HUABIO

HUABIO RESEARCH INC

Human Thrombomodulin ELISA Instructions

Cat:EH0328

Content

	CAT	Volume
❶ CP (Coated Plate)	EH0328CP	96 well
❷ S (Standard)	EH0328S,S1~S7,S0	9 vial
❸ SD (Sample Diluent)	ESD01	15 ml/bottle
❹ DA (Detect Antibody)	EH0328DA	6 ml/bottle
❺ SH (Streptavidin-HRP)	ESH01	12 ml/bottle
❻ AB (Assay Buffer 1×)	EAB01	12 ml/bottle
❼ TS (TMB Substrate)	ETS01	12 ml/bottle
❽ SS (Stop Solution)	ESS01	12 ml/bottle
❾ WB (Wash Buffer 10×)	EWB01	50 ml/bottle
❿ SF (Sealer Film)	ESF01	6 pieces

NOTE: After the kit is opened, the stabilization period of each content is 30 days, so please use the kit within 30 days after opening.

Sample Dilution

Samples such as serum、plasma require at least a 5-fold dilution into Sample Diluent. A suggested 5-fold dilution is 40 µl of sample + 160 µl of Sample Diluent.

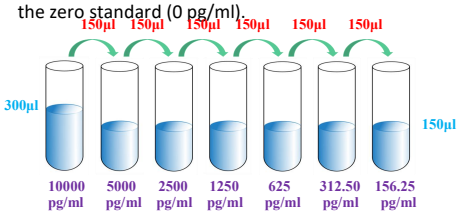
REAGENT PREPARATION

Standard Curve Preparation:

S1 to S7 and S0 is ready to use for serum and plasma.

Other sample type, prepare the standard curve with whatever buffer (SPB, Sample Prepared Buffer) is used to prepare the sample, such as cell culture supernatant, tissue grinding liquid, cell lysate, etc. Urine sample use AB (Assay Buffer) prepare standard curve.

The human Thrombomodulin Standard EH0328S 100000 pg/ml 30 µl + 270 µl SPB serves as the high standard (10000 pg/ml). Pipette 150 µl of SPB into each tube. Use the high standard to produce a 1:1 dilution series. Mix each tube thoroughly before the next transfer. SPB serves as the zero standard (0 pg/ml).



Washing Buffer (1×) Preparation

Pour entire contents (50 ml) of the **Washing Buffer Concentrate** (10×) into a clean 500 ml graduated cylinder. Bring to final volume of 500 ml with glass-distilled or deionized water. Transfer to a clean wash bottle and store at 2 to 25℃.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use.

❶ Prepare all reagents and working standards as directed in the previous sections.

❷ Remove excess **CP** (Coated Plate) strips from the plate frame, return them to the foil pouch and reseal.

❸ Add 50 µl or 10 µl of **AB** (Assay Buffer) to each well.

❹ Add 50 µl of **Standard or sample** per well. Ensure reagent addition is uninterrupted and completed within 15 minutes.

❺ Add 50 µl of **DA** (Detect Antibody) to each well.

❻ Cover with an **SF** (Sealer Film). Incubate at room temperature (18 to 25℃) for 1 hour on a microplate **shaker** set at 500 rpm.

❼ Aspirate each well and **wash**, repeating the process four times. Wash by filling each well with WB (Washing Buffer 300 µl). Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining WB (Washing Buffer) by aspirating or decanting. Invert the plate and **blot** it against clean paper towels.

❽ Add 100 µl of **SH** (Streptavidin-HRP) to each well.

❾ Cover with a new **SF** (Sealer Film). Incubate at room temperature (18 to 25℃) for 30 min on a microplate **shaker** set at 500 rpm.

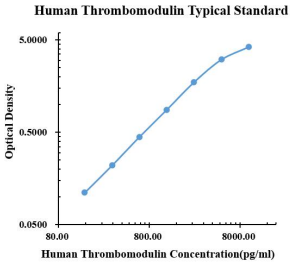
❿ Repeat aspiration/**wash** as in step 7.

⓫ Add 100 µl of **TS** (TMB Substrate) to each well. Incubate for 5-30 minutes at room temperature.

⓬ Add 100 µl of **SS** (Stop Solution) to each well.

⓭ Determine the optical density within 30 minutes, using microplate **reader** set to 450 nm corrected with 570 nm or 630 nm.

TYPICAL DATA



pg/ml	O.D.		Average	Corrected
0.00	0.0511	0.0490	0.0501	
156.25	0.1620	0.1604	0.1612	0.1112
312.50	0.2721	0.2656	0.2689	0.2188
625.00	0.4935	0.4966	0.4951	0.4450
1250.00	0.9292	0.9211	0.9252	0.8751
2500.00	1.7800	1.8000	1.7900	1.7400
5000.00	3.1980	3.0140	3.1060	3.0560
10000.00	4.1872	4.2956	4.2414	4.1914

SENSITIVITY

The minimum detectable dose (MDD) of human Thrombomodulin is typically less than 3.73 pg/ml (50 µl of sample volume) or 4.40 pg/ml (10 µl of sample volume) .

The MDD was determined by adding two standard deviations to the mean optical density value of ten zero standard replicates and calculating the corresponding concentration.

PRECISION

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested twenty-two times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

	Intra-assay Precision			Inter-assay Precision		
Sample Number	S1	S2	S3	S1	S2	S3
	22	22	22	6	6	6
Average (pg/ml)	175.0	901.0	2768.8	192.8	960.4	3060.5
Standard Deviation	7.4	35.0	166.9	12.4	48.9	93.9
Coefficient of Variation (%)	4.2	3.9	6.0	6.4	5.1	3.1

RECOVERY

The spike recovery was evaluated by spiking 3 levels of human Thrombomodulin into health human serum sample. The un-spiked serum was used as blank in this experiment.

The recovery ranged from 98% to 112% with an overall mean recovery of 105%.

LINEARITY

To assess the linearity of the assay, five samples were spiked with high concentration of Thrombomodulin in human serum and diluted with Sample Diluent to produce samples with values within the dynamic range of the assay.

The linearity ranged from 101% to 110% with an overall mean recovery of 106%.

SAMPLE VALUES

Serum/Plasma – Thirty samples from apparently healthy volunteers were evaluated for the presence of human Thrombomodulin in this assay. No medical histories were available for the donors.

Sample Matrix	Sample Evaluated	Range (pg/ml)	Detectable %	Mean of Detectable (pg/ml)
Serum	30	5246.47-10627.71	100	8020.10

n.d. = non-detectable. Samples measured below the sensitivity are considered to be non-detectable.