

ab277712 – Human PD-L1 SimpleStep ELISA® Kit (28-8)

For the quantitative measurement of PD-L1 in human serum, plasma (citrate), plasma (EDTA), plasma (heparin), cell culture supernatant, urine, and cell extract.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab277712

This kit is available in a 384-well plate format. This plate utilises smaller volumes of standards and samples per well. Directions for using this format can be found on pages 6-7.

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.

Materials Supplied

Item	Quantity 1 x 96 tests	Storage Condition
Human PD-L1 Capture Antibody 10X	600 µL	+4°C
Human PD-L1 Detector Antibody 10X	600 µL	+4°C
Human PD-L1 Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent CPI2	6 mL	+4°C
Cell Extraction Buffer PTR 5X	10 mL	+4°C
Sample Diluent NS	12 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
TMB Development Solution	12 mL	+4°C
Stop Solution	12 mL	+4°C
SimpleStep Pre-Coated 96-Well Microplate	96 wells	+4°C
Plate Seal	1	+4°C

Note: Antibody Diluent CPI2- This buffer has been reformulated to enhance stability after freeze-thaw cycles while producing data equivalent to the original formulation of antibody diluent CPI previously used in this kit. While we run stock down, you may receive kits containing antibody diluent CPI. This does not affect the way you should use the kit. If you have any questions, please contact Abcam Scientific Support.

Materials Required, Not Supplied

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

Microplate reader capable of measuring absorbance at 450 or 600 nm.
Method for determining protein concentration (BCA assay recommended).
Deionized water.
Multi- and single-channel pipettes.
Tubes for standard dilution.
Plate shaker for all incubation steps.
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 Cell Extraction Buffer PTR (For cell and tissue extracts only): Prepare 1X Cell Extraction Buffer PTR by diluting Cell Extraction Buffer PTR 5X to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR, combine 8 mL deionized water and 2 mL Cell Extraction Buffer PTR 5X. Mix thoroughly and gently. If required protease inhibitors can be added.

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 CPI2. 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 CPI2. Mix thoroughly and gently.

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).

- IMPORTANT:** If the protein standard vial has a volume identified on the label, reconstitute the PD-L1 by adding that volume of Diluent indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the PD-L1 standard by adding 500 µL Diluent. Hold at room temperature for 10 minutes and mix gently. This is the 10,000 pg/mL Stock Standard Solution.
For **serum, plasma, cell culture supernatant** and **urine samples measurements**, use Sample Diluent NS. For **cell extract samples measurements**, use 1X Cell Extraction Buffer PTR.
- Label eight tubes, Standards 1–8.
- Use the same Sample Diluent as used to resuspend the Stock Standard to prepare the standard curve. Add 380 µL of Sample Diluent into tube number 1 and 150 µL of Sample Diluent into numbers 2-8.
- Use the **Stock Standard** to prepare the following dilution series. Standard #8 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	20	380	10,000	500
2	Standard#1	150	150	500	250
3	Standard#2	150	150	250	125
4	Standard#3	150	150	125	62.5
5	Standard#4	150	150	62.5	31.25
6	Standard#5	150	150	31.25	15.63
7	Standard#6	150	150	15.63	7.81
8	Blank Control	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	Range
Serum	11.88 – 95%
Plasma – Citrate*	≤ 95%
Plasma – EDTA*	≤ 95%
Plasma – Heparin*	≤ 50%
Stimulated PBMC Cell Culture Supernatant	3.13 – 100%
Urine*	≤ 25%
HDLM-2 Cell Extract	0.31 – 10 µg/mL

*Based on spiked sample.

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. Assay, or dilute samples into Sample Diluent NS and assay. Store un-diluted serum at -20°C 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. Assay plasma (citrate or EDTA), or dilute samples into Sample Diluent NS and assay. Dilute plasma (heparin) samples at least 1:2 into Sample Diluent NS and assay. Store un-diluted plasma samples at -20°C 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 NS and assay. Store un-diluted samples at -20°C 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 1:4 into Sample Diluent NS and assay. Store un-diluted urine samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Preparation of extracts from cell pellets Collect non-adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 5 minutes at 4°C. Rinse cells twice with PBS. Solubilize pellet at 2×10^7 cell/mL in chilled 1X Cell Extraction Buffer PTR. Incubate on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

Preparation of extracts from adherent cells by direct lysis (alternative protocol) Remove growth media and rinse adherent cells 2 times in PBS. Solubilize the cells by addition of chilled 1X Cell Extraction Buffer PTR directly to the plate (use 750 µL - 1.5 mL 1X Cell Extraction Buffer PTR per confluent 15 cm diameter plate). Scrape the cells into a microfuge tube and incubate the lysate on ice for 15 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

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 4°C.

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 4°C 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 1 hour at room temperature on a plate shaker set to 400 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 10 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 100 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.
Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.
Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.
8. Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.
9. Alternative to 7 – 8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode	Kinetic
Wavelength:	600 nm
Time:	up to 20 min
Interval:	20 sec - 1 min
Shaking:	Shake between readings

Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

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

www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus

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Additional information

ASSAY SPECIFICITY

This kit is designed for the quantification of human PD-L1.

The standard protein in this kit is the extracellular domain of human PD-L1.

Native signal was detected in serum, cell culture supernatant, and cell extract sample types.

Spiked protein experiments were used to validate serum, plasma (citrate), plasma (EDTA), plasma (heparin), cell culture supernatant, and urine sample types.

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

CROSS REACTIVITY

Recombinant human PD-L2, human PD-1, and mouse PD-L1 were each prepared at 50 ng/mL and 500 pg/mL and assayed for cross reactivity. No cross-reactivity was observed.

INTERFERENCE

Recombinant human PD-L2 and human PD-1 were each prepared at 50 ng/mL and 500 pg/mL and tested for interference. No interference with was observed.

SPECIES REACTIVITY

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

50% Rhesus macaque serum interpolated as 134.95 pg/ml on the human curve.

Other species reactivity not determined.

CALCULATION

- Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.
- Create a standard curve by plotting the average blank control subtracted absorbance 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 microplate 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 absorbance 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 absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at absorbance 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)	O.D. 450 nm		Mean O.D.
	1	2	
0	0.056	0.057	0.057
7.81	0.113	0.113	0.113
15.63	0.181	0.171	0.176
31.25	0.285	0.277	0.281
62.50	0.495	0.489	0.492
125	0.896	0.887	0.891
250	1.697	1.686	1.691
500	3.096	3.109	3.103

Table 1. Example of human PD-L1 standard curve in Sample Diluent NS. The PD-L1 standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

Standard Curve Measurements			
Concentration (pg/mL)	O.D. 450 nm		Mean O.D.
	1	2	
0	0.057	0.066	0.062
7.81	0.111	0.113	0.112
15.63	0.170	0.174	0.172
31.25	0.278	0.263	0.271
62.50	0.490	0.472	0.481
125	0.866	0.800	0.833
250	1.672	1.644	1.658
500	3.064	3.030	3.047

Table 2. Example of human PD-L1 standard curve in 1X Cell Extraction PTR. The PD-L1 standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

TYPICAL SAMPLE VALUES

Sensitivity:

The minimal detectable dose (MDD) was determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentration.

Sample Diluent Buffer	N=	Minimal Detectable Dose
Sample Diluent NS	16	3.75 pg/mL
1X Cell Extraction Buffer PTR	16	1.77 pg/mL

Recovery

Three concentrations of PD-L1 were spiked into the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
95% Serum	96	94 - 97
95% Plasma – Citrate	90	87 - 95
95% Plasma – EDTA	90	89 - 92
50% Plasma – Heparin	101	90 - 111
12.5% Stimulated PBMC Cell Culture Supernatant	93	82 - 100
25% Pooled Gender Urine	93	86 - 97
1 µg/mL HDLM-2 Cell Extract	102	99 - 106

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 Sample Diluent NS.

Dilution Factor	Interpolated Value	95% Human Serum	100% Stimulated PBMC Supernatant
Undiluted	pg/mL	109	393
	% Expected value	100	100
2	pg/mL	55	192
	% Expected value	101	97
4	pg/mL	26	90
	% Expected value	95	91
8	pg/mL	13	45
	% Expected value	95	91
16	pg/mL	NL	21
	% Expected value	NL	86

NL – Nonlinear

100% unstimulated PBMC supernatant was measured in duplicate. All values were below the detectable range of the assay.

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

Dilution Factor	Interpolated Value	95% Serum	95% Plasma (Citrate)	50% Plasma (Heparin)	95% Plasma (EDTA)	25% Urine
Undiluted	pg/mL	359	283	334	324	262
	% Expected value	100	100	100	100	100
2	pg/mL	183	145	169	167	137
	% Expected value	102	102	101	103	104
4	pg/mL	90	75	83	83	70
	% Expected value	100	106	99	103	107
8	pg/mL	57	41	50	42	35
	% Expected value	108	116	119	103	108
16	pg/mL	23	19	20	20	17
	% Expected value	104	109	98	100	104

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

Dilution Factor	Interpolated Value	10 µg/mL HDLM-2 Extract
Undiluted	pg/mL	412
	% Expected value	100
2	pg/mL	196
	% Expected value	95
4	pg/mL	96
	% Expected value	94
8	pg/mL	48
	% Expected value	93
16	pg/mL	24
	% Expected value	94

Precision

Mean coefficient of variations of interpolated values of PD-L1 from two concentrations of PBMC stimulated with 1.5% PHA-M within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	3
CV (%)	2.7	4.3

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

www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus

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DIRECTIONS FOR 384-WELL PLATE FORMAT:

Materials Supplied for 384-well Format

Item	Quantity	Storage Condition
Human PD-L1 Capture Antibody 10X	600 µL	+4°C
Human PD-L1 Detector Antibody 10X	600 µL	+4°C
Human PD-L1 Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent CPI2	6 mL	+4°C
Cell Extraction Buffer PTR 5X	50 mL	+4°C
Sample Diluent NS	2 x 50 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
TMB Development Solution	2 x 12 mL	+4°C
Stop Solution	2 x 12 mL	+4°C
SimpleStep Pre-Coated 384-Well Microplate	384 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:

Microplate reader capable of measuring absorbance at 450 or 600 nm in a 384-well plate.

Method for determining protein concentration (BCA assay recommended).

Deionized water.

Multi- and single-channel pipettes.

Tubes for standard dilution.

Plate shaker for all incubation steps.

Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

Optional: Automated liquid handler.

Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for one full plate. The sample volumes below are sufficient for running all 384 wells; adjust volumes as needed for the number of samples and dilution scheme for 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 Cell Extraction Buffer PTR (For cell and tissue extracts only): Prepare 1X Cell Extraction Buffer PTR by diluting Cell Extraction Buffer PTR 5X to 1X with deionized water. To make 250 mL 1X Cell Extraction Buffer PTR combine 200 mL deionized water and 50 mL Cell Extraction Buffer PTR 5X.

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 CPI2. To make 6 mL of the Antibody Cocktail combine 600 µL 10X Capture Antibody and 600 µL 10X Detector Antibody with 4.8 mL Antibody Diluent CPI2. Mix thoroughly and gently.

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).

- IMPORTANT:** If the protein standard vial has a volume identified on the label, reconstitute the PD-L1 by adding that volume of Diluent indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the PD-L1 standard by adding 500 µL Diluent. Hold at room temperature for 10 minutes and mix gently. This is the 10,000 pg/mL Stock Standard Solution.

For **serum, plasma, cell culture supernatant** and **urine samples measurements**, use Sample Diluent NS. For **cell extract samples measurements**, use 1X Cell Extraction Buffer PTR.
- Label seven tubes, Standards 1–7.
- Use the same Sample Diluent as used to resuspend the Stock Standard to prepare the standard curve. Add 190 µL of Sample Diluent into tube number 1 and 75 µL of Sample Diluent into numbers 2-7.
- Use the **Stock Standard** to prepare the following dilution series. Standard #7 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	10	190	10,000	500
2	Standard#1	75	75	500	250
3	Standard#2	75	75	250	125
4	Standard#3	75	75	125	62.5
5	Standard#4	75	75	62.5	31.25
6	Standard#5	75	75	31.25	15.63
7	Blank Control	0	75	0	0

Plate Preparation

The 384-well plate included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.

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 for 384-well Plate Format

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. Add 12.5 μL of all sample or standard to appropriate wells.
3. Add 12.5 μL of the Antibody Cocktail to each well.
4. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 700 rpm.
5. Wash each well with 3 x 100 μL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 100 μL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 10 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.
6. Add 25 μL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 700 rpm.

Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.

Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.₆₀₀ equal to 1.0.

7. Add 25 μL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading. Proper mixing of the Stop Solution is required for proper measurement.
8. Alternative to 6 – 7: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode	Kinetic
Wavelength:	600 nm
Time:	up to 20 min
Interval:	20 sec – 1 min
Shaking:	Shake between readings

Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 25 μL Stop Solution to each well and recording the OD at 450 nm.

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

www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus