ab326305 – Human PD-L2 SimpleStep ELISA® Kit, Chemiluminescent

For the quantitative measurement of PD-L2 in human serum, plasma (heparin), plasma (EDTA), plasma (citrate), cell culture supernatant, saliva, urine, and milk.
For research use only - not intended for diagnostic use.
Patent pending.

For overview, typical data and additional information please visit: www.abcam.com/ab326305

Storage and Stability: Store kit at 2-8°C immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Standard Preparation and Reagent preparation sections.

Limitations: All data, except typical standard curve and sensitivity were collected using the colorimetric version of this kit (ab231928).

Materials Supplied

Item	Quantity 1 x 96 tests	Storage Condition
Human PD-L2 Capture Antibody 10X	600 µL	+4°C
Human PD-L2 Detector Antibody 10X	600 µL	+4°C
Human PD-L2 Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent 4BI	6 mL	+4°C
Sample Diluent NS	50 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
ChemiHRP Reagent A	3 mL	+4°C
ChemiHRP Reagent B	3 mL	+4°C
SimpleStep Pre-Coated Black 96-Well Microplate	96 Wells	+4°C
Plate Seal	1	+4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

Luminometer with the following settings: 0.5-1 second/well read time; summation mode (all wavelengths).
Deionized water.
Multi- and single-channel pipettes.
Tubes for standard dilution.
Orbital microplate shaker for all incubation steps: capable of 750 rpm shaking speed.
Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.

Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

1X Wash Buffer PT: Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 4BI. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent 4BI. Mix thoroughly and gently.

Lumi HRP Development Solution: Just prior to use, prepare Lumi HRP Development Solution by mixing equal volume of the ChemiHRP Reagent A and the ChemiHRP Reagent B. To make 3 mL of the Lumi HRP Development Solution combine 1.5 mL of ChemiHRP Reagent A and 1.5 mL of ChemiHRP Reagent B. Mix thoroughly and gently by inversion or slow pipetting (Avoid shaking or vortexing). Protect the prepared solution from light until use.

Standard Preparation

Always prepare a fresh set of standards for every use. Discard working standard dilutions after use as they do not store well. The following section describes the preparation of a standard curve for duplicate measurements (recommended).

1. Reconstitute the PD-L2 standard sample by adding the volume of Sample Diluent NS indicated on the protein vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 60,000 pg/mL **Stock Standard** Solution.
2. Label nine tubes, Standards 1– 9.
3. Add 257 µL of Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent NS into numbers 2-9.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #9 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	**Stock Standard**	43	257	60,000	8,600
2	Standard#1	75	150	8,600	2,867
3	Standard#2	75	150	2,867	955.6
4	Standard#3	75	150	955.6	318.5
5	Standard#4	75	150	318.5	106.2
6	Standard#5	75	150	106.2	35.4
7	Standard#6	75	150	35.4	11.8
8	Standard#7	75	150	11.8	3.93
9	Blank Control	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum	1: 320 – 1:20
Plasma – Citrate	1: 160 – 1:10
Plasma – EDTA	1: 320 – 1:20
Plasma – Heparin	1: 320 – 1:20
Urine	6.25 - 50%
Milk	6.25 - 50%
Saliva	≤ 50%
Cell Culture Media	≤ 100%

Serum Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Dilute samples at least 1:20 into Sample Diluent NS and assay. Store un-diluted serum at -20ºC or below. Avoid repeated freeze-thaw cycles.

Plasma Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute plasma citrate samples at least 1:10, and dilute plasma EDTA and Heparin at least 1:20 into Sample Diluent NS and assay. Store un-diluted plasma samples at -20ºC or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Cell Culture Supernatants Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants. Assay, or dilute samples into Sample Diluent NSand assay. un-diluted samples at -20ºC or below. Avoid repeated freeze-thaw cycles.

Urine Centrifuge urine at 2,000 x g for 10 minutes to remove debris. Dilute samples at least 1:2 into Sample Diluent NS and assay. Store un-diluted urine samples at -20ºC or below. Avoid repeated freeze-thaw cycles.

Saliva Centrifuge saliva at 800 x g for 10 minutes to remove debris. Collect supernatants. Dilute samples at least 1:2 into Sample Diluent NS and assay. Store un-diluted samples at -20ºC or below. Avoid repeated freeze-thaw cycles.

Milk De-fat milk samples as follows. Centrifuge milk samples at 500 x g for 15 minutes at 4ºC and collect the aqueous fraction using syringe attached to needle. Centrifuge the aqueous fraction at 3,000 x g for 15 minutes at 4ºC and collect the final aqueous fraction (de-fatted milk) using syringe attached to needle. Dilute samples at least 1:2 into Sample Diluent NS and assay. Store un-diluted de-fatted milk at -20ºC or below. Avoid repeated freeze-thaw cycles.

Plate Preparation

The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.

Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4ºC.

For each assay performed, a minimum of two wells must be used as the zero control.

For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

Differences in well absorbance or "edge effects" have not been observed with this assay.

Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.

We recommend that you assay all standards, controls and samples in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4ºC storage.
3. Add 50 µL of all sample or standard to appropriate wells.
4. Add 50 µL of the Antibody Cocktail to each well.
5. Seal the plate and incubate for 30 minutes at room temperature on a plate shaker set to 750 rpm.
6. Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 30 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.
7. Add 50 µL of prepared Lumi HRP Development Solution to each well and incubate for 1 minute in the dark on a plate shaker set to 750 rpm. Further optimization of incubation time vs signal strength can be performed if needed. Avoid introducing bubbles into the wells.
8. Measure the produced light of each well using a microplate luminometer with the following settings: 0.5-1 second/well read time in summation mode (all wavelengths). Relative light unit (RLU) readings may vary between luminometer models. It is recommended to configure instrument settings according to the manufacturer's specifications. Note: Relative light unit (RLU) values may change over the course of the 15-minute reading window.
9. Analyze the data as described below.

Mode:	Luminescence
Instrument settings:	Endpoint
Detection Mode:	All wavelengths
Read Time:	0.5-1 sec
Read:	Top

Note For microplate readers with Pre-Read Optimization option, the Read Height as well as Microplate Optimization is recommended before the first read.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
https://www.abcam.com/en-us/technical-resources/guides/elisa-guide

Technical Support

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For all technical or commercial enquiries please go to:
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Additional information

ASSAY SPECIFICITY
This kit is designed for the quantification of human PD-L2.

The standard protein in this kit is the extracellular domain of human PD-L2.

Native signal was detected in serum, plasma (citrate), plasma (EDTA), plasma (heparin), cell culture supernatant, urine, and milk sample types.

Spiked protein experiments were used to validate cell culture supernatant, and saliva sample types.

CSF, cell extract, and tissue extract samples have not been tested with this kit.

CROSS REACTIVITY
Recombinant PD-L1 was prepared at 50 ng/mL and 1 ng/mL and assayed for cross reactivity. No cross-reactivity was observed.

INTERFERENCE
Recombinant PD-L1 was prepared at 50 ng/mL and 1 ng/mL and tested for interference. No interference with was observed.

SPECIES REACTIVITY
Other species reactivity was determined by measuring 10% 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

Other species reactivity not determined.

CALCULATION
- Preconfigured protocols are available when using SoftMax Pro software from Molecular Devices.
- Calculate the average chemiluminescence value for the blank control (zero) standards. Subtract the average blank control standard chemiluminescence value from all other chemiluminescence values.
- Create a standard curve by plotting the average blank control subtracted chemiluminescence value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.
- Note: Most chemiluminescence reader software or graphing software will plot these values and fit a curve to the data. A four-parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4-parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.
- Determine the concentration of the target protein in the sample by interpolating the blank control subtracted chemiluminescence values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.
- Samples generating chemiluminescence values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at chemiluminescence values less than that of the lowest standard should be retested in a less dilute form.

TYPICAL DATA
Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

Standard Curve Measurements			
Concentration (pg/mL)	RLU		Mean RLU
	1	2	
0	1,294	1,294	1,294
3.93	2,603	2,673	2,638
11.8	5,538	5,476	5,507
35.4	15,976	16,053	16,015
106.2	57,708	57,991	57,850
318.5	196,660	206,400	201,530
955.6	653,680	629,830	641,755
2,867	2,030,000	2,020,500	2,025,250
8,600	6,036,600	6,079,400	6,058,000

Table 1. Example of human PD-L2 standard curve in Sample Diluent NS. The PD-L2 standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

TYPICAL SAMPLE VALUES
Sensitivity:
The calculated minimal detectable dose (MDD) is 1.23 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=16) and adding 2 standard deviations then extrapolating the corresponding concentration.

Recovery

Three concentrations of PD-L2 were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
1% Human Serum	103	101 - 104
1% Human Plasma – EDTA	92	89 - 96
1% Human Plasma – Heparin	100	95 - 107
1% Human Plasma – Citrate	100	92 - 106
1% Human Urine	97	92 - 100
50% Human Saliva	102	101 - 103
25% Human Milk	82	77 - 85
50% Cell Culture Media	99	99 - 99

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-L2 was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	5% Human Serum	10% Human Plasma (Citrate)	5% Human Plasma (EDTA)	5% Human Plasma (Heparin)
Undiluted	pg/mL	490	1080	368	411
	% Expected value	100	100	100	100
2	pg/mL	243	551	197	205
	% Expected value	99	102	107	100
4	pg/mL	117	267	85	107
	% Expected value	96	99	93	104
8	pg/mL	61	142	50	55
	% Expected value	99	105	109	107
16	pg/mL	31	72	24	28
	% Expected value	101	107	105	108

Native PD-L2 was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	50% Human Urine	50% Human Milk
Undiluted	pg/mL	980	80.5
	% Expected value	100	100
2	pg/mL	519	38.8
	% Expected value	106	96
4	pg/mL	262	19.2
	% Expected value	107	95
8	pg/mL	144	8.3
	% Expected value	118	83

Recombinant PD-L2 was spiked into the following biological samples and then diluted in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	50% Human Saliva	50% Cell Culture Media
Undiluted	pg/mL	639	479
	% Expected value	100	100
2	pg/mL	313	234
	% Expected value	98	98
4	pg/mL	153	116
	% Expected value	96	97
8	pg/mL	77	57
	% Expected value	96	95
16	pg/mL	41	28
	% Expected value	103	95

Precision

Mean coefficient of variations of interpolated values of PD-L2 from three concentrations of human serum within the working range of the assay.

	Intra-assay	Inter-assay
N=	5	3
CV (%)	2.9	6.0

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
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