Mouse Thrombopoietin Sandwich ELISA Kit Datasheet

For the quantitative detection of mouse Thrombopoietin in serum and plasma samples.

General Information

Catalogue Number	KE10059
Product Name	Mouse Thrombopoietin Sandwich ELISA Kit
Species cross-reactivity	Mouse
Range (calibration Range)	62.5-4000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	22018
SwissProt	P35419

Kit Components & Storage

Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	**Unopened Kit:** Store at 2-8°C for 6 months or -20°C for 12 months. **Opened Kit:** All reagents stored at 2-8°C for 7 days. **Please use a new standard for each assay.**
Protein standard - 4000 pg/bottle; lyophilized*	2 bottles	
Detection antibody, biotinylated (100X) - 120 µL/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 µL/vial	1 vial	
Sample Diluent PT 3 - 30 mL/bottle.	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	3 pieces	

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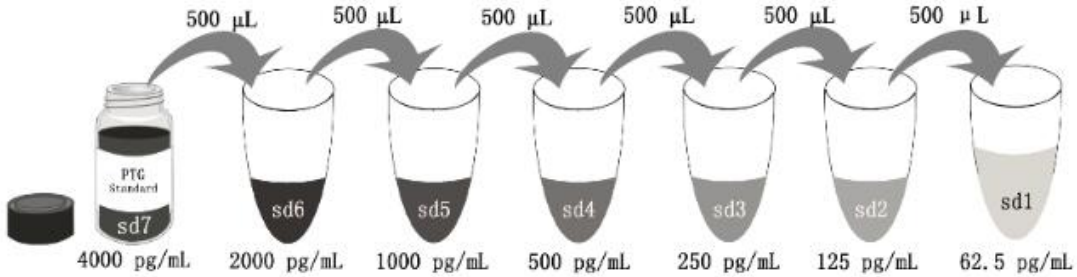
NB: Do not use the kit after the expiration date.
Sample Diluent PT 3 is for protein standard and mouse serum.
Detection Diluent is for Detection antibody and Streptavidin-HRP.
*Add **1 mL** Sample Diluent PT 3 in protein standard. This reconstitution gives a stock solution of 4000 pg/mL.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 3	**1000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE10059 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The mouse Thrombopoietin ELISA kit is to be used to detect and quantify protein levels of endogenous mouse Thrombopoietin. The assay recognizes mouse Thrombopoietin. An antibody specific for mouse Thrombopoietin has been pre-coated onto the microwells. The mouse Thrombopoietin protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for mouse Thrombopoietin is added to detect the captured mouse Thrombopoietin protein. For signal development, Streptavidin-HRP is added, followed by Tetramethyl-benzidine (TMB) reagent. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450 nm with the correction wavelength set at 630 nm.

Background

Thrombopoietin (TPO) is produced in the liver by both parenchymal cells and sinusoidal endothelial cells, as well as in the kidney by proximal convoluted tubule cells. It is a primary regulator of megakaryocyte development and platelet production in mammals. TPO binds the TPO receptor, activates JAK and STAT pathways, thus stimulating megakaryocyte growth and platelet production. Additionally, as TPO is vital for the maintenance of haematopoietic stem cells, it can truly be described as a pan-haematopoietic cytokine.

Sample Preparation

Different samples may require proper dilution to fall within the range of the assay. The mouse serum is better to be diluted 1:2.5 or 1:5 before assay.

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Safety Notes

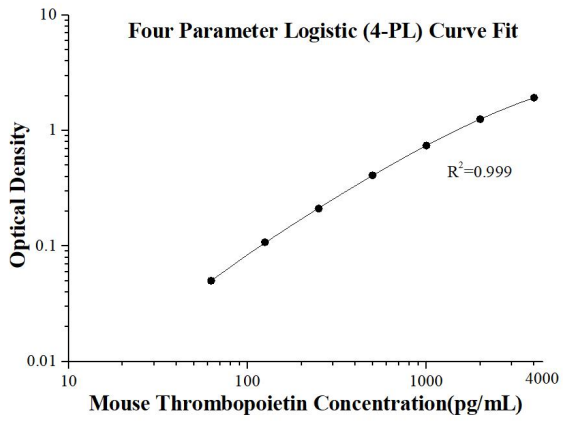
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

Assay Procedure Summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 min	Do not wash	Incubate in the dark at 37°C
5	Stop Solution	100 µL	0 min	Do not wash	-
6	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

Example data

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



(pg/mL)	O.D	Average	Corrected
0	0.103 0.107	0.105	-
62.5	0.152 0.158	0.155	0.050
125	0.214 0.212	0.213	0.108
250	0.315 0.316	0.316	0.211
500	0.514 0.515	0.515	0.41
1000	0.853 0.842	0.848	0.743
2000	1.352 1.372	1.362	1.257
4000	2.03 2.04	2.035	1.930

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Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

Intra-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	20	1,961.9	55.0	2.8
2	20	467.5	13.5	2.9
3	20	92.0	5.0	5.4

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	1,891.6	42.2	2.2
2	24	457.5	11.5	2.5
3	24	110.5	4.4	3.9

Recovery

The recovery of Thrombopoietin spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Mouse serum	1:5	85	78-97
	1:10	85	79-92

Sample Values

Serum - Mouse serum samples were evaluated for the presence of mouse Thrombopoietin in this assay.

Sample Type	Mean of Detectable (pg/mL)	Range (pg/mL)
Mouse serum (n=16)	607.7	525.9-712.6

Sensitivity

The minimum detectable dose of mouse Thrombopoietin is 8.0 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

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Linearity

To assess the linearity of the assay, mouse serum were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

		Mouse serum
1:2.5	Average% of Expected	100
	Range (%)	-
1:5	Average% of Expected	112
	Range (%)	104-118
1:10	Average% of Expected	114
	Range (%)	108-121

References

1. David J Kuter. et al. (2013). Int J Hematol. 98(1):10-23.
2. Matthew Decker. et al. (2018) Science. 360(6384):106-110.
3. Kenneth Kaushansky. et al. (2006) N Engl J Med. 354(19):2034-45.
4. D Prow. et al. (1998) Oncology (Williston Park). 12(11):1597-604, 1607-8.

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