Code No. HAK-HELPDL2-1

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For Research Use Only

PD-L2 ELISA Kit, Human

【Ⅰ】About this kit

【Ⅰ－1】Background and Measurement Principal

Immune checkpoint blockade therapies targeting programmed cell death protein 1 (PD-1) achieve marked clinical benefits in cancer patients harboring programmed death ligand-1 (PD-L1) expression tumors. PD-L2 was confirmed highly expressed in HNSCC, salivary gland cancer (SGC), prostate cancer, gastric cancer, colorectal cancer, oesophageal adenocarcinoma, and bladder cancer. Although the structure of PD-L2 is similar to PD-L1, the binding affinity between PD-L2 and PD-1 is two- to sixfold higher than that with PD-L1, suggesting PD-L2 is an important molecule in immune escape as the strong interaction inhibits cytokines secretion and proliferation of T cells. These findings open a new era to understand the function of PD-L2 and provide new insights for improving the efficiency of anti-PD-1 therapy. [1)]

This kit detects human PD-L2 by a two-step sandwich method using the high-performance antibody against human PD-L2, an immune checkpoint related molecule.

【Ⅰ－2】Features

- Directly quantitate PD-L2 in the cell culture supernatant.
- No special equipment is required. Standard microplate reader capable of reading at 450nm will do the job.
- Detects high sensitivity human PD-L2 with rapidly by a two-step sandwich method using solid phase anti human PD-L2 antibody and HRP conjugated anti human PD-L2 antibody.

【Ⅰ－3】Kit Principle

This ELISA kit uses Two-step Sandwich ELISA principle. The ELISA plate provided in this kit has been pre-coated with an anti-human PD-L2 antibody.

First, the sample is added into the wells of the ELISA plate with HRP conjugated anti-PD-L2 antibody and allowed to react. Next, after washing, substrate is added to the HRP conjugated anti-PD-L2 antibody reacted with PD-L2. Finally, HRP color development is read with a plate reader to quantify human PD-L2 in the sample.

【Ⅰ－4】Kit Component

Storage temperature： 2～8℃

	Reagent	Volume	Quantity
1	Anti-PD-L2 Antibody Immobilized Plate	96well (8well x 12 strips)	1 plate
2	PD-L2 Standard	150µL	1tube[*1]
3	Assay Buffer	25mL	1vial
4	Washing Buffer (10X)[*2]	25mL	1vial
5	HRP Conjugated Anti-PD-L2 Antibody (500X)[*3]	20µL	1tube
6	Substrate Solution	12mL	1vial
7	Stop Solution (2N H_2SO_4)	6mL	1vial
8	Plate Seals		2sheets

[*1] Sufficient to create 3 standard curves with n=2.

[*2] Crystals may precipitate in the Washing Buffer (10x) during refrigerated storage. Warm the buffer to dissolve it at 45℃ before use.

[*3] If the kit is not going to be used immediately, remove the labeled antibody from the kit and store it at -20℃.

Required Materials Not Included in the Kit

・Micropipettes (10 ~ 1000 µL)
・Multichannel micropipette
・Multichannel micropipette Reservoir
・Plate shaker
・Microplate reader (enable to measure at wavelength 450nm)

・Plate washer

【Ⅱ】Preparation of Reagents and Samples

【Ⅱ－1】Preparation of Washing Buffer(Prepare 2 wells for each assay)

・Dilute Washing Buffer (10×) to 10 folds with purified water.
e.g. For 1 plate, add 225 mL of purified water to 25mL of Washing Buffer (10 x) and mix well.

【Ⅱ－2】Preparation of Standard Protein solution

	Concentration (pg/mL)	PD-L2 Standard	Assay Buffer	Dilution factor
A	20000			
B	2000	50μL of A	450μL	10
C	1000	250μL of B	250μL	2
D	500	250μL of C	250μL	2
E	250	250μL of D	250μL	2
F	125	250μL of E	250μL	2
G	62.5	250μL of F	250μL	2
H	31.25	250μL of G	250μL	2

・To prepare Solution B, add 450μL of Assay Buffer into 50μL of PD-L2 Standard (Solution A), and then mix well (10 times dilution). To prepare Solution C, add 250μL of Assay Buffer into 250μL of Solution B, and then mix well (2 times dilution). Similarly, 2 times dilution series for Solution D through H should be prepared.
・Use 200μL for measurement, using 2 wells for each solution (n=2).
・Diluted PD-L2 Standard Solution(31.25～2000 ng/mL) should be freshly prepared at each time before use.

【Ⅱ－3】Preparation of antibody solution

・Dilute HRP conjugated anti-PD-L2 antibody (500x) to 500 folds using Assay Buffer.

e.g. For 1 plate, add 20μL of antibody (500x) into 10mL of Assay Buffer. Mix by inverting the tube.

* Diluted antibody solution should be freshly prepared at each time before use.

【Ⅱ－4】Preparation of Samples (For cell culture medium supernatant)

Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants and assay.

Samples generating absorbance values greater than that of the highest standard should be further diluted using Assay Buffer and reanalyzed.

【Ⅱ－5】Sample Storage

After sample preparation, store at 2-8°C until measurement.

【Ⅲ】Sample measurement procedure

1. Bring anti-PD-L2 antibody solid phased plate and the reagents to the room temperature.
2. Prepare PD-L2 Standard solution by serial dilution. (from step【Ⅱ－2】)
3. Add 100μL each of serial diluted PD-L2 Standard solution (32.5～2000 pg/mL) or Sample solution into the well.
4. Seal the microplate with Plate Seals.
5. Incubate at room temperature for 1 hour on a plate shaker set to 800 rpm.
6. Discard all the reaction solution, and then rinse each well with 300μL of Washing Buffer (from step 【II-1】). Repeat this step for 3 times.
7. Add 100μL each of diluted HRP conjugated anti-PD-L2 antibody (from step 【II-3】) to the well.
8. Seal the microplate with Plate Seals.
9. Incubate at room temperature for 1 hour on a plate shaker set to 800 rpm.
10. Discard the reaction solution, and then rinse each well with 300μL of Washing Buffer(from step 【II-1】). Repeat this step for 3 times.
11. Add 100μL of Substrate Solution into each well, and then incubate at room temperature protected from light for 20min for static reaction.
12. Visually confirm the coloring, and then add 50μL each of Stop Solution.
13. Place into the Plate-reader, and read the absorbance of each well on a spectrophotometer at the wavelength of 450nm.

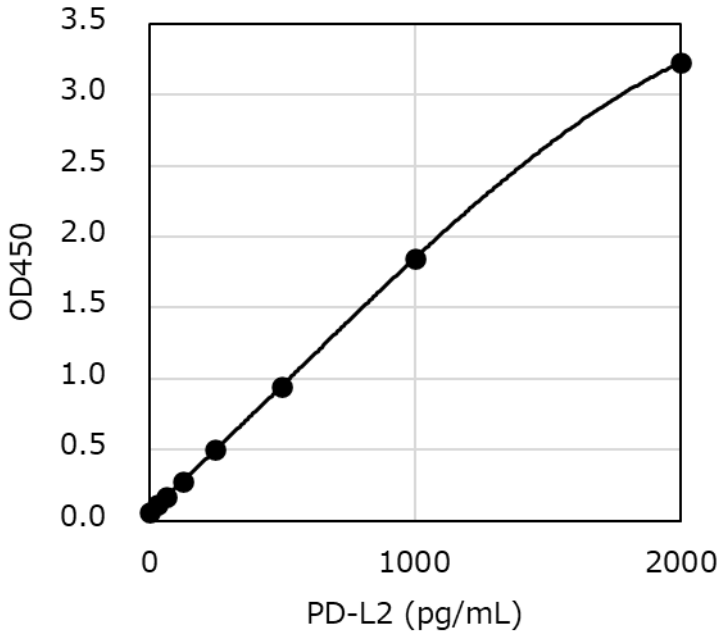
14. Create a standard curve by plotting the absorbance value (y axis) against the Standard Protein concentration (x axis).
15. Calculate the concentration by comparing the absorbance obtained from the sample solution to the standard curve. Multiply the resulting value by the appropriate sample dilution factor, to obtain the concentration of PD-L2 in the sample.

【Ⅳ】Measurement example

【Ⅳ－１】Standard curve

As an example, the graph of absorbance (OD450) against standard PD-L2 concentration is drawn as shown in Figure 1.

However, draw a new standard curve for each assay to calculate the concentration in the sample.

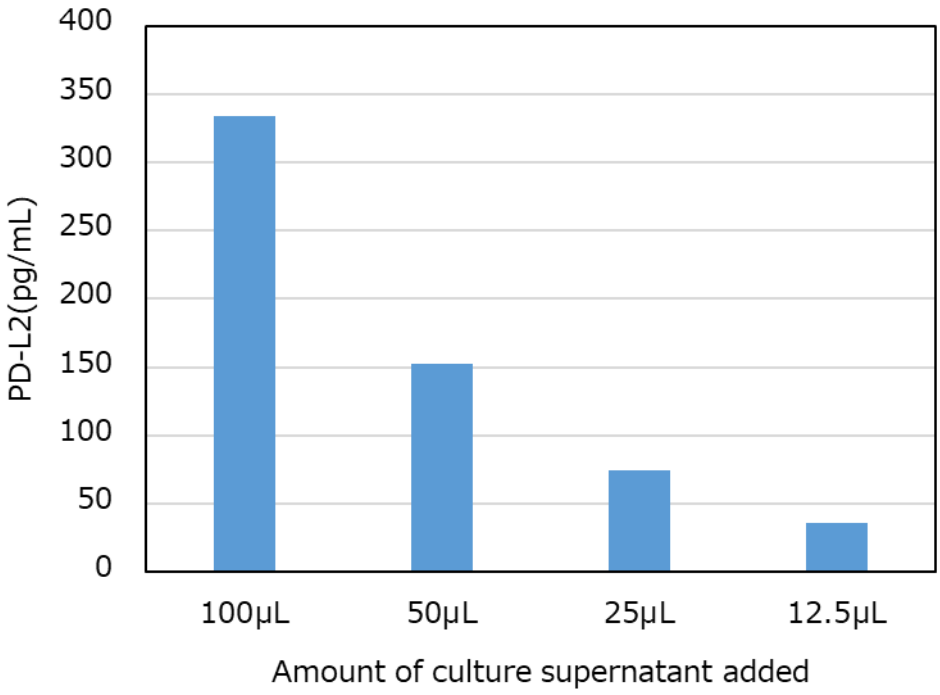


PD-L2 (pg/mL)	Absorbance (450nm)		Average
	1	2	
0	0.061	0.062	0.062
31.25	0.115	0.118	0.117
62.5	0.169	0.167	0.168
125	0.275	0.281	0.278
250	0.498	0.509	0.504
500	0.939	0.957	0.948
1000	1.850	1.851	1.851
2000	3.228	3.226	3.227

Fig.1 Standard curve

【Ⅳ−2】Sample measurement example

The results of adding 12.5, 25, 50, and 100μL of PD-L2 high-expression cell line (human renal cell carcinoma) culture supernatant to each well and measuring are shown in Figure 2.



【V】Kit expiry date and storage

Expiry date : indicated on the kit box

Storage : Refrigeration (2-8℃)

【Reference】

1) Y. Wang, et al.: Br J Cancer., 128, 1196 (2022).