ab214029 – Human Thrombomodulin SimpleStep ELISA® Kit

Instructions for use:

For the quantitative measurement of human Thrombomodulin in serum, plasmas, urine, cell culture supernatants, and cell and tissue extract samples.

This product is for research use only and is not intended for diagnostic use.

Version 5 - Last Updated 24 August 2022

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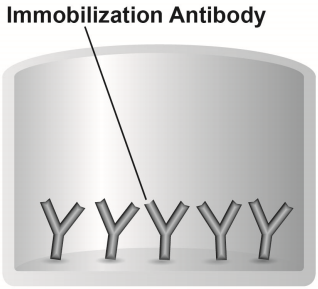
1. BACKGROUND

Thrombomodulin *in vitro* SimpleStep ELISA® (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of Thrombomodulin protein in serum, plasmas, urine, cell culture supernatants, cell and tissue extract samples.

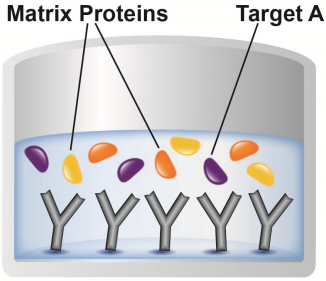
The SimpleStep ELISA® employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. TMB Development Solution is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

Human Thrombomodulin is a 557 amino acid type I transmembrane protein, consisting of six epidermal growth factor (EGF)-like domains and is highly glycosylated. Thrombomodulin is an important component in the anti-coagulation system by functioning as a cell surface receptor for thrombin, and has also been shown to play a role in anti-inflammation, cell adhesion, and proliferation. The Thrombomodulin - thrombin complex activates protein C and thrombin-activatable fibrinolysis inhibitor (TAFI); protein C degrades coagulation factors Va and VIIIa, which regulates thrombin resulting in inhibition of coagulation. The extracellular portion of Thrombomodulin has been shown to be released from the cell surface due to protein cleavage during vascular endothelial cell injury.

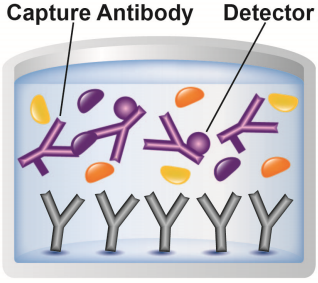
2. ASSAY SUMMARY



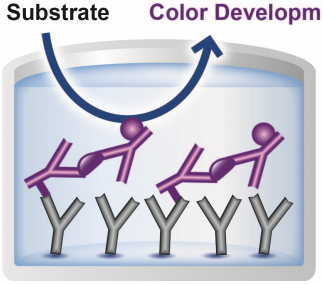
Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all reagents, samples, and standards as instructed.



Add standard or sample to appropriate wells.



Add Antibody Cocktail to all wells. Incubate at room temperature.



Aspirate and wash each well. Add TMB Development Solution to each well and incubate. Add Stop Solution at a defined endpoint.

Alternatively, record color development kinetically after TMB substrate addition.

INTRODUCTION

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at +4ºC immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent and Standard Preparation sections.

5. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Human Thrombomodulin Capture Antibody (lyophilized)	1 Vial	+4ºC
20X Human Thrombomodulin Detector Antibody	300 µL	+4ºC
Human Thrombomodulin Lyophilized Recombinant Protein	2 Vials	+4ºC
Antibody Diluent 4BI	6 mL	+4ºC
10X Wash Buffer PT	20 mL	+4ºC
5X Cell Extraction Buffer PTR	10 mL	+4ºC
50X Cell Extraction Enhancer Solution	1 mL	+4ºC
TMB Development Solution	12 mL	+4ºC
Stop Solution	12 mL	+4ºC
Sample Diluent NS	50 mL	+4ºC
Pre-Coated 96 Well Microplate (12 x 8 well strips)	96 Wells	+4ºC
Plate Seal	1	+4ºC

7. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 or 600 nm.
- Method for determining protein concentration (BCA assay recommended).
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for standard dilution.
- Plate shaker for all incubation steps.
- Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

8. TECHNICAL HINTS

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 11).
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.
- When generating positive control samples, it is advisable to change pipette tips after each step.
- The provided 50X Cell Extraction Enhancer Solution may precipitate when stored at + 4ºC. To dissolve, warm briefly at + 37ºC and mix gently. The 50X Cell Extraction Enhancer Solution can be stored at room temperature to avoid precipitation.
- **To avoid high background always add samples or standards to the well before the addition of the antibody cocktail.**
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. REAGENT PREPARATION

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. **The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment**.
- Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X and 20X formulations.

9.1 1X Cell Extraction Buffer PTR (For cell and tissue extracts only)

Prepare 1X Cell Extraction Buffer PTR by diluting 5X Cell Extraction Buffer PTR and 50X Cell Extraction Enhancer Solution to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 7.8 mL deionized water, 2 mL 5X Cell Extraction Buffer PTR and 200 µL 50X Cell Extraction Enhancer Solution Mix thoroughly and gently. If required protease inhibitors can be added.

Alternative – Enhancer may be added to 1X Cell Extraction Buffer PTR after extraction of cells or tissue. Refer to note in the Troubleshooting section.

9.2 1X Wash Buffer PT

Prepare 1X Wash Buffer PT by diluting 10X Wash Buffer PT with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL 10X Wash Buffer PT with 45 mL deionized water. Mix thoroughly and gently.

9.3 10X Capture Antibody

Reconstitute the Thrombomodulin Capture Antibody in 660 µL Sample Diluent NS and gently mix on rotator for 10 minutes at room temperature. Unused reconstituted antibody can be stored frozen at -20ºC. Avoid repeated freeze-thaw cycles.

9.4 Antibody Cocktail

Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 4BI. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 150 µL 20X Detector Antibody with 2.55 mL Antibody Diluent 4BI. Mix thoroughly and gently.

10. STANDARD PREPARATION

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of positive controls for every use.

The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 **IMPORTANT:** If the protein standard vial has a volume identified on the label, reconstitute the human Thrombomodulin standard by adding that volume of Diluent indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the human Thrombomodulin standard by adding 500 µL Diluent.

For **serum, plasma, urine, and cell culture supernatant sample** measurements, reconstitute the human Thrombomodulin protein standard by adding Sample Diluent NS.

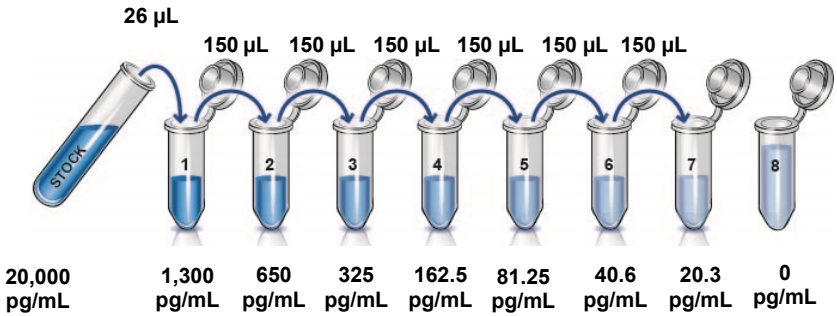
For **cell and tissue extract sample** measurements, reconstitute the human Thrombomodulin protein standard by adding 1X Cell Extraction Buffer PTR.

Hold at room temperature for 10 minutes and mix thoroughly and gently. This is the 20,000 pg/mL **Stock Standard** Solution.

10.2 Label eight tubes, Standards 1– 8.

10.3 Add 374 µL of appropriate diluent (see step 10.1) into tube number 1 and 150 µL of appropriate diluent into numbers 2-8.

10.4 Use the Stock Standard to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:



11. SAMPLE PREPARATION

TYPICAL SAMPLE DYNAMIC RANGE	
Sample Type	**Range**
Human Plasma - Heparin	0.78% - 25%
Human Plasma - EDTA	0.78% - 25%
Human Plasma - Citrate	0.78% - 25%
Human Serum	0.78% - 25%
Human Urine	0.39% - 6.25%
HepG2 Cell Culture Supernatant	2.8% - 45%
HUVEC Extract	31.25 µg/mL - 500 µg/mL
Human Liver Homogenate Extract	7.8 µg/mL - 125 µg/mL

11.1 **Plasma**

Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples into Sample Diluent NS and assay. Store un-diluted plasma samples at -20ºC or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.2 **Serum**

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Dilute samples into Sample Diluent NS and assay. Store un-diluted serum at -20ºC or below. Avoid repeated freeze-thaw cycles.

11.3 **Cell Culture Supernatants**

Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants, dilute samples into

Sample Diluent NS, and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

11.4 **Urine**

Centrifuge urine at 2,000 x g for 10 minutes to remove debris. Collect supernatants, dilute in Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

11.5 **Preparation of extracts from cell pellets**

11.5.1 Collect non-adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 5 minutes at 4ºC.

11.5.2 Rinse cells twice with PBS.

11.5.3 Solubilize pellet at $2x10^7$ cell/mL in chilled 1X Cell Extraction Buffer PTR.

11.5.4 Incubate on ice for 20 minutes.

11.5.5 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.5.6 Transfer the supernatants into clean tubes and discard the pellets.

11.5.7 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.5.8 Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

11.6 **Preparation of extracts from adherent cells by direct lysis (alternative protocol)**

11.6.1 Remove growth media and rinse adherent cells 2 times in PBS.

11.6.2 Solubilize the cells by addition of chilled 1X Cell Extraction Buffer PTR directly to the plate (use 750 µL - 1.5 mL 1X Cell Extraction Buffer PTR per confluent 15 cm diameter plate).

11.6.3 Scrape the cells into a microfuge tube and incubate the lysate on ice for 15 minutes.

11.6.4 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.6.5 Transfer the supernatants into clean tubes and discard the pellets.

11.6.6 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.6.7 Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

11.7 **Preparation of extracts from tissue homogenates**

11.7.1 Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended).

11.7.2 Homogenize 100 to 200 mg of wet tissue in 500 µL – 1 mL of chilled 1X Cell Extraction Buffer PTR. For lower amounts of tissue adjust volumes accordingly.

11.7.3 Incubate on ice for 20 minutes.

11.7.4 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.7.5 Transfer the supernatants into clean tubes and discard the pellets.

11.7.6 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.7.7 Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
- For each assay performed, a minimum of two wells must be used as the zero control.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Differences in well absorbance or “edge effects” have not been observed with this assay.

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

13.1. Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4ºC storage.

13.3. Add 50 µL of all sample or standard to appropriate wells.

13.4. Add 50 µL of the Antibody Cocktail to each well.

13.5. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.

13.6. Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 10 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.

13.7. Add 100 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.

Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.

Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.

13.8. Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.

Alternative to 13.7 – 13.8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode:	Kinetic
Wavelength:	600 nm
Time:	up to 20 min
Interval:	20 sec - 1 min
Shaking:	Shake between readings

Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

13.9. Analyze the data as described below.

14. CALCULATIONS

14.1 Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.

14.2 **Create a standard curve** by plotting the average blank control subtracted absorbance value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.

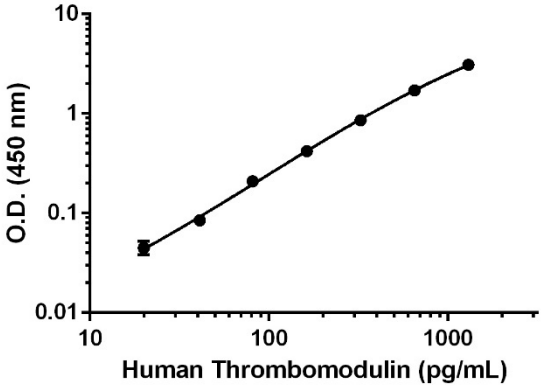
Note: Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4 parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.

14.3 Determine the concentration of the target protein in the sample by interpolating the blank control subtracted **absorbance values against the standard curve**. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.

14.4 Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at an absorbance values less than that of the lowest standard should be retested in a less dilute form.

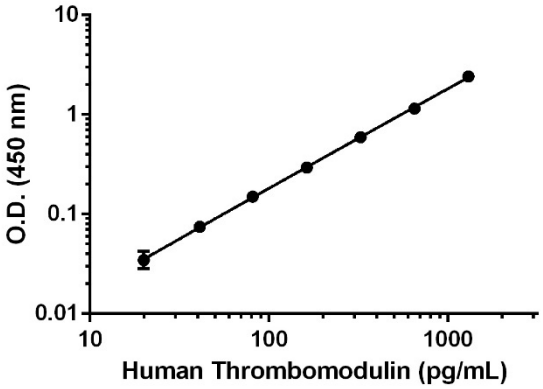
15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.



Standard Curve Measurements			
Conc. (pg/mL)	O.D. 450 nm		Mean O.D.
	1	2	
0	0.12	0.14	0.13
20.3	0.17	0.18	0.17
40.6	0.22	0.21	0.22
81.25	0.34	0.34	0.34
162.5	0.55	0.55	0.55
325	0.99	0.99	0.99
650	1.83	1.85	1.84
1,300	3.21	3.25	3.23

Figure 1. Example of human Thrombomodulin standard curve in Sample Diluent NS. The Thrombomodulin standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.



Standard Curve Measurements			
Conc. (pg/mL)	O.D. 450 nm		Mean O.D.
	1	2	
0	0.07	0.08	0.08
20.3	0.12	0.11	0.11
40.6	0.15	0.15	0.15
81.25	0.22	0.23	0.23
162.5	0.36	0.38	0.37
325	0.66	0.69	0.67
650	1.17	1.28	1.23
1,300	2.48	2.52	2.50

Figure 2. Example of human Thrombomodulin standard curve in 1X Cell Extraction Buffer PTR. The Thrombomodulin standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

16. TYPICAL SAMPLE VALUES

SENSITIVITY –

The calculated minimal detectable dose (MDD) is determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentration.

Sample Diluent Buffer	n=	Minimal Detectable Dose
Sample Diluent NS	16	11 pg/mL
1X Cell Extraction Buffer PTR	16	3 pg/mL

RECOVERY –

Three concentrations of recombinant human Thrombomodulin were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
Human Plasma - Heparin	99	98 - 100
Human Plasma - EDTA	96	94 - 98
Human Plasma - Citrate	96	92 - 98
Human Serum	97	96 - 99
Human Urine	106	102 - 112
HepG2 Cell Culture Supernatant	90	88 - 91
HUVEC Extract	94	92 - 96
Human Liver Homogenate Extract	105	101 - 110

LINEARITY OF DILUTION –

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native Thrombomodulin was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	25% Human Serum	25% Human Plasma (Citrate)	25% Human Plasma (EDTA)	25% Human Plasma (Heparin)	6.25% Human Urine
Undiluted	pg/mL	1186	921	950	1024	701
	% Expected value	**100**	**100**	**100**	**100**	**100**
2	pg/mL	612	486	470	532	346
	% Expected value	**103**	**105**	**99**	**104**	**99**
4	pg/mL	304	245	233	267	164
	% Expected value	**102**	**107**	**98**	**104**	**94**
8	pg/mL	150	123	119	139	83
	% Expected value	**102**	**107**	**100**	**108**	**94**
16	pg/mL	74	60	64	72	41
	% Expected value	**100**	**105**	**107**	**112**	**94**

Recombinant Thrombomodulin was spiked into the following biological sample and diluted in a 2-fold dilution series in Sample Diluent NS.

Dilution Factor	Interpolated value	45% HepG2 Cell Culture Supernatant
Undiluted	pg/mL	287
	% Expected value	**100**
2	pg/mL	150
	% Expected value	**105**
4	pg/mL	77
	% Expected value	**107**
8	pg/mL	39
	% Expected value	**108**
16	pg/mL	18
	% Expected value	**101**

Native Thrombomodulin was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in 1X Cell Extraction Buffer PTR.

Dilution Factor	Interpolated value	500 µg/mL HUVEC Extract	125 µg/mL Liver Homogenate Extract
Undiluted	pg/mL	564	985
	% Expected value	**100**	**100**
2	pg/mL	284	500
	% Expected value	**101**	**102**
4	pg/mL	147	257
	% Expected value	**104**	**104**
8	pg/mL	76	128
	% Expected value	**107**	**104**
16	pg/mL	40	62
	% Expected value	**114**	**100**

PRECISION –

Mean coefficient of variations of interpolated values of Thrombomodulin in 3 concentrations of pooled normal human serum within the working range of the assay.

	Intra-Assay	Inter-Assay
n=	8	3
CV (%)	3.1	9.4

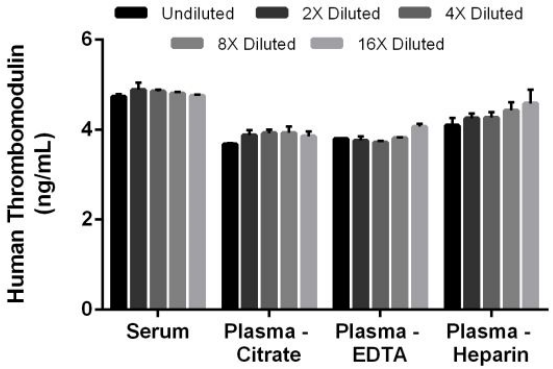


Figure 3. Interpolated concentrations of native Thrombomodulin in human serum, and plasma samples. The concentrations of Thrombomodulin were measured in duplicate, interpolated from the Thrombomodulin standard curve and corrected for sample dilution. Undiluted samples are as follows: serum 25%, plasma (citrate) 25%, plasma (EDTA) 25% and plasma (heparin) 25%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Thrombomodulin concentration was determined to be 4.8 ng/mL in serum, 3.9 ng/mL in plasma (citrate), 3.8 ng/mL in plasma (EDTA) and 4.3 ng/mL in plasma (heparin).

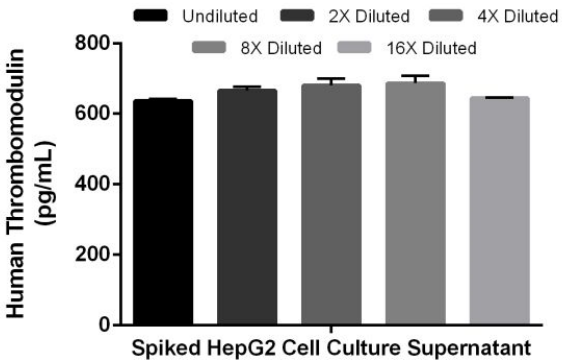


Figure 4. Interpolated concentrations of spiked recombinant Thrombomodulin in HepG2 cell culture supernatant samples. The concentrations of Thrombomodulin were measured in duplicate, interpolated from the Thrombomodulin standard curve and corrected for sample dilution. Undiluted sample is at 45%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The native Thrombomodulin concentration was undetectable in the null spike control run in parallel with the spiked sample.

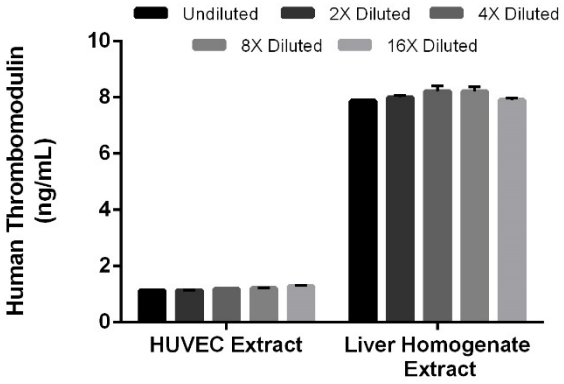


Figure 5. Interpolated concentrations of native Thrombomodulin in HUVEC and human liver homogenate extracts based on a 1,000 µg/mL extract load. The concentrations of Thrombomodulin were measured in duplicate and interpolated from the Thrombomodulin standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Thrombomodulin concentration was determined to be 1.2 ng/mL in HUVEC extract and 8.0 ng/mL in liver homogenate extract.

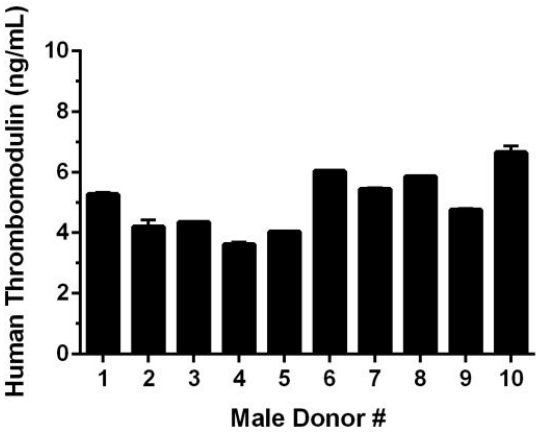


Figure 6. Serum from ten individual healthy human male donors was measured in duplicate. Interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Thrombomodulin concentration was determined to be 5.1 ng/mL with a range of 3.6 – 6.8 ng/mL.

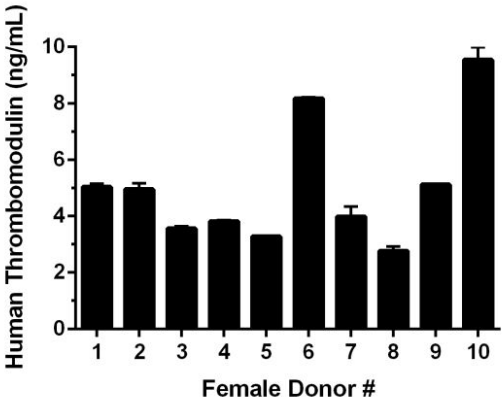


Figure 7. Serum from ten individual healthy human female donors was measured in duplicate. Interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean target concentration was determined to be 5.1 ng/mL with a range of 2.7 – 9.9 ng/mL.

17. ASSAY SPECIFICITY

This kit recognizes both native and recombinant human Thrombomodulin protein in serum, plasma, urine, cell culture supernatant, and cell and tissue extract samples only.

CROSS REACTIVITY

Purified human prothrombin, purified human protein C, and recombinant mouse Thrombomodulin were prepared at 30 ng/mL and 1.3 ng/mL and assayed for cross reactivity. No cross-reactivity was observed.

INTERFERENCE

The following proteins and complexes were tested for interference.

Protein	Concentration	Interference Observed?
Purified human prothrombin	1:1 molar ratio to Thrombomodulin standard	No
Purified human prothrombin	10:1 molar ratio to Thrombomodulin standard	No
Purified human protein C	1:1 molar ratio to Thrombomodulin standard	No
Purified human protein C	10:1 molar ratio to Thrombomodulin standard	No
Purified human prothrombin mixed with protein C	1:1:1 molar ratio to Thrombomodulin standard	No
Purified human prothrombin mixed with protein C	10:10:1 molar ratio to Thrombomodulin standar	No

18. SPECIES REACTIVITY

This kit recognizes human Thrombomodulin protein.

Other species reactivity was determined by measuring 3% serum samples of various species, interpolating the protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in human serum assayed at the same dilution.

Reactivity < 3% was determined for the following species:

- Mouse
- Rat
- Cow

Please contact our Technical Support team for more information.

19. TROUBLESHOOTING

Problem	Cause	Solution
Difficulty pipetting lysate; viscous lysate.	Genomic DNA solubilized	Prepare 1X Cell Extraction Buffer PTR (without enhancer). Add enhancer to lysate after extraction.
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; increase to 2 or 3 hour standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Incubation times with TMB too brief	Ensure sufficient incubation time until blue color develops prior addition of Stop solution
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your reconstituted standards at -80°C, all other assay components 4°C. Keep TMB Development Solution protected from light.
Precipitate in Diluent	Precipitation and/or coagulation of components within the Diluent.	Precipitate can be removed by gently warming the Diluent to 37ºC.

20. NOTES

RESOURCES

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)