BioLegend®

Enabling Legendary Discovery™

LEGEND MAX™

ELISA Kit

Human PD-L2

Cat. No. 447607

ELISA Kit for Accurate Quantitation of Human PD-L2 from Serum, Plasma and Other Biological Fluids

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It is highly recommended that this manual be read in its entirety before using this product. Do not use this kit beyond the expiration date.

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Introduction:

PD-L2 (CD273, PDCD1LG2, B7-DC) and its related protein PD-L1 are ligands for the Programmed Cell Death Protein 1 (PD1). A wide variety of immune and non-immune cells can express PD-L2, but it is mainly expressed in dendritic cells. Through its interaction with PD1, PD-L2 can act as an immune checkpoint molecule by negatively regulating T cell proliferation and cytokine production. However, many studies reported that PD-L2 can also stimulate T cell proliferation through interaction with receptors other than PD1. High level of PD-L2 expression can promote tumor metastasis because of its ability to suppress T cells-mediated anti-tumor activity. Moreover, increased PD-L2 expression might dampen the effectiveness of anti-PD-L1 treatments.

The LEGEND MAX™ Human PD-L2 ELISA Kit is a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with a 96-well strip plate that is pre-coated with anti-human PD-L2 monoclonal antibody. The detection antibody is a biotinylated anti-human PD-L2 monoclonal antibody. This kit is specifically designed for the accurate quantitation of human PD-L2 from serum and plasma. It is analytically validated with ready-to-use reagents.

Materials Provided:

Description	Quantity	Volume	Part #
Human PD-L2 pre-coated 96-well Strip Microplate	1 plate		750002215
Human PD-L2 Detection Antibody	1 bottle	12 mL	750002214
Human PD-L2 Standard	1 vial	lyophilized	750002218
Avidin HRP	1 bottle	12 mL	77897
Assay Buffer B	1 bottle	25 mL	79128
Wash Buffer (20x)	1 bottle	50 mL	78233
Substrate Solution F	1 bottle	12 mL	79132
Stop Solution	1 bottle	12 mL	79133
Plate Sealers	4 sheets		78101

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Materials to be Provided by the End-User:

- Microplate reader able to measure absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 1 µL to 1,000 µL
- Deionized water
- Wash bottle or automated microplate washer
- Log-Log graph paper or software for data analysis
- Polypropylene tubes to prepare standard dilutions
- Timer
- Plate Shaker
- Polypropylene vials

Storage Information:

Store unopened kit components between 2°C and 8°C. Do not use this kit beyond its expiration date.

Opened or Reconstituted Components	
Microplate wells	If not all microplate strips are used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal. Store between 2°C and 8°C for up to one month.
Standard	The remaining reconstituted standard stock solution can be aliquoted into polypropylene vials and stored at -70°C for up to one month. Avoid repeated freeze-thaw cycles.
Avidin-HRP	Store opened reagent bottles at 2° - 8°C and use within 1 month
Assay Buffer B	
Wash Buffer (20X)	
Substrate Solution F	
Stop Solution	

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online at BioLegend's website for details (www.biolegend.com/msds).
2. Substrate Solution F is harmful if inhaled or ingested. Avoid skin, eye and clothing contact.
3. To reduce the likelihood of blood-borne transmission of infectious agents,

handle all serum, plasma and other biological fluids in accordance with NCCLS regulations.

4. Stop Solution contains strong acid. *Wear eye, hand, and face protection.*
5. Before disposing of the plate, rinse it with an excess amount of tap water.

Specimen Collection and Handling:

Specimens should be clear and non-hemolyzed. If possible, unknown samples should be run at a number of dilutions to determine the optimal dilution factor that will ensure accurate quantitation.

Cell Culture Supernatant: If necessary, centrifuge all samples to remove debris prior to analysis. It is recommended that samples be stored at < -70°C. Avoid repeated freeze-thaw cycles.

Serum: Use a serum separator tube and allow clotting for at least 30 minutes, then centrifuge for 10 minutes at 1,000 x *g*. Remove serum layer and assay immediately or store serum samples at < -70°C. Avoid repeated freeze-thaw cycles.

Plasma: Collect blood samples in citrate, heparin or EDTA containing tubes. Centrifuge for 10 minutes at 1,000 x *g* within 30 minutes of collection. Assay immediately or store plasma samples at < -70°C. Avoid repeated freeze-thaw cycles.

Reagent and Sample Preparation:

Note: All reagents should be diluted immediately prior to use.

1. Dilute the 20X Wash Buffer to 1X with deionized water. For example, make 1 liter of 1X Wash Buffer by adding 50 mL of 20X Wash Buffer to 950 mL of deionized water. If crystals have formed in the 20X Wash Buffer, bring to room temperature and vortex until dissolved.
2. Reconstitute the lyophilized Human PD-L2 Standard by adding the volume of Assay Buffer B to make the 10,000 pg/mL standard stock solution (Refer to LEGEND MAX Kit Lot-Specific Certificate of Analysis/LEGEND MAX Kit Protocol). Allow the reconstituted standard to sit at room temperature for 15-20 minutes, then briefly vortex to mix completely.
3. In general, a 800-fold dilution in Assay Buffer B is recommended for healthy serum and plasma samples, but samples can be diluted further to fit within the range of the assay as determined by the end user.

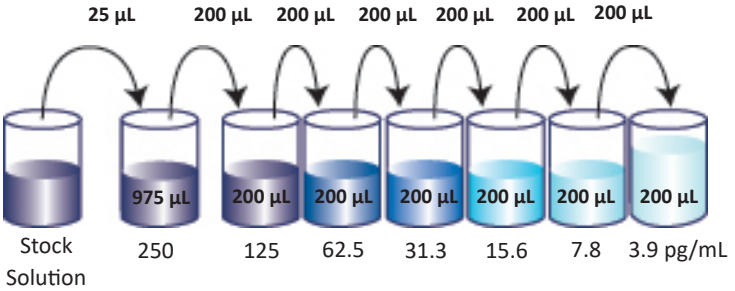
Assay Procedure:

Note: Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this kit.

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.

2. If not all microplate strips will be used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal.

3. Prepare 1000 µL of the 250 pg/mL top standard by adding 25 uL of the 10,000 pg/mL standard stock solution into 975 µL Assay Buffer B. Perform six two-fold serial dilutions of the 250 pg/mL top standard in separate tubes using Assay Buffer B as the diluent. Thus, the hPD-L2 standard concentrations in the tubes are 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.3 pg/mL, 15.6 pg/mL, 7.8 pg/mL and 3.9 pg/mL, respectively. Assay Buffer B serves as the zero standard (0 pg/mL).



4. Wash the plate 4 times with at least 300 µL of 1X Wash Buffer per well and blot any residual buffer by firmly tapping the plate upside down on absorbent paper. All subsequent washes should be performed similarly.

5. Add 50 µL of Assay Buffer B to each well that will contain either standard dilutions or samples. Then add 50 µL of standard dilutions or samples to the appropriate wells.

6. Seal the plate with a Plate Sealer included in the kit and incubate the plate for 2 hours at room temperature with shaking.

7. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.

8. Add 100 µL of Human PD-L2 Detection Antibody solution to each well, seal the plate and incubate at room temperature for 1 hour while shak-

ing.

9. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.

10. Add 100 µL of Avidin-HRP solution to each well, seal the plate and incubate at room temperature for 30 minutes while shaking.

11. Discard the contents of the plate into a sink, then wash the plate 5 times with 1X Wash Buffer as in step 4. For this final wash, soak wells in 1X Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.

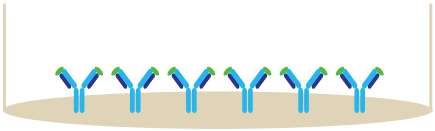
12. Add 100 µL of Substrate Solution F to each well and incubate for 15 minutes in the dark. Wells containing Human PD-L2 should turn blue in color with an intensity proportional to its concentration. It is not necessary to seal the plate during this step.

13. Stop the reaction by adding 100 µL of Stop Solution to each well. The solution color should change from blue to yellow.

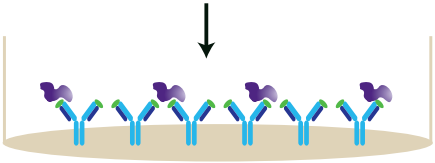
14. Read absorbance at 450 nm within 30 minutes. If the reader is capable of reading at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

Assay Procedure Summary

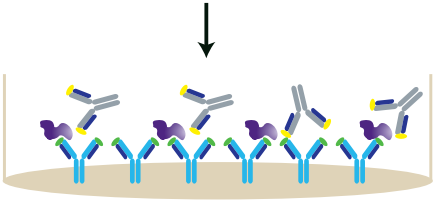
1. Wash 4 times.
   Add 50 µL Assay Buffer B to standard wells and sample wells



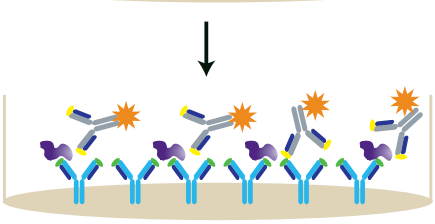
2. Add 50 µL of standard or sample, incubate 2 hr, RT, shaking



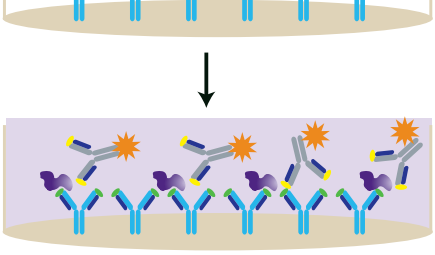
3. Wash 4 times
   Add 100 µL of Detection Antibody solution. Incubate 1 hr, RT, shaking



4. Wash 4 times
   Add 100 µL Avidin-HRP solution
   Incubate 30 min, RT, shaking



5. Wash 5 times
   Add 100 µL Substrate Solution F
   Incubate 15 min, RT, in the dark



6. Add 100 µL Stop Solution

7. Read absorbance at 450 nm and 570 nm

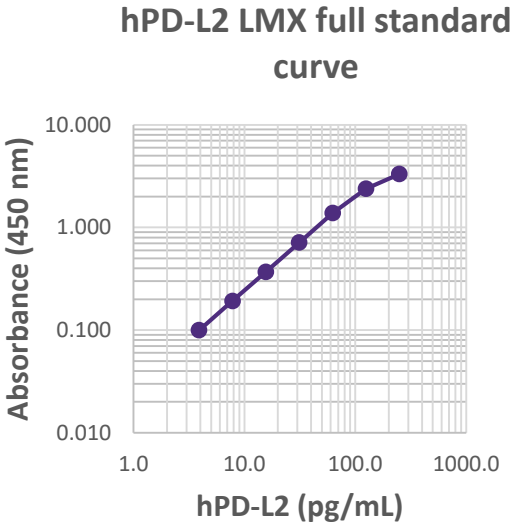
Calculation of Results:

The data can be best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If an appropriate software is not available, use log-log graph paper to determine sample concentrations. Determine the mean absorbance for each set of duplicate or triplicate standards, controls, and samples. Plot the standard curve on log-log graph paper with cytokine concentration on the X-axis and absorbance on the Y-axis. Draw a best fit line through the standard points. To determine the unknown cytokine concentrations, find the mean absorbance value of the unknown concentration on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the cytokine concentration.

If samples were diluted, multiply the concentration by the appropriate dilution factor. If a test sample's absorbance value falls outside the linear portion of the standard curve, the test sample needs to be re-analyzed at a higher (or lower) dilution as appropriate.

Typical Data:

This standard curve was generated at BioLegend for demonstration purposes only. A standard curve must be run with each assay.



Performance Characteristics:

Specificity: This kit recognizes natural and recombinant Human PD-L2. No cross reactivity was observed when this kit was used to analyze the following recombinant proteins at 40 ng/mL.

Mouse	PD-L2
Human	PD-L1, PD1, TGF-β1, CD86, TIM-3, 4-1BB, TGF-β2

Sensitivity: The minimum detectable concentration of Human PD-L2 is 0.35 ± 0.26 pg/mL (n=6).

Recovery: Recombinant or mouse PD-L2 at 3 different concentrations was spiked into 3 different human samples each of Serum, Citrate Plasma, EDTA Plasma, and Heparin Plasma. Sample recovery was then analyzed with the LEGEND MAX™ Human PD-L2 Kit.

Sample Type	N	% Recovery
Serum	4	91.0%
Citrate Plasma	3	109.0%
EDTA Plasma	3	87.7%
Heparin Plasma	3	81.3%

Linearity: Natural human samples were first diluted to 200 folds, then diluted 2 fold in serial to produce samples within the dynamic range of the kit. Samples were then assayed to determine the dilutional linearity.

Sample Type	N	% Linearity
Serum	4	107.5%
Citrate Plasma	3	92.7%
EDTA Plasma	3	106.0%
Heparin Plasma	4	104.3%

Intra-Assay Precision: Two samples containing different human PD-L2 concentrations were tested on one plate with 12 replicates.

Concentration	Sample 1	Sample 2
Number of Replicates	12	12
Mean Concentration (pg/mL)	45.8	12.6
Standard Deviation	1.9	1.1
%CV	4%	9%

Inter-Assay Precision: Two samples containing different human PD-L2 concentrations were tested in ten independent assays.

Concentration	Sample 1	Sample 2
Number of Assays	10	10
Mean Concentration (pg/mL)	48.1	13.5
Standard Deviation	4.0	1.2
%CV	8%	9%

Biological Samples: Human serum, Citrate Plasma, EDTA Plasma, Heparin Plasma were assayed for natural PD-L2. Serum and plasma range detected was 5788 - 11082 pg/mL.

	Serum	Citrate Plasma	EDTA Plasma	Heparin Plasma
N	8	6	6	7
Min (pg/mL)	6763	7435	6453	5788
Max (pg/mL)	11082	10319	9767	10346
Mean (pg/mL)	8402	8772	8401	7511
Standard Dev.	1780	903	928	1435

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Troubleshooting Guide:

Problem	Probable Cause	Solution
High Background	Background wells were contaminated	Avoid cross-well contamination by using the provided plate sealers. Use multichannel pipettes and change tips between pipetting samples and reagents.
	Insufficient washes	Increase number of washes. Increase soaking time between washes prior to addition of substrate solution.
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells. Use a clean container prior to pipetting substrate solution into wells.
No or poor signal	Detection Antibody, Avidin-HRP or Substrate solution were NOT added	Rerun the assay and follow the protocol.
	Wrong reagent or reagents were added in wrong sequential order	
	Insufficient plate agitation	The plate should be agitated during all incubation steps using a plate shaker at a speed where solutions in wells are within constant motion without splashing.
	The wash buffer contains Sodium Azide (NaN3)	Avoid Sodium Azide contamination in the wash buffer as it inhibits HRP activity.
	Incubations were done at an inappropriate temperature, timing or without agitation	Rerun the assay and follow the protocol.
Low or poor standard curve signal	The standard was incorrectly reconstituted or diluted	Adjust the calculations and follow the protocol.
	Standard was inappropriately stored	Store the reconstituted standard stock solution in polypropylene vials at -70°C. Avoid repeated freeze-thaw cycles.
	Reagents added to wells with incorrect concentrations	Check for pipetting errors and the correct reagent volume.

Problem	Probable Cause	Solution
Signal is high, standard curves have saturated signal	Standard reconstituted with less volume than required	Reconstitute new lyophilized standard with the correct volume of solution recommended in the protocol.
	Standards/samples, detection antibody, Avidin-HRP or substrate solution were incubated for too long	Rerun the assay and follow the protocol.
Sample readings are out of range	Samples contain no or below detectable levels of the analyte	If samples are below detectable levels, it may be possible to use a larger sample volume. Contact technical support for appropriate protocol modifications.
	Samples contain analyte concentrations greater than highest standard point	Samples may require dilution and analysis.
High variation in samples and/or standards	Multichannel pipette errors	Confirm that pipette calibrations are accurate.
	Plate washing was not adequate or uniform	Ensure pipette tips are tightly secured. Ensure uniformity in all wash steps.
	Non-homogenous samples	Thoroughly mix samples before assaying.
	Samples may have high particulate matter	Remove particulate matter by centrifugation.
	Cross-well contamination	Do not reuse plate sealers. Always change tips for reagent additions. Ensure that pipette tips do not touch the reagents on the plate.

ELISA Plate Template

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

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