Human PD-L1 Sandwich ELISA Kit Datasheet

For the quantitative detection of human PD-L1 concentrations in serum, plasma, cell culture supernatants and cell lysates.

General Information

Catalogue Number	KE00074
Product Name	Human PD-L1 Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	0.156-10 ng/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	29126
SwissProt	Q9NZQ7

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 - 20 ng/bottle; lyophilized*	2 bottles	
Detection antibody (100X) - 120 μL/vial	1 vial	
HRP-conjugated antibody (100X) - 120 μL/vial	1 vial	
Sample Diluent PT 1-ef - 30 mL/bottle. For cell culture supernatants sample	1 bottle	
Sample Diluent PT 3-ef - 30 mL/bottle. For serum, plasma and cell lysates samples	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20X) - 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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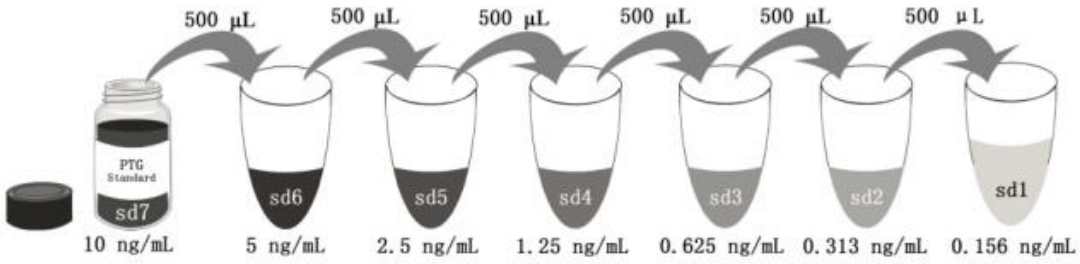
NB: Do not use the kit after the expiration date.
Sample Diluent PT 1-ef is for protein standard and cell culture supernatants sample.
Sample Diluent PT 3-ef is for protein standard, serum, plasma and cell lysate samples.
Detection Diluent is for Detection antibody and HRP-conjugated antibody.
*Add **2 mL** Sample Diluent PT 1-ef or PT 3-ef in protein standard. This reconstitution gives a stock solution of 10 ng/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 or PT 3-ef	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00074 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The PD-L1 ELISA kit is to be used to detect and quantify protein levels of endogenous PD-L1. The assay recognizes human PD-L1. An antibody specific for PD-L1 has been pre-coated onto the microwells. The PD-L1 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody specific for PD-L1 is added to detect the captured PD-L1 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

PD-L1 (programmed cell death ligand 1, also known as CD274 or B7-H1) is a 290 aa type I transmembrane protein. PD-L1 is expressed constitutively on murine T cells, B cells, DCs, macrophages, mesenchymal stem cells and cultured bone marrow-derived mast cells. In addition, PD-L1 is also expressed on many nonhematopoietic cell types, including vascular endothelial cells, epithelial cells, muscle cells, hepatocytes, pancreatic islet cells, astrocytes in the brain, placental syncytiotrophoblasts, and cells in cornea, iris-ciliary body and retina of eye. PD-L1 is frequently upregulated in a wide variety of solid tumors, including melanoma, ovarian, lung, glioblastoma, breast, and pancreatic cancers. PD-L1 and PD-L2 are two ligands of PD-1. Engagement of PD-1 by 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.

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Sample Preparation

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.				

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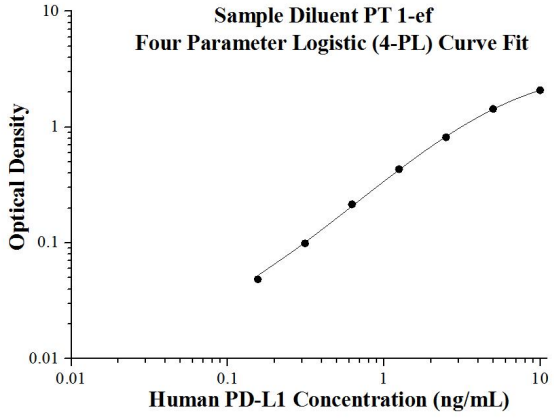
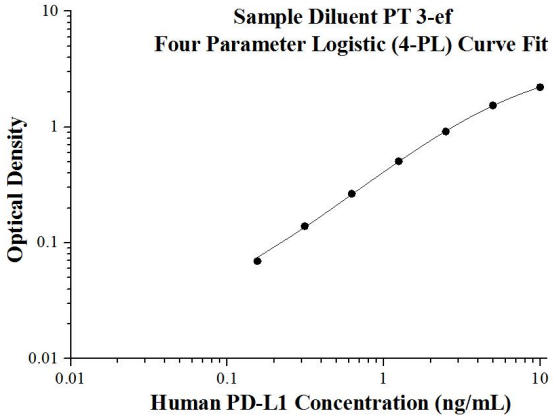
Example data

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



(ng/mL)	O.D	Average	Corrected
0	0.02 0.024	0.022	-
0.156	0.072 0.069	0.0705	0.0485
0.313	0.123 0.119	0.121	0.099
0.625	0.242 0.232	0.237	0.215
1.25	0.466 0.444	0.455	0.433
2.5	0.851 0.824	0.8375	0.8155
5	1.49 1.422	1.456	1.434
10	2.117 2.084	2.1005	2.0785



(ng/mL)	O.D	Average	Corrected
0	0.025 0.023	0.024	-
0.156	0.096 0.091	0.0935	0.0695
0.313	0.167 0.16	0.1635	0.1395
0.625	0.275 0.304	0.2895	0.2655
1.25	0.524.0.536	0.53	0.506
2.5	0.969 0.906	0.9375	0.9135
5	1.604 1.514	1.559	1.535
10	2.267 2.188	2.2275	2.2035

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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 (ng/mL)	SD	CV%
1	20	8.26	0.64	7.8
2	20	2.02	0.14	6.8
3	20	0.49	0.02	4.6

Inter-assay Precision				
Sample	n	Mean (ng/mL)	SD	CV%
1	24	7.43	0.38	5.1
2	24	1.90	0.12	6.1
3	24	0.47	0.03	7.0

Recovery

The recovery of PD-L1 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	92	74-125
	1:4	95	75-120
Cell culture supernatants	1:2	108	95-127
	1:4	102	87-127
Cell lysates	1:2	102	79-126
	1:4	98	74-116

Sample Values

Sixty-four serum and plasma samples from healthy volunteers were evaluated for human PD-L1 in this assay. Sixty samples measured less than the lowest standard, 0.156 ng/mL. Four samples measured between 0.25 and 2 ng/mL. No medical histories were available for the donors used in this study.

Sample Type	Concentration (ng/mL)
Jurkat cell lysates (1 x 10^7cell)	1.6

Sensitivity

The minimum detectable dose of human PD-L1 is 0.04 ng/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 PD-L1 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 (Sample Diluent PT 1-ef)	Cell culture supernatants (Sample Diluent PT 3-ef)	Cell lysates (Sample Diluent PT 1-ef)
1:2	Average% of Expected	79	108	94
	Range (%)	76-82	91-124	79-109
1:4	Average% of Expected	86	110	101
	Range (%)	78-95	98-127	86-116
1:8	Average% of Expected	84	107	100
	Range (%)	75-97	96-121	86-114
1:16	Average% of Expected	94	108	101
	Range (%)	86-106	100-120	91-110

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).
2. Keir ME, et al. PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol. 26:677-704 (2008).
3. Riley JL, et al. PD-1 signaling in primary T cells. Immunol Rev. 229(1):114-25 (2009).

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