Human PD-L1 ELISA Kit

Enzyme-linked Immunosorbent Assay for quantitative detection of human PD-L1

Catalog Numbers BMS2327 and BMS2327TEN

Pub. No. MAN0026033 **Rev.** A.0 (30)

⚠️ **WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Human PD-L1 ELISA Kit is an enzyme-linked immunosorbent assay for the quantitative detection of human PD-L1.

Summary

Programmed T cell death ligand 1 (PD-L1) is a transmembrane protein ligand that binds to and activates programmed T cell death 1 (PD-1) receptors on the surface of T cells. The binding of PD-L1 to PD-1 results in suppression of T cell activity and reduction of T cell-mediated cytotoxicity. Thus, PD-L1 and PD-1 are immune down-regulators or immune checkpoint *off switches*.

It is a 40 kDa protein that has been speculated to play a major role in suppressing the immune system during particular events such as pregnancy, tissue allografts, autoimmune disease, and other disease states such as hepatitis. Normally the immune system reacts to foreign antigens where there is some accumulation in the lymph nodes or spleen that triggers a proliferation of antigen-specific CD8+ T cell. The formation of PD-1 receptor / PD-L1 or B7.1 receptor /PD-L1 ligand complex transmits an inhibitory signal that reduces the proliferation of these CD8+ T cells at the lymph nodes, and supplementary to that, PD-1 is also able to control the accumulation of foreign antigen specific T cells in the lymph nodes through apoptosis, which is further mediated by a lower regulation of the gene Bcl-2.

For literature updates, refer to our website.

Principles of the test

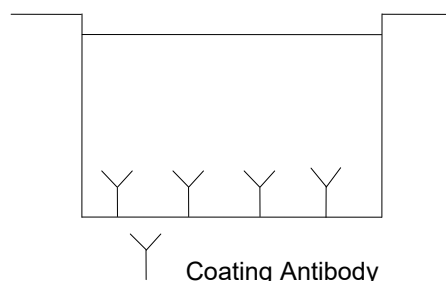An anti-human PD-L1 coating antibody is adsorbed onto microwells.

Coating Antibody

Fig. 1 Coated microwell

Human PD-L1 present in the sample or standard binds to antibodies adsorbed to the microwells.
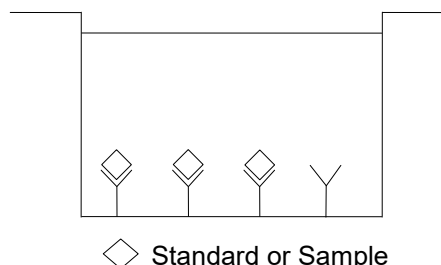

◇ Standard or Sample

Fig. 2 First incubation

Following incubation unbound biological components are removed during a wash step and a biotin-conjugated anti-human PD-L1 antibody is added and binds to human PD-L1 captured by the first antibody.
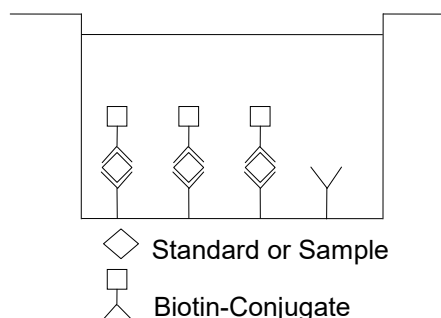

◇ Standard or Sample

Biotin-Conjugate

Fig. 3 Second incubation

Following incubation unbound biotin-conjugated anti-human PD-L1 antibody is removed during a wash step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-human PD-L1 antibody.
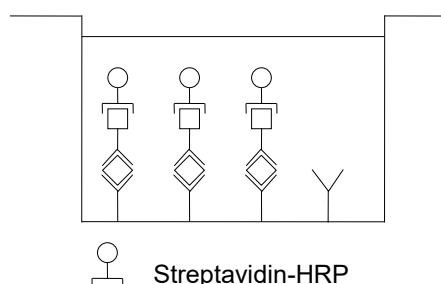

Streptavidin-HRP

Fig. 4 Third incubation

Following incubation unbound Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

For Research Use Only. Not for use in diagnostic procedures.
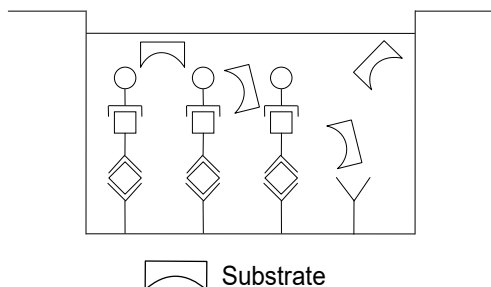
Thermo Fisher
SCIENTIFIC

Substrate

Fig. 5 Fourth incubation

A colored product is formed in proportion to the amount of human PD-L1 present in the sample or standard. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from 7 human PD-L1 standard dilutions and human PD-L1 sample concentration determined.
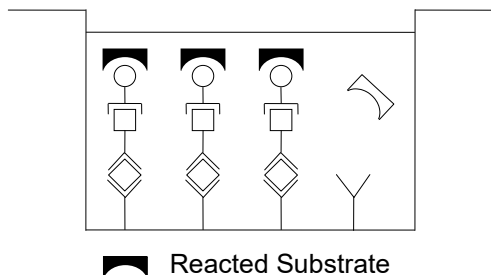

Reacted Substrate

Fig. 6 Stop reaction

Reagents provided

Table 1   Reagents provided in the Human PD-L1 ELISA Kit

Contents	Cat. No. BMS2327 (96 tests)	Cat. No. BMS2327TEN (10 x 96 tests)
Aluminum pouch with a Microwell Plate coated with polyclonal antibody to human PD-L1	1 pouch	10 pouches
Biotin-Conjugate anti-human PD-L1 monoclonal antibody (120 µL)	1 vial	10 vials
Streptavidin-HRP (150 µL)	1 vial	10 vials
Human PD-L1 Standard lyophilized, 600 pg/mL upon reconstitution	2 vials	10 vials
Sample Diluent (12 mL)	1 bottle	6 bottles
Assay Buffer Concentrate 20x (PBS with 1% Tween™ 20, 10% BSA) (5 mL)	1 vial	3 vials
Wash Buffer Concentrate 20x (PBS with 1% Tween™ 20) (50 mL)	1 bottle	6 bottles
Substrate Solution (tetramethyl-benzidine) (15 mL)	1 vial	10 vials
Stop Solution (1M Phosphoric acid)	1 vial (15 mL)	1 vial (100 mL)
Adhesive film	6 films	30 films

Storage instructions – ELISA kit

Store kit reagents between 2°C and 8°C. Immediately after use remaining reagents should be returned to cold storage (2–8°C). Expiry of the kit and reagents is stated on labels.

Expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, this reagent is not contaminated by the first handling.

Sample collection and storage instructions

Cell lysates, cell culture supernatant, serum, and plasma (citrate, heparin, EDTA) were tested with this assay. Other biological samples might be suitable for use in the assay.

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic samples.

Samples should be aliquoted and must be stored frozen at –20°C to avoid loss of bioactive human PD-L1.

Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

Materials required but not provided

- 5 mL and 10 mL graduated pipettes
- 5 µL to 1,000 µL adjustable single channel micropipettes with disposable tips
- 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microplate shaker
- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform regression analysis

Precautions for use

- All reagents should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses, and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.
- Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Do not mix or substitute reagents with those from other lots or other sources.
- Do not use kit reagents beyond expiration date on label.
- Do not expose kit reagents to strong light during storage or incubation.
- Do not pipet by mouth.
- Do not eat or smoke in areas where kit reagents or samples are handled.
- Avoid contact of skin or mucous membranes with kit reagents or samples.
- Rubber or disposable latex gloves should be worn while handling kit reagents or samples.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.

- Exposure to acid inactivates the conjugate.
- Glass-distilled water or deionized water must be used for reagent preparation.
- Substrate solution must be at room temperature prior to use.
- Decontaminate and dispose samples and all potentially contaminated materials as if they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.
- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

Preparation of reagents

1. Buffer Concentrates should be brought to room temperature and should be diluted before starting the test procedure.
2. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved.

Wash buffer (1x)

1. Pour entire contents (50 mL) of the Wash Buffer Concentrate (20x) into a clean 1,000 mL graduated cylinder. Bring to final volume of 1,000 mL with glass-distilled or deionized water. Mix gently to avoid foaming.
2. Transfer to a clean wash bottle and store at 2–25°C. Please note that Wash Buffer (1x) is stable for 30 days.
3. Wash Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Wash Buffer Concentrate (20x) (mL)	Distilled Water (mL)
1 - 6	25	475
1 - 12	50	950

Assay buffer (1x)

1. Pour the entire contents (5 mL) of the Assay Buffer Concentrate (20x) into a clean 100 mL graduated cylinder. Bring to final volume of 100 mL with distilled water. Mix gently to avoid foaming.
2. Store at 2–8°C. The Assay Buffer (1x) is stable for 30 days.
3. Assay Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Assay Buffer Concentrate (20x) (mL)	Distilled Water (mL)
1–6	2.5	47.5
1–12	5.0	95.0

Biotin-Conjugate

Note: The Biotin-Conjugate should be used within 30 minutes after dilution.

Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

Number of Strips	Biotin-Conjugate (mL)	Assay Buffer (1x) (mL)
1 - 6	0.06	5.94
1 - 12	0.12	11.88

Streptavidin-HRP

Note: The Streptavidin-HRP should be used within 30 minutes after dilution.

Make a 1:200 dilution of the concentrated Streptavidin-HRP solution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

Number of Strips	Streptavidin-HRP (mL)	Assay Buffer (1x) (mL)
1 - 6	0.03	5.97
1 - 12	0.06	11.94

Human PD-L1 standard

1. Reconstitute human PD-L1 standard by addition of distilled water. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 600 pg/mL).
2. Allow the standard to reconstitute for 10-30 minutes. Mix well prior to making dilutions.
3. The standard has to be used immediately after reconstitution and cannot be stored.
4. Standard dilutions can be prepared directly on the microwell plate (see "Test protocol" on page 4) or alternatively in tubes (see "External standard dilution" on page 3).

External standard dilution

1. Label 7 tubes, one for each standard point: S1, S2, S3, S4, S5, S6, S7.
2. Prepare 2-fold serial dilutions for the standard curve as follows: Pipette 225 µL of Sample Diluent into each tube.
3. Pipette 225 µL of reconstituted standard (concentration = 600 pg/mL) into the first tube, labeled S1, and mix (concentration of S1 = 300 pg/mL).
4. Pipette 225 µL of this dilution into the second tube, labeled S2, and mix thoroughly before the next transfer.
5. Repeat serial dilutions 5 more times thus creating the points of the standard curve (see Figure 7).
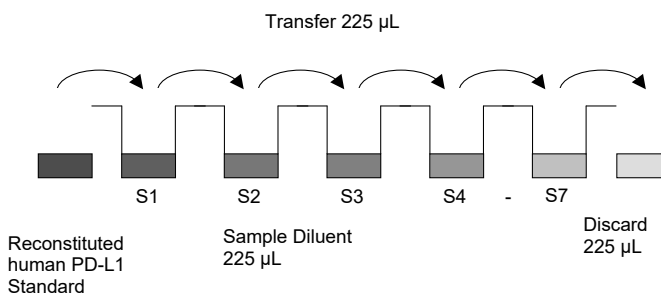
   Sample Diluent serves as blank.

Transfer 225 µL

Reconstituted human PD-L1 Standard

Sample Diluent 225 µL

Discard 225 µL

Fig. 7 Dilute standards - tubes

Test protocol

1. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank and optional control sample should be assayed in duplicate. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2–8°C sealed tightly.

2. Wash the microwell strips twice with approximately 400 µL Wash Buffer per well with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about 10 – 15 seconds before aspiration. Take care not to scratch the surface of the microwells.

   After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing. Alternatively microwell strips can be placed upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.

3. Standard dilution on the microwell plate (Alternatively the standard dilution can be prepared in tubes – see "External standard dilution" on page 3):

   Add 100 µL of Sample Diluent in duplicate to all standard wells. Pipette 100 µL of prepared standard (see Preparation of Standard "Human PD-L1 standard" on page 3, concentration = 600 pg/mL), in duplicate, into well A1 and A2 (see Table 2). Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1 S1 = 300 pg/mL), and transfer 100 µL to wells B1 and B2, respectively (see Figure 8). Take care not to scratch the inner surface of the microwells. Continue this procedure 5 times, creating two rows of human PD-L1 standard dilutions, ranging from 300 pg/mL to 4.7 pg/mL. Discard 100 µL of the contents from the last microwells (S7) used.

Transfer 100 µL

S1   S2   S3   S4   -   S7

Reconstituted human PD-L1 Standard
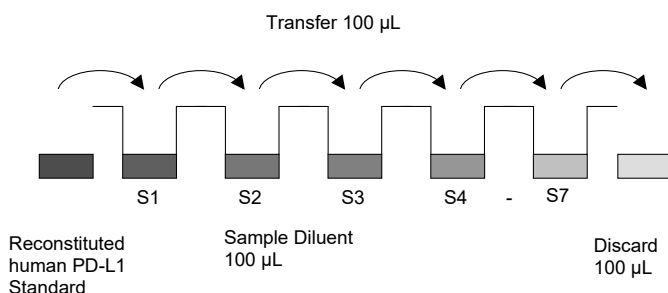
Sample Diluent 100 µL

Discard 100 µL

Fig. 8 Dilute standards - microwell plate

Table 2 Table depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

	1	2	3	4
A	Standard 1 (300.0 pg/mL)	Standard 1 (300.0 pg/mL)	Sample 1	Sample 1
B	Standard 2 (150.0 pg/mL)	Standard 2 (150.0 pg/mL)	Sample 2	Sample 2
C	Standard 3 (75.0 pg/mL)	Standard 3 (75.0 pg/mL)	Sample 3	Sample 3
D	Standard 4 (37.5 pg/mL)	Standard 4 (37.5 pg/mL)	Sample 4	Sample 4
E	Standard 5 (18.8 pg/mL)	Standard 5 (18.8 pg/mL)	Sample 5	Sample 5
F	Standard 6 (9.4 pg/mL)	Standard 6 (9.4 pg/mL)	Sample 6	Sample 6
G	Standard 7 (4.7 pg/mL)	Standard 7 (4.7 pg/mL)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

In case of an external standard dilution (see "External standard dilution" on page 3), pipette 100 µL of these standard dilutions (S1 – S7) in the standard wells according to Table 2.

4. Add 100 µL of Sample Diluent in duplicate to the blank wells.
5. Add 50 µL of Sample Diluent in duplicate to the sample wells.
6. Add 50 µL of sample in duplicate to the sample wells.
7. Cover with an adhesive film and incubate at room temperature (18–25°C) for 2 hours, on a microplate shaker.
8. Prepare Biotin-Conjugate (see Preparation of Biotin-Conjugate "Biotin-Conjugate" on page 3).
9. Remove adhesive film and empty wells. Wash microwell strips 4 times according to point 3 of the test protocol. Proceed immediately to the next step.
10. Add 100 µL of diluted Biotin-Conjugate to all wells, including the blank wells.
11. Cover with an adhesive film and incubate at room temperature (18–25°C) for 1 hour, on a microplate shaker.
12. Prepare Streptavidin-HRP (see Preparation of Streptavidin-HRP "Streptavidin-HRP" on page 3).
13. Remove adhesive film and empty wells. Wash microwell strips 4 times according to point 3 of the test protocol. Proceed immediately to the next step.
14. Add 100 µL of diluted Streptavidin-HRP to all wells, including the blank wells.
15. Cover with an adhesive film and incubate at room temperature (18–25°C) for 30 minutes, on a microplate shaker.
16. Remove adhesive film and empty wells. Wash microwell strips 4 times according to point 3 of the test protocol. Proceed immediately to the next step.
17. Pipette 100 µL of TMB Substrate Solution to all wells.
18. Incubate the microwell strips at room temperature (18–25°C) for 30 minutes. Avoid direct exposure to intense light.

    The color development on the plate should be monitored and the substrate reaction stopped (see next point of this protocol) before positive wells are no longer properly recordable. Determination of the ideal time period for color development has to be done individually for each assay.

    It is recommended to add the stop solution when the highest standard has developed a dark blue color. Alternatively the color development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9 – 0.95.

19. Stop the enzyme reaction by quickly pipetting 100 µL of Stop Solution into each well. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2–8°C in the dark.

20. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the standards.

Note: If instructions of this protocol have been followed samples have been diluted 1:2, the concentration read from the standard curve must be multiplied by the dilution factor (x2).

Calculation of results

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 percent of the mean value.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human PD-L1 concentration on the abscissa. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).
- To determine the concentration of circulating human PD-L1 for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding human PD-L1 concentration.
- If instructions in this protocol have been followed, samples have been diluted 1:2, the concentration read from the standard curve must be multiplied by the dilution factor (x2).
- Calculation of samples with a concentration exceeding standard 1 may result in incorrect human PD-L1 levels. Such samples require further external predilution (according to expected human PD-L1 values) with Sample Diluent in order to precisely quantitate the actual human PD-L1 level.
- It is suggested that each testing facility establishes a control sample of known human PD-L1 concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.
- A representative standard curve is shown in Figure 9.

  Note: Do not use this standard curve to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.
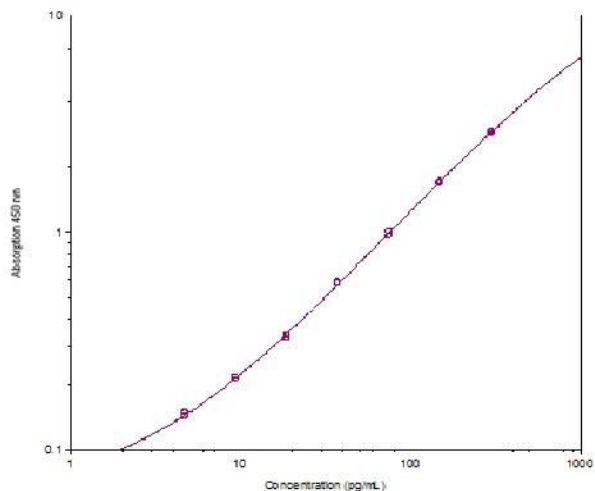

Fig. 9 Representative standard curve for human PD-L1 ELISA. Human PD-L1 was diluted in serial 2-fold steps in Sample Diluent.

Table 3   Typical data using the human PD-L1 ELISA.

Measuring wavelength: 450 nm

Reference wavelength: 620 nm

Standard	human PD-L1 Concentration (pg/mL)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V. (%)
1	300.0	3.009 3.000	3.005	0.2
2	150.0	1.831 1.882	1.857	1.9
3	75.0	1.111 1.095	1.103	1.0
4	37.50	0.606 0.620	0.613	1.6
5	18.75	0.377 0.355	0.366	4.3
6	9.38	0.227 0.223	0.225	1.3
7	4.69	0.158 0.160	0.159	0.8
Blank	0.0	0.084 0.080	0.082	3.1

The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). Furthermore, shelf life of the kit may affect enzymatic activity and thus color intensity. Values measured are still valid.

Limitations

- Since exact conditions may vary from assay to assay, a standard curve must be established for every run.
- Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.
- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Empty wells completely before dispensing fresh wash solution, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.

Performance characteristics

Sensitivity
The limit of detection of human PD-L1 defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 0.60 pg/mL (mean of 3 independent assays).

Reproducibility

Intra-assay

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of serum samples containing different concentrations of human PD-L1. 2 standard curves were run on each plate. Data below show the mean human PD-L1 concentration and the coefficient of variation for each

sample (see Table 4). The calculated overall intra-assay coefficient of variation was 2.1%.

Table 4   The mean human PD-L1 concentration and the coefficient of variation for each sample

Sample	Experiment	Mean human PD-L1 Concentration (pg/mL)	Coefficient of Variation (%)
1	1	458.3	1.2
	2	461.3	0.9
	3	480.7	0.5
2	1	281.7	1.0
	2	287.0	1.2
	3	296.2	1.4
3	1	135.9	2.2
	2	144.0	1.8
	3	144.1	1.8
4	1	78.4	1.7
	2	77.5	3.0
	3	85.7	2.5
5	1	63.0	2.9
	2	60.8	1.9
	3	65.7	2.2
6	1	46.1	1.6
	2	46.1	2.2
	3	44.7	2.4
7	1	39.6	2.4
	2	39.0	2.4
	3	36.9	6.0
8	1	27.1	3.2
	2	26.4	1.6
	3	25.0	2.8

Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of serum samples containing different concentrations of human PD-L1. 2 standard curves were run on each plate. Data below show the mean human PD-L1 concentration and the coefficient of variation calculated on 18 determinations of each sample (see Table 5). The calculated overall inter-assay coefficient of variation was 3.4%.

Table 5   The mean human PD-L1 concentration and the coefficient of variation of each sample

Sample	Mean human PD-L1 Concentration (pg/mL)	Coefficient of Variation (%)
1	466.8	2.6
2	288.3	2.5
3	141.3	3.3
4	80.5	5.6
5	63.2	3.9
6	45.7	1.8
7	38.5	3.7
8	26.1	4.0

Spike recovery

The spike recovery was evaluated by spiking 3 levels of human PD-L1 into serum, plasma (EDTA, heparin, citrate), and cell culture supernatant. Recoveries were determined with 2 replicates each. The amount of endogenous human PD-L1 in unspiked samples was subtracted from the spike values.

Sample matrix	Spike high (%)		Spike medium (%)		Spike low (%)	
	Mean	Range	Mean	Range	Mean	Range
Serum	108	99–118	108	97–116	128	110–138
Plasma (EDTA)	76	69–82	80	73–86	83	74–91
Plasma (citrate)	90	82–102	91	81–103	101	89–116
Plasma (heparin)	93	90–98	99	95–104	110	103–122
Cell culture supernatant	105	104–106	105	104–107	99	98–99

Dilution parallelism

Serum and plasma (EDTA, citrate, heparin) samples with different levels of human PD-L1 were analyzed at serial 2-fold dilutions with 4 replicates each.

Sample matrix	Recovery of exp. val.		
	Dilution	Mean (%)	Range (%)
Serum	1:4	94	86–103
	1:8	99	95–102
	1:16	101	94–117
Plasma (EDTA)	1:4	110	99–1123
	1:8	112	106–117
	1:16	110	94–132
Plasma (citrate)	1:4	97	90–110
	1:8	96	89–101
	1:16	97	81–111
Plasma (heparin)	1:4	89	78–96
	1:8	96	92–100
	1:16	104	96–116

Sample stability

Freeze-Thaw stability

Aliquots of serum samples (spiked or unspiked) were stored at -20°C and thawed 3 times, and the human PD-L1 levels determined.

There was no significant loss of human PD-L1 immunoreactivity detected by freezing and thawing.

Storage stability

Aliquots of serum samples (spiked or unspiked) were stored at –20°C, 2–8°C, room temperature, and at 37°C, and the human PD-L1 level determined after 24 hours. There was no significant loss of PD-L1 immunoreactivity detected during storage under above conditions.

Specificity

The assay detects both natural and recombinant human PD-L1. The cross-reactivity and interference of PD-1, PD-L2, and LAG-3 was tested by spiking these proteins into a human serum sample No cross-reactivity or interference was detected.

Expected values

Panels of 40 serum as well as plasma samples (EDTA, citrate, heparin), from randomly selected healthy donors (males and females) were tested for PD-L1 The detected PD-L1 levels ranged between 50 pg/mL and not detectable. The levels measured may vary with the sample collection used.

Sample matrix	Number of samples evaluated	Mean (pg/mL)	Range (pg/mL)	Standard deviation (pg/mL)
Serum	40	26.5	11.5–50.8	8.1
Plasma (EDTA)	40	14.8	5.0–32.2	6.1
Plasma (citrate)	40	19	6.6–41.2	7.6
Plasma (heparin)	40	20.3	nd[1]–56.6	8.8

[1] Nondetectable

Reagent preparation summary

Wash buffer (1x)
Add Wash Buffer Concentrate 20x (50 mL) to 950 mL distilled water.

Number of Strips	Wash Buffer Concentrate (mL)	Distilled Water (mL)
1 - 6	25	475
1 - 12	50	950

Assay buffer (1x)
Add Assay Buffer Concentrate 20x (5 mL) to 95 mL distilled water.

Number of Strips	Assay Buffer Concentrate (mL)	Distilled Water (mL)
1–6	2.5	47.5
1–12	5.0	95.0

Biotin-Conjugate
Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

Number of Strips	Biotin-Conjugate (mL)	Assay Buffer (1x) (mL)
1 - 6	0.06	5.94
1 - 12	0.12	11.88

Streptavidin-HRP
Make a 1:200 dilution of the concentrated Streptavidin-HRP solution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

Number of Strips	Streptavidin-HRP (mL)	Assay Buffer (1x) (mL)
1 - 6	0.03	5.97
1 - 12	0.06	11.94

Human PD-L1 standard
Reconstitute human PD-L1 standard with distilled water. (Reconstitution volume is stated on the label of the standard vial.)

Test protocol summary

1. Determine the number of microwell strips required.
2. Wash microwell strips twice with Wash Buffer.

3. Standard dilution on the microwell plate: Add 100 µL Sample Diluent, in duplicate, to all standard wells. Pipette 100 µL prepared standard into the first wells and create standard dilutions by transferring 100 µL from well to well. Discard 100 µL from the last wells.

   Alternatively external standard dilution in tubes (see "External standard dilution" on page 3): Pipette 100 µL of these standard dilutions in the microwell strips.
4. Add 100 µL of Sample Diluent in duplicate to the blank wells.
5. Add 50 µL of Sample Diluent to the sample wells.
6. Add 50 µL of sample in duplicate to the sample wells.
7. Cover microwell strips and incubate 2 hours at room temperature (18–25°C) on a microplate shaker.
8. Prepare Biotin-Conjugate.
9. Empty and wash microwell strips 4 times with Wash Buffer.
10. Add 100 µL diluted Biotin-Conjugate to all wells.
11. Cover microwell strips and incubate 1 hour at room temperature (18–25°C) on a microplate shaker.
12. Prepare Streptavidin-HRP.
13. Empty and wash microwell strips 4 times with Wash Buffer.
14. Add 100 µL diluted Streptavidin-HRP to all wells.
15. Cover microwell strips and incubate 30 minutes at room temperature (18–25°C) on a microplate shaker.
16. Empty and wash microwell strips 4 times with Wash Buffer.
17. Add 100 µL of TMB Substrate Solution to all wells.
18. Incubate the microwell strips for about 30 minutes at room temperature (18–25°C).
19. Add 100 µL Stop Solution to all wells.
20. Blank microwell reader and measure color intensity at 450 nm.

Note: If instructions of this protocol have been followed, samples have been diluted 1:2 and the concentration read from the standard curve must be multiplied by the dilution factor (x2).

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