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Antibodies | ELISA kits | Proteins

Human PD1 Sandwich ELISA Kit Datasheet

For the quantitative detection of human PD1 concentrations in serum, plasma, cell culture supernatants and cell lysates.

General Information

Catalogue Number	KE00075
Product Name	Human PD1 Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	125-8000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	5133
SwissProt	Q15116

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 - 16000 pg/bottle; lyophilized*	2 bottles	
Detection antibody (100×) - 120 µL/vial	1 vial	
HRP-conjugated antibody (100×) - 120 µL/vial	1 vial	
Sample Diluent PT 1-ef - 30 mL/bottle	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20×) - 30 mL/bottle	1 bottle	
Extraction Reagent - 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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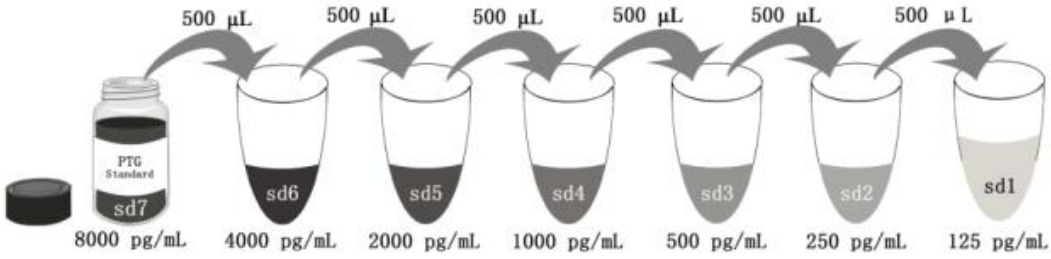
NB: Do not use the kit after the expiration date.
Sample Diluent PT 1-ef is for protein standard and samples.
Detection Diluent is for Detection antibody and HRP-conjugated antibody.
*Add **2 mL** Sample Diluent PT 1-ef in protein standard. This reconstitution gives a stock solution of 8000 pg/mL.



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

Product Description

KE00075 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The PD1 ELISA kit is to be used to detect and quantify protein levels of endogenous PD1. The assay recognizes human PD1. An antibody specific for PD1 has been pre-coated onto the microwells. The PD1 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody specific for PD1 is added to detect the captured PD1 protein. For signal development, horseradish peroxidase (HRP)-conjugated antibody 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

Programmed cell death 1 (PD-1, also known as CD279) is an immunoinhibitory receptor that belongs to the CD28/CTLA-4 subfamily of the Ig superfamily. It is a 288 amino acid (aa) type I transmembrane protein composed of one Ig superfamily domain, a stalk, a transmembrane domain, and an intracellular domain containing an immunoreceptor tyrosine-based inhibitory motif (ITIM) as well as an immunoreceptor tyrosine-based switch motif (ITSM). PD-1 can be expressed on T cells, B cells, natural killer T cells, activated monocytes, and dendritic cells (DCs). Engagement of PD-1 by its ligands PD-L1 or PD-L2 transduces a signal that inhibits T-cell proliferation, cytokine production, and cytolytic function. It is critical for the regulation of T cell function during tolerance, autoimmunity and infection. Blockade of PD-1 can overcome immune resistance and also has been shown to have antitumor activity.

Sample Preparation

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The serum or plasma samples may require proper dilution to fall within the range of the assay. A range of dilutions like 1:2, 1:4 is suggested according to the individual samples.

Safety Notes

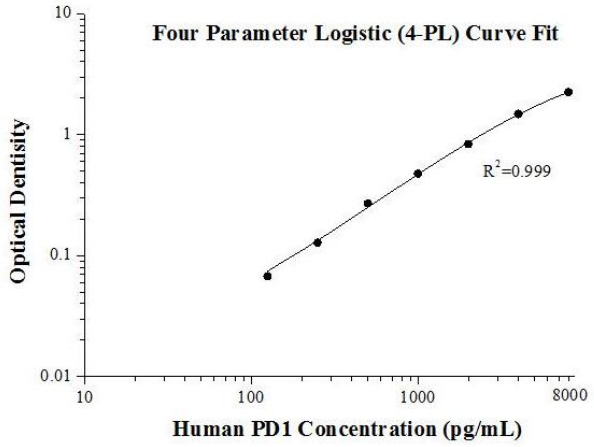
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

Assay Procedure Summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	60 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	0.068 0.065	0.0665	-
125	0.132 0.136	0.134	0.0675
250	0.198 0.19	0.194	0.1275
500	0.361 0.311	0.336	0.2695
1000	0.556 0.527	0.5415	0.475
2000	0.919 0.881	0.9	0.8335
4000	1.541 1.549	1.545	1.4785
8000	2.265 2.355	2.312	2.2455

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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	7,390	191	2.6
2	20	2,176	81	3.7
3	20	542	14	2.6

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	7,796	382	4.9
2	24	2,399	139	5.8
3	24	539	27	5.0

Recovery

The recovery of PD1 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 (%)
Human plasma	1:2	93	80-110
	1:4	98	81-116
Cell culture supernatants	1:2	95	79-118
	1:4	94	79-120
Cell lysates	1:2	99	83-125
	1:4	103	84-120

Sample Values

Sample Type	Concentration (pg/mL)
293 cell lysates (1 x 10^7cells)	4,000
Over expression samples in 293 cell lysates (1 x 10^7cells)	24,000

Sensitivity

The minimum detectable dose of human PD1 is 43.0 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, three samples were spiked with high concentrations of PD1 in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay.

(The samples were initially diluted 1:4)

		Human plasma	Cell culture supernatants	Cell lysates
1:2	Average% of Expected	89	102	105
	Range (%)	76-102	88-115	97-121
1:4	Average% of Expected	88	93	96
	Range (%)	79-105	77-115	78-110
1:8	Average% of Expected	84	90	103
	Range (%)	77-98	78-114	80-119
1:16	Average% of Expected	88	89	108
	Range (%)	79-100	77-110	87-125

References

1. Sharpe AH, et al. The function of programmed cell death 1 and its ligands in regulating autoimmunity and infection. Nat Immunol. 8(3):239-45 (2007).
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4. Francisco LM, et al. The PD-1 pathway in tolerance and autoimmunity. Immunol Rev. 236:219-42 (2010).
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