abcam

Product datasheet

Human PD-L1 ELISA Kit [28-8] ab214565

SimpleStep ELISA

1 Abreviews 21 References 11 Images

Overview

Product name Human PD-L1 ELISA Kit [28-8]

Detection method Colorimetric

Precision

Intra-assay

Sample	n	Mean	SD	CV%
Overall	8			5.4%

Inter-assay

Sample	n	Mean	SD	CV%
Overall	3			4.1%

Sample type Cell culture supernatant, Urine, Serum, Plasma, Cell culture extracts, Tissue Extracts

Assay type Sandwich (quantitative)

Sensitivity 2.91 pg/ml

Range 21.87 pg/ml - 1400 pg/ml

Recovery

Sample specific recovery

Sample type	Average %	Range
Cell culture supernatant	103	102% - 105%
Urine	91	86% - 96%
Serum	86	82% - 91%
Cell culture extracts	102	100% - 103%
Tissue Extracts	105	104% - 107%
Hep Plasma	92	89% - 93%

Sample type	Average %	Range
EDTA Plasma	85	81% - 88%
Cit plasma	84	79% - 88%

Assay time

1h 30m

Assay duration

One step assay

Species reactivity

Reacts with: Human
Does not react with: Cow, Cynomolgus monkey, Macaque monkey

Product overview

Human PD-L1 ELISA Kit [28-8] (ab214565) has been re-developed with a new recombinant antibody to provide improved consistency and security of supply. This version, ab214565, will be discontinued when remaining inventory is depleted.

The new version is available as ab277712. It retains the recombinant PD-L1 antibody clone [28-8].

Human PD-L1 ELISA Kit [28-8] (ab214565) is a single-wash 90 min sandwich ELISA designed for the quantitative measurement of PD-L1 [28-8] protein in cell culture extracts, cell culture supernatant, serum, tissue extracts, urine, and plasma. It uses our proprietary SimpleStep ELISA® technology. Quantitate Human PD-L1 [28-8] with 2.91 pg/ml sensitivity.

SimpleStep ELISA® technology employs capture antibodies conjugated to an affinity tag that is recognized by the monoclonal antibody used to coat our SimpleStep ELISA® plates. This approach to sandwich ELISA allows the formation of the antibody-analyte sandwich complex in a single step, significantly reducing assay time. See the SimpleStep ELISA® protocol summary in the image section for further details. Our SimpleStep ELISA® technology provides several benefits:

- Single-wash protocol reduces assay time to 90 minutes or less
- High sensitivity, specificity and reproducibility from superior antibodies
- Fully validated in biological samples
- 96-wells plate breakable into 12 x 8 wells strips

A 384-well SimpleStep ELISA® microplate (ab203359) is available to use as an alternative to the 96-well microplate provided with SimpleStep ELISA® kits.

ASSAY SPECIFICITY

This kit recognizes both native and recombinant human PD-L1 protein in human serum, plasma, and cell culture supernatant, cell and tissue extract samples only.

CROSS REACTIVITY

Recombinant mouse PD-L1 was prepared at 50 ng/mL and 1250 pg/mL and assayed for cross reactivity. No cross-reactivity was observed.

INTERFERENCE

Recombinant PD-1 was prepared at 50 ng/mL and 1250 pg/mL and tested for interference. No interference with was observed.

SPECIES REACTIVITY

This kit recognizes human PD-L1 protein.

Other species reactivity was determined by measuring 50% serum samples of various species, interpolating the protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in human serum assayed at the same dilution.

Reactivity < 3% was determined for the following species: Mouse, Rat, Cow

Notes

PD-L1 (also known as CD274 or B7-H1) is a membrane bound glycoprotein involved in regulation of the immune system. PD-L1 is expressed on a variety of inflammatory-activated cells as well as some carcinomas and in melanoma. PD-L1 binds to PD-1 and CD80, where it can suppress T cell activation and proliferation as well as induce apoptosis. Levels of PD-L1 are increased in the plasma of cancer patients as well as in cerebrospinal fluid of gliomas. PD-L1 can bind PD-1 in order to regulate T cell apoptosis.

Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances.
It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.

Platform

Pre-coated microplate (12 x 8 well strips)

Properties

Storage instructions

Store at +4°C. Please refer to protocols.

Components	1 x 96 tests
10X Wash Buffer PT (ab206977)	1 x 20ml
5X Cell Extraction Buffer PTR (ab193970)	1 x 10ml
Antibody Diluent CPI - HAMA Blocker (ab193969)	1 x 6ml
10X Human PD-L1 Capture Antibody	1 x 600µl
Human PD-L1 Lyophilized Recombinant Protein	2 vials
Plate Seals	1 unit
10X Human PD-L1 Detector Antibody (RabMab clone 28-8)	1 x 600µl

Components	1 x 96 tests
Sample Diluent NS (ab193972)	1 x 50ml
SimpleStep Pre-Coated 96-Well Microplate (ab206978)	1 unit
Stop Solution	1 x 12ml
TMB Development Solution	1 x 12ml

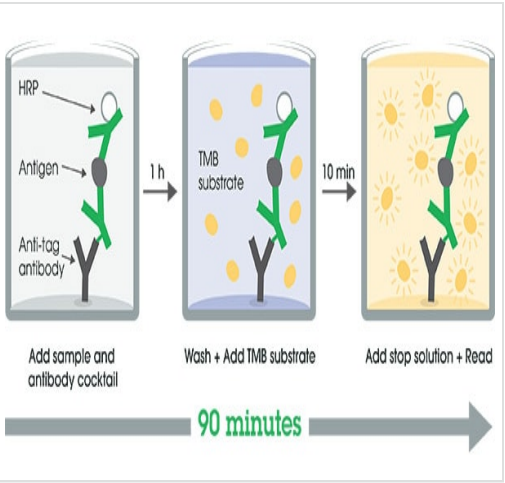
Function Involved in the costimulatory signal, essential for T-cell proliferation and production of IL10 and IFNG, in an IL2-dependent and a PDCD1-independent manner. Interaction with PDCD1 inhibits T-cell proliferation and cytokine production.

Tissue specificity Highly expressed in the heart, skeletal muscle, placenta and lung. Weakly expressed in the thymus, spleen, kidney and liver. Expressed on activated T- and B-cells, dendritic cells, keratinocytes and monocytes.

Sequence similarities Belongs to the immunoglobulin superfamily. BTN/MOG family.
Contains 1 Ig-like C2-type (immunoglobulin-like) domain.
Contains 1 Ig-like V-type (immunoglobulin-like) domain.

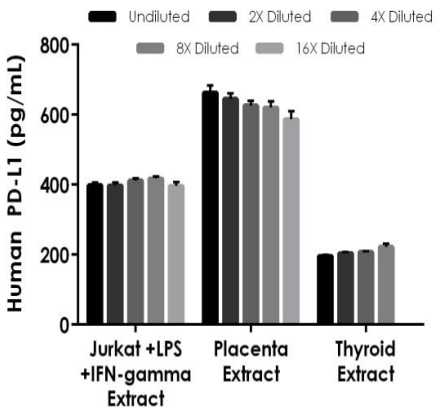
Cellular localization Cell membrane and Endomembrane system.

Images



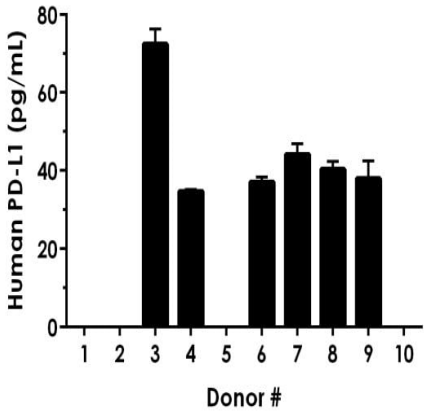
Other - Human PD-L1 ELISA Kit [28-8] (ab214565)

SimpleStep ELISA technology allows the formation of the antibody-antigen complex in one single step, reducing assay time to 90 minutes. Add samples or standards and antibody mix to wells all at once, incubate, wash, and add your final substrate. See protocol for a detailed step-by-step guide.



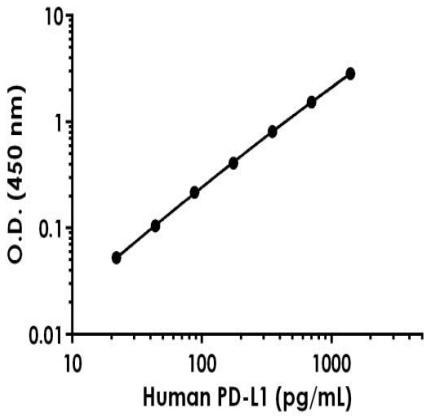
Interpolated concentrations of native PD-L1 in human Jurkat stimulated with LPS and IFN-gamma, placenta and thyroid based on a 1,000 µg/mL extract load.

The concentrations of PD-L1 were measured in duplicate and interpolated from the PD-L1 standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean PD-L1 concentration was determined to be 404.3 pg/mL in Jurkat stimulated with LPS and IFN-gamma, 628.4 pg/mL in placenta and 207.5 pg/mL in thyroid extracts.



Serum from ten individual healthy human male donors was measured in duplicate.

Interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). PD-L1 was measured in 6 donor serum samples and the remaining 4 samples measured less than the lowest point of the PD-L1 standard curve. Of those measured, the mean PD-L1 concentration was determined to be 44.5 pg/mL with a range of 34.4 – 75.3 pg/mL.



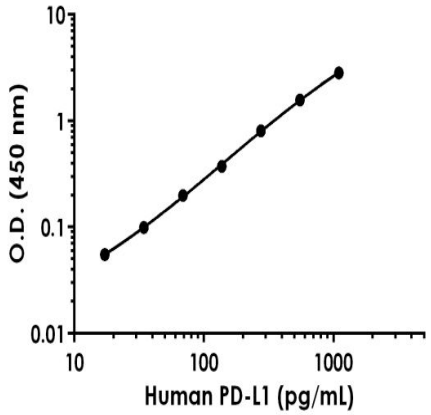
Example of human PD-L1 standard curve in 1X Cell Extraction Buffer PTR.

Background-subtracted data values (mean +/- SD) are graphed.

Standard Curve Measurements			
Concentration (pg/mL)	O.D 450 nm		Mean O.D
	1	2	
0	0.051	0.056	0.053
21.87	0.102	0.110	0.106
43.75	0.154	0.163	0.158
87.5	0.274	0.270	0.272
175	0.461	0.470	0.465
350	0.856	0.881	0.869
700	1.558	1.644	1.601
1400	2.868	2.908	2.888

Example of human PD-L1 standard curve in 1X Cell Extraction Buffer PTR.

The PD-L1 standard curve was prepared as described. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.



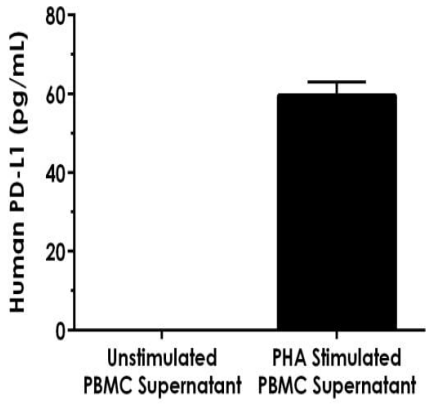
Example of human PD-L1 standard curve in Sample Diluent NS.

Background-subtracted data values (mean +/- SD) are graphed.

Standard Curve Measurements			
Concentration (pg/mL)	O.D 450 nm		Mean O.D
	1	2	
0	0.058	0.059	0.058
17.18	0.110	0.117	0.114
34.37	0.157	0.159	0.158
68.75	0.258	0.258	0.258
137.5	0.406	0.463	0.434
275	0.864	0.878	0.871
550	1.610	1.661	1.636
1100	2.807	2.982	2.895

Example of human PD-L1 standard curve in Sample Diluent NS.

The PD-L1 standard curve was prepared as described. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.



Human peripheral blood mononuclear cells were cultured unstimulated or stimulated with 10 µg/mL PHA.

Conditioned media was harvested after 48 hours. PD-L1 was measured in 100% unstimulated and PHA stimulated PBMC supernatant. The concentrations of PD-L1 were measured in duplicate and interpolated from the PD-L1 standard curves. The interpolated values are plotted (mean +/- SD, n=2). The mean PD-L1 concentration was determined to be 59.7 pg/mL in PHA stimulated PBMC supernatant. There was no detectable signal in unstimulated supernatant.

Dilution Factor	Interpolated value	1000 µg/mL Jurkat + LPS + IFN-gamma	1000 µg/mL Human Placenta Extract	1000 µg/mL Human Thyroid Extract
Undiluted	pg/mL	399	663	196
	% Expected value	100	100	100
2	pg/mL	199	322	102
	% Expected value	100	97	104
4	pg/mL	103	157	51.9
	% Expected value	103	94	106
8	pg/mL	52.2	77.5	27.8
	% Expected value	105	94	113
16	pg/mL	24.8	36.7	NL
	% Expected value	99	89	NL

Linearity of dilution.

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native PD-L1 was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in 1X Cell Extraction Buffer PTR.

Dilution Factor	Interpolated value	50% Human Serum	50% Human Plasma (Citrate)	50% Human Plasma (EDTA)	50% Human Plasma (Heparin)	50% Jurkat Supernatant
Undiluted	pg/mL	515	521	563	552	585
	% Expected value	100	100	100	100	100
2	pg/mL	303	310	323	319	306
	% Expected value	118	119	115	115	105
4	pg/mL	150	157	166	162	147
	% Expected value	116	121	118	118	101
8	pg/mL	73.9	77.8	78.9	79.8	74.2
	% Expected value	115	120	116	112	101
16	pg/mL	35.0	37.5	38.5	39.9	36.5
	% Expected value	109	115	116	110	100

Linearity of dilution.

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Recombinant PD-L1 was spiked into the following biological samples and diluted in a 2-fold dilution series in Sample Diluent NS.

Dilution Factor	Interpolated value	50% Human Urine
Undiluted	pg/mL	459
	% Expected value	100
2	pg/mL	246
	% Expected value	107
4	pg/mL	127
	% Expected value	110
8	pg/mL	65.3
	% Expected value	114
16	pg/mL	30.9
	% Expected value	108

Linearity of dilution.

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Recombinant PD-L1 was spiked into the following biological sample and diluted in a 2-fold dilution series in Sample Diluent NS.

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