Human t-Plasminogen activator Sandwich ELISA Kit Datasheet

For the quantitative detection of human t-Plasminogen activator concentrations in serum, plasma and cell culture supernatants.

General Information

Catalogue Number	KE00180
Product Name	Human t-Plasminogen activator Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	156.3 - 10000 pg/mL, 78.1 - 5000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	5327
SwissProt	P00750

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 - 20000 pg/bottle; lyophilized* Reconstitution by Sample Diluent PT 1-ec	2 bottles	
Protein standard - 10000 pg/bottle; lyophilized* Reconstitution by Sample Diluent PT 3-ef	2 bottles	
Detection antibody (100X) - 120 µL/vial	1 vial	
HRP-conjugated antibody (100X) - 120 µL/vial	1 vial	
Sample Diluent PT 1-ec - 30 mL/bottle. For serum and plasma	1 bottle	
Sample Diluent PT 3-ef - 30 mL/bottle. For cell culture supernatants	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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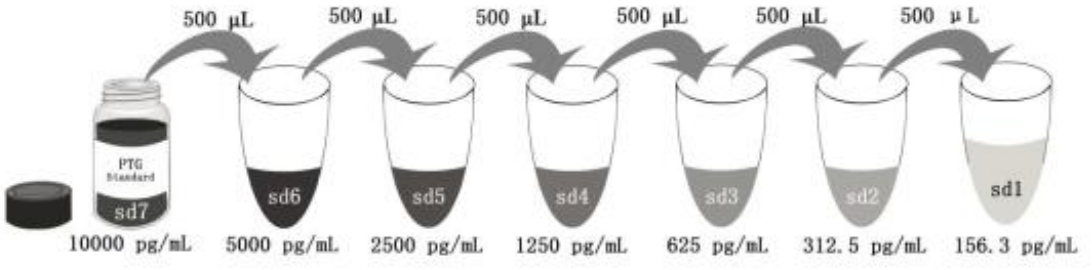
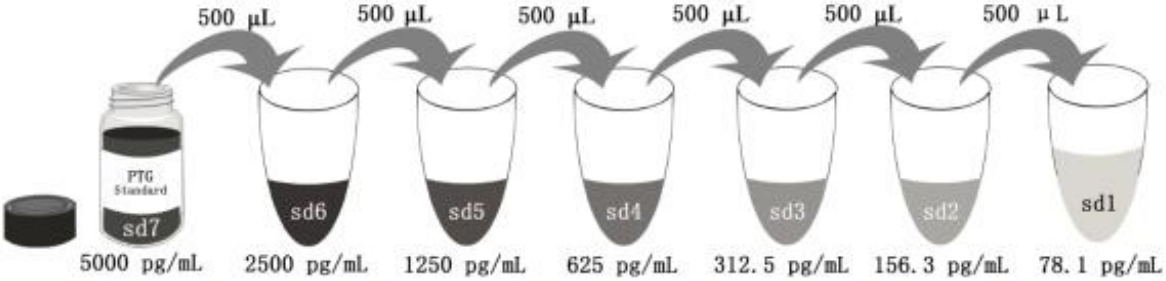
NB: Do not use the kit after the expiration date.
Sample Diluent PT 1-ec is for protein standard, serum and plasma.
Sample Diluent PT 3-ef is for protein standard and cell culture supernatants.
Detection Diluent is for Detection antibody and HRP-conjugated antibody.
*Add **2 mL** Sample Diluent PT 1-ec in standard (20000 pg/bottle). This reconstitution gives a stock solution of 10000 pg/mL.
*Add **2 mL** Sample Diluent PT 3-ef in standard (10000 pg/bottle). This reconstitution gives a stock solution of 5000 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 1-ec**	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"



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-ef**	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

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Product Description

KE00180 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The t-Plasminogen activator ELISA kit is to be used to detect and quantify protein levels of endogenous t-Plasminogen activator. The assay recognizes human t-Plasminogen activator. An antibody specific for t-Plasminogen activator has been pre-coated onto the microwells. The t-Plasminogen activator protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody specific for t-Plasminogen activator is added to detect the captured t-Plasminogen activator protein. For signal development, horseradish peroxidase (HRP)-conjugated 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

Plasminogen activator, tissue (PLAT, synonyms: TPA, T-PA) is a tissue-type plasminogen activator, a secreted serine protease which converts the proenzyme plasminogen to plasmin, a fibrinolytic enzyme. Tissue-type plasminogen activator is synthesized as a single chain which is cleaved by plasmin to a two chain disulfide linked protein (33 kDa and 32 kDa). PLAT enzyme plays a role in cell migration and tissue remodeling. Increased enzymatic activity causes hyperfibrinolysis, which manifests as excessive bleeding; decreased activity leads to hypofibrinolysis which can result in thrombosis or embolism. tPA has 4 isoforms produced by alternative splicing with the MW of 63 kDa, 33 kDa, 57 kDa and 44 kDa.

Sample Preparation

The samples may require proper dilution to fall within the range of the assay. A minimum 1:4 dilution is recommended for serum or plasma and cell culture supernatants.

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.				

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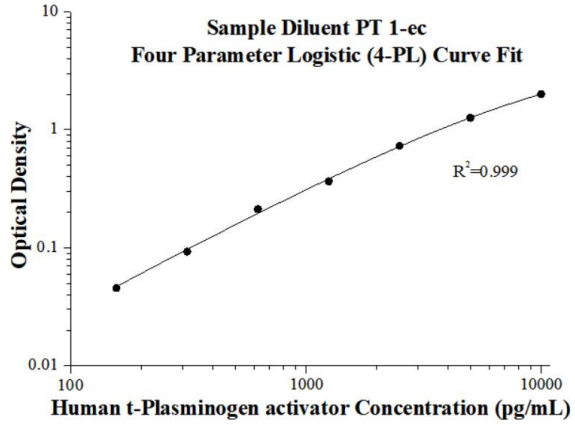
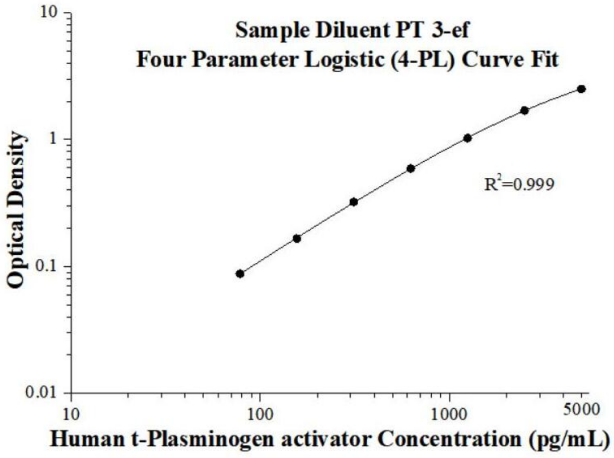
Example data

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	0.025 0.025	0.025	-
156.3	0.069 0.072	0.071	0.046
312.5	0.118 0.117	0.118	0.093
625	0.235 0.239	0.237	0.212
1250	0.406 0.373	0.390	0.365
2500	0.754 0.756	0.755	0.730
5000	1.295 1.275	1.285	1.260
10000	2.073 1.981	2.027	2.002



(pg/mL)	O.D	Average	Corrected
0	0.052 0.050	0.051	-
78.1	0.140 0.137	0.138	0.087
156.3	0.221 0.212	0.217	0.166
312.5	0.378 0.369	0.373	0.322
625	0.645 0.637	0.641	0.590
1250	1.094 1.059	1.076	1.025
2500	1.748 1.734	1.741	1.690
5000	2.588 2.516	2.552	2.501

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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	2076.2	110.5	5.3
2	20	797.3	56.7	7.1
3	20	538.5	49.2	9.1

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	2214.8	168.9	7.6
2	24	814.1	71.9	8.8
3	24	539.8	34.2	6.3

Recovery

The recovery of t-Plasminogen activator 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 (%)
Serum	1:8	85	75-93
	1:16	82	74-89
Plasma	1:4	95	78-125
	1:8	89	81-105
Cell culture supernatants	1:8	87	79-103
	1:16	87	76-100

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Sample Values

Serum and plasma samples from healthy volunteers were evaluated for t-Plasminogen activator in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)
Human serum (n=16)	6,100	1,300-13,700
Human plasma (n=16)	6,500	3,300-12,100

cell culture supernatants

HUVEC human umbilical vein endothelial cells were cultured in EGM-2. Aliquots of the cell culture supernatants were removed and assayed for levels of t-Plasminogen activator.

Condition	2 day (pg/mL)	3 days (pg/mL)
concentration	2,931	1,760

Sensitivity

The minimum detectable dose of t-Plasminogen activator is 8.9 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

Linearity

To assess the linearity of the assay, serum, plasma and cell culture supernatants were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

Sample Type		Average% of Expected	Range (%)
Serum (Sample Diluent PT 1-ec)	1:8	89	80-98
	1:16	100	-
	1:32	115	110-120
Plasma (Sample Diluent PT 1-ec)	1:4	100	-
	1:8	105	97-111
	1:16	100	78-121
	1:32	100	84-115
Cell culture supernatants (Sample Diluent PT 3-ef)	1:4	100	-
	1:8	118	113-123
	1:16	99	78-121

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References

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2. Chen S. et al. (2017) Sci Rep.7(1):6871.
3. Jiang H. et al. (2017) Transl Psychiatry. 7(4): e1079.

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