 plate
CEA292-C02	Human TIMP-1 Standard	5 μg/mL×2
CEA292-C03	Biotin-Anti-TIMP-1 Antibody Con. Solution	100 μL
CEA292-C04	Biotin-Antibody Dilution Buffer	8 mL
CEA292-C05	Streptavidin-HRP Con. Solution	500 μL
CEA292-C06	Streptavidin-HRP Dilution Buffer	15 mL
CEA292-C07	20× Washing Buffer	50 mL
CEA292-C08	Sample Dilution Buffer	15 mL
CEA292-C09	Substrate Solution	12 mL
CEA292-C10	Stop Solution	6 mL
CEA292-C11	2× Standard Dilution Buffer	15 mL

NOTE: Bubbles in microplate wells do not affect the experiment and require no action. Proceed with the experimental procedures and methods described below.

Storage

Keep the unopened kit stored at 2-8 °C. Avoid using the kit beyond its expiration date.
For opened kit and reconstituted reagents, with the exception of the two contents listed in following table, others can be stored for up to 30 days at 2-8 °C.

Contents	Storage conditions
Pre-coated Anti-TIMP-1 Antibody Microplate	Return unused wells to the foil pouch, reseal along entire edge. May be stored for up to 1 month at 2-8°C.
Human TIMP-1 Standard	Prepare fresh before use. Avoid freeze-thaw cycles.

NOTE: Streptavidin-HRP Con. Solution and Substrate Solution should avoid light.

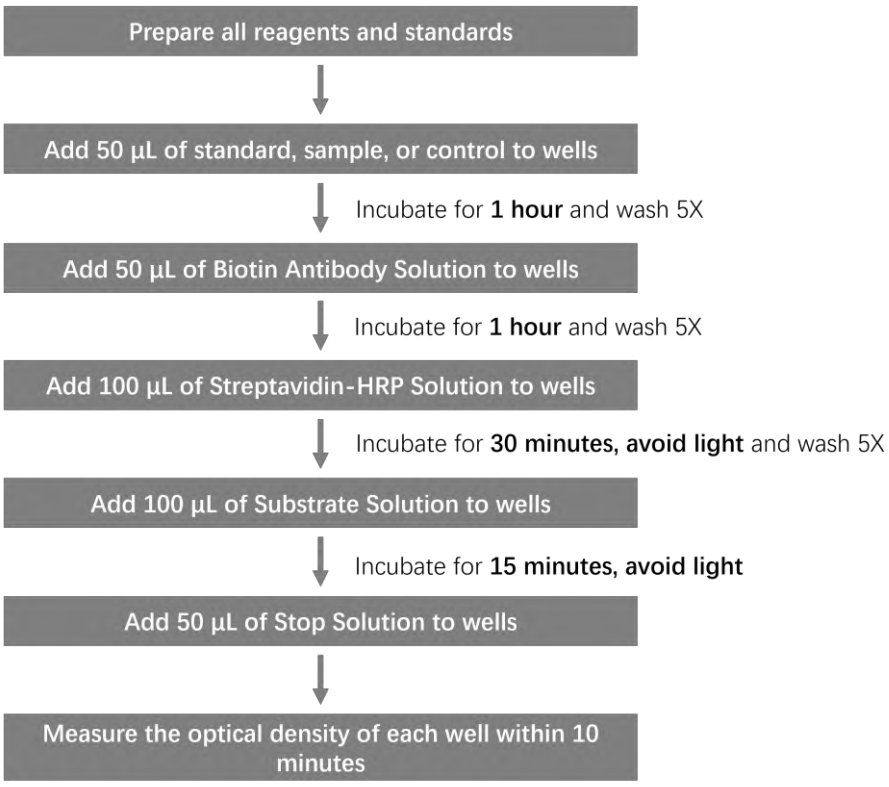
IMPORTANT: Bring all reagents to room temperature before use. If crystals have formed in buffer solution, place the buffer solution in a 37°C incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.

Required materials not supplied.

Instrument	Microplate reader capable of measuring absorbance at 450 nm
Reagents	Deionized, ultrapure or distilled water
Consumables	50 mL and 500 mL graduated cylinders
	Pipettes and pipette tips
	Tubes to prepare standard dilutions.

Workflow

Analyte: TIMP-1



NOTE: Incubation temperature is 18~25℃

Prepare the working buffers and standard dilutions.

Prepare the working buffers.

1. 1×Washing Buffer: Dilute 50 mL 20×Washing Buffer with deionized or distilled water to 1000 mL.
2. Biotin Antibody Solution: Add 60 µL of Biotin-Anti-TIMP-1 Antibody Con. Solution to 6 mL Biotin-Antibody Dilution Buffer, thoroughly mix. The solution was freshly prepared just before use.
3. Streptavidin-HRP Solution: Add 240 µL of Streptavidin-HRP Con. Solution to 12 mL of Streptavidin-HRP Dilution Buffer, thoroughly mix. The solution was freshly prepared just before use.
4. 1×Standard Dilution Buffer: Dilute 15 mL 2×Standard Dilution Buffer with deionized or distilled water to 30 mL.

Prepare the reconstituted standard.

Reconstitute the provided lyophilized product（CEA292-C02）with deionized or distilled water **(Refer to the vial label for reconstitution volume)**, dissolve at room temperature for 15-30 minutes, and mix by gently pipetting. The concentration of reconstituted TIMP-1 Standard is 5 µg/mL.

NOTE: *Avoiding vigorous shaking or vortexing. Avoid freeze-thaw cycles.*

Prepare the standard serial dilutions.

1. Label a tube "**Cm**". Add 50 µL of the reconstituted human TIMP-1 Standard and 450 µL of 1×Standard Dilution Buffer to tube Cm, gently mix well.
2. Label 7 tubes, one for each standard point: Std.-1, Std.-2, Std.-3, Std.-4, Std.-5, Std.-6, Std.-7.
3. Add 80 µL of the liquid from **Cm** and 920 µL of 1×Standard Dilution Buffer to tube Std.-1, thoroughly mix (Std.-1 =40 ng/mL).
4. Prepare serial dilutions for the standard curve as follows: Add 350 µL of 1×Standard Dilution Buffer to each tube (Std.-2, Std.-3, Std.-4, Std.-5, Std.-6, Std.-7).
5. Transfer 500 µL of liquid from Std.-1 to the tube Std.-2, and thoroughly mix (Std.-2 = 24 ng/mL).
6. Continue to transfer 500 µL of liquid from previous dilution tube to the next dilution tube until add liquid to tube Std.-7.
7. 1×Standard Dilution Buffer serves as zero standard (blank).

Prepare the serum / plasma specimen

To minimize matrix interference in samples, it is necessary to determine the Minimum Required Dilution (MRD) of the samples. It is recommended to begin validation with an MRD of 3. Based on the target dilution, the dilution factor can be gradually increased, and the accuracy of the results can be analyzed until the optimal dilution factor is identified.

PROCEDURE OF ASSAY

1. Add 50 µL of TIMP-1 Standard, sample, or control to wells. Incubate at room temperature (18-25 °C) for **1 hour.**
2. Aspirate each well and add 300 µL of 1×Washing Buffer to each well, gently tap the plate for **1 minute**. Remove any remaining Washing Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels. Repeat the wash process four times for a total of five washes.
3. Add 50 µL Biotin Antibody Solution to each well, Seal the plate with microplate sealing film. Incubate at room temperature (18-25 °C) for **1 hour.**
4. Repeat step 2.
5. Add 100 µL of Streptavidin-HRP Solution to each well. Seal the plate with microplate sealing film. Incubate at room temperature (18-25 °C) for **30 minutes, avoid light.**
6. Repeat step 2.
7. Add 100 µL of Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature (18-25 °C) for **15 minutes, avoid light**.
8. Add 50 µL of Stop Solution to each well. Tap the plate gently to ensure thorough mixing. ***Note:*** *the color in the wells should change from blue to yellow.*
9. Read the absorbance at 450nm and 630nm using Microplate reader within 10minutes. ***Note:*** *To reduce the background noise, subtract the readings at 630nm from the readings at 450nm.*

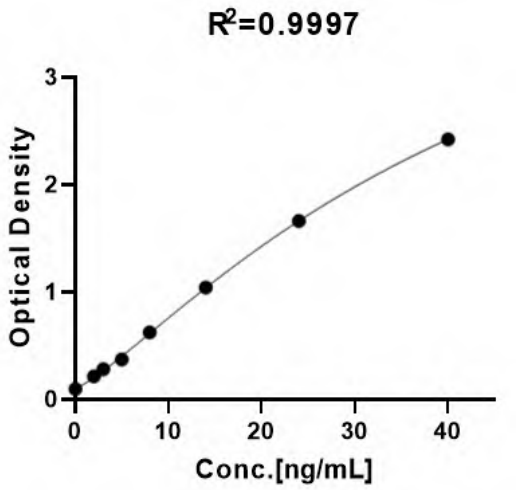
CALCULATION OF RESULTS

1. Compute the average of the duplicated readings for every standard, control, and sample. Then, subtract the average optical density (O.D.) of the zero standard(blank).
2. Establish a standard curve by processing the data using computer software capable of executing a **four-parameter logistic (4-PL)** curve fitting.
3. Normal range of Standard curve: $R^2 \geqslant 0.9900$.
4. If the OD value of the sample to be tested is higher than the highest standard, the sample shall be diluted with dilution buffer and assay repeated.

Typical data

Note: *For each experiment, a standard curve needs to be set for each microplate, and the specific OD value may vary depending on different laboratories, testers, or equipment. The following example data is for reference only. The sample concentration was calculated based on the results of the standard curve.*

Human TIMP-1 Standard(ng/mL)	$OD_{450nm-630nm}$
40	2.425
24	1.663
14	1.045
8.1	0.625
4.8	0.373
2.8	0.285
1.7	0.217
Blank	0.099



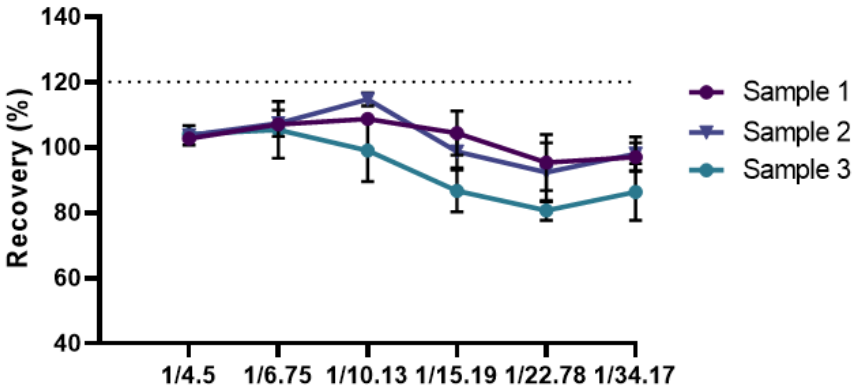
PERFORMANCE CHARACTERISTICS

1. Sensitivity

The minimum detectable concentration (MDC) of TIMP-1 is typically less than 1.5 ng/mL. The MDC was determined by adding two standard deviations to the mean optical density value of twenty standard replicates and calculating the corresponding concentration.

2. Linearity

Three serum samples with high TIMP-1 concentrations were serially diluted with dilution buffer to produce samples with values within the dynamic range of the assay and then assayed. The average recovery of TIMP-1 for serum samples is 101.1%.



3. Intra-Assay Precision

Ten replicates of each of 3 samples containing different TIMP-1 concentrations were tested in one assay. Acceptable criteria: CV < 10%.

Sample Concentration (ng/mL)	Mean (ng/mL)	SD	Numbers	CV (%)
14	13.3	0.8	10	5.7
8.1	7.5	0.4	10	5.5
4.8	4.5	0.3	10	5.9

4. Inter-Assay Precision

3 samples containing different concentrations of TIMP-1 were tested in independent assays. Acceptable criteria: CV<15%.

Sample Concentration (ng/mL)	Mean (ng/mL)	SD	Numbers	CV (%)
14	14.1	0.5	9	3.6
8.1	8.1	0.3	9	4.0
4.8	4.9	0.5	9	9.5

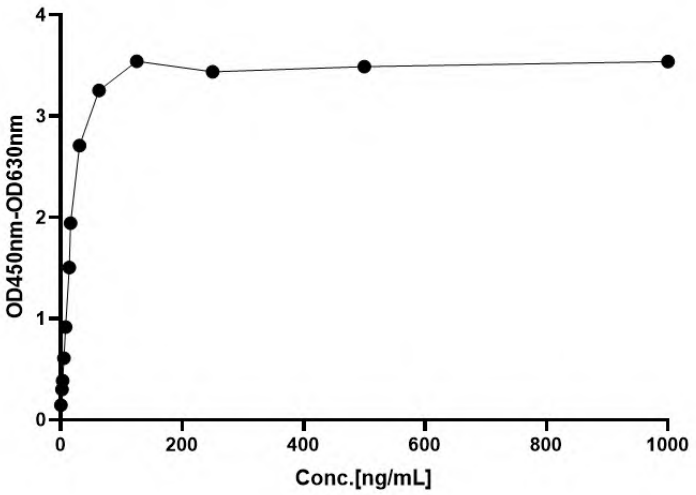
5. Recovery

Recombinant TIMP-1 was spiked into 3 human serum samples, and then analyzed. The average recovery of TIMP-1 for serum samples is 114.4%.

Sample ID	Conc Measured (ng/mL)	Conc Added (ng/mL)	Conc Recovered (ng/mL)	Recovery (%)
1	27.6	24	24.5	104.0
	19.4	14	16.3	117.5
	12.0	8.1	8.9	109.0
	3.5	-	-	-
2	29.6	24	27.5	117.0
	18.5	14	16.5	118.9
	11.1	8.1	9.1	111.7
	2.3	-	-	-
3	28.0	24	24.5	104.0
	21.2	14	17.7	127.6
	13.3	8.1	9.8	120.3
	3.9	-	-	-

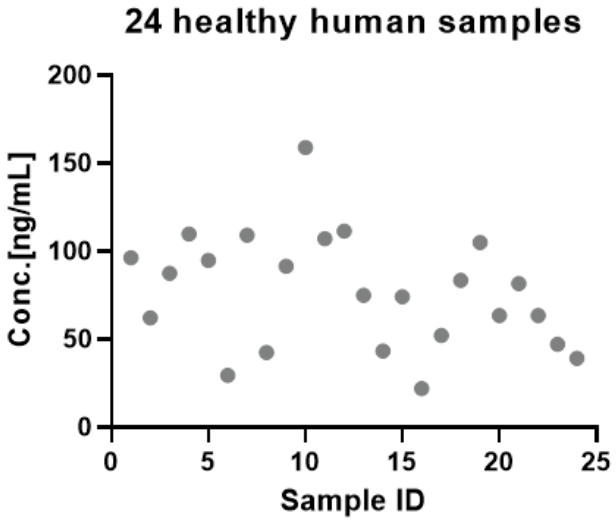
6. Hook Effect

Not be affected by the concentration of TIMP-1 up to 63 ng/mL.



7. Sample Values

Healthy donor serum samples (n=24) were evaluated for the concentrations of human TIMP-1 in the assay. The detectability rate of the samples was 100%, with a mean detectable concentration of 77 ng/mL and a concentration range of 22 to 159 ng/mL.



TROUBLESHOOTING GUIDE

Problem	Cause	Solution
Poor standard curve	* Inaccurate pipetting	* Check pipettes
Large CV	* Inaccurate pipetting * Air bubbles in wells	* Check pipettes * Remove bubbles in wells
High background	* Plate is insufficiently washed * Contaminated wash buffer	* Review the manual for proper wash. * Make fresh wash buffer
Very low readings across the plate	* Incorrect wavelengths * Insufficient development time	* Check filters/reader * Increase development time
Samples are reading too high, but standard curve looks fine	* Samples contain cytokine levels above assay range	* Dilute samples and run again
Drift	* Interrupted assay set-up * Reagents not at room temperature	* Assay set-up should be continuous - have all standards and samples prepared appropriately before commencement of the assay * Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts