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QuickDetect™ PD-L1/B7-H1 (Human) ELISA Kit

rev 02/20

(Catalog # K4416-100, 100 assays, Store at 4°C)

I. Introduction:

Involved in the costimulatory signal, essential for T-cell proliferation and production of IL10 and IFNG, in an IL2-dependent and a PDCD1-independent manner. Interaction with PDCD1 inhibits T-cell proliferation and cytokine production. BioVision's QuickDetect™ PDL1 ELISA kit is a sandwich ELISA assay for measuring the quantity of human PDL1 in serum, plasma and cell culture and other biological fluid. The entire process can be performed within 90 minutes with only a single incubation and a wash step respectively, resulting in fewer handling steps, fewer errors and more consistent results.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Human PDL1.
Detection Range: 12.5 – 800 ng/ml

III. Sample Type:

Human serum, plasma, cell culture supernatant, tissue homogenate and other biological fluid.

IV. Kit Contents:

Components	K4416-100	Part No.
Micro ELISA Plate	8 X 12 strips	K4416-100-1
Standard (800 ng/ml)	0.6 ml	K4416-100-2
Standard diluent	6 ml	K4416-100-3
Special diluent	6 ml	K4416-100-4
HRP-Conjugate reagent	6 ml	K4416-100-5
Chromogen Solution A	6 ml	K4416-100-6
Chromogen Solution B	6 ml	K4416-100-7
Stop Solution	6 ml	K4416-100-8
Wash buffer (20X)	20 ml	K4416-100-9
Plate Sealers	2	K4416-100-10

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- 37°C incubator
- Precision pipettes with disposable tips
- Distilled or deionized water
- Clean eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

VI. Storage and Handling:

The entire kit may be stored at 4°C for up to 6 months from the date of shipment.

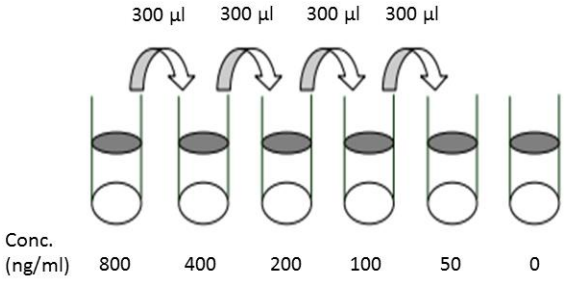
VII. Reagent and Sample Preparation:

Note: Prepare reagents within 30 minutes before the experiment.
Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Wash Buffer:** Dilute 20 mL of Concentrated Wash Buffer into 400 mL of Wash Buffer with deionized or distilled water. Put unused solution back at 4°C. If crystals have formed in the concentrate, warm it with 40°C water bath and mix it gently until the crystals have completely dissolved. The solution should be cooled to room temperature before use.

2. Standard Preparation:

- Prepare 0.6 ml of 400 ng/ml standard by adding 0.3 ml of the 800 ng/ml stock solution in 0.3 ml of Standard Buffer. Perform 2-fold serial dilutions of the top standards to make the standard curve within the range of this assay. Use 0.3 ml standard diluent as blank control.
- Suggested standard points are: 800, 400, 200, 100, 50, 0 ng/ml



3. Sample Preparation:

Note: Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination. Avoid multiple freeze-thaw cycles. Samples that contain NaN_3 cannot be used for this assay. Do not use heat-treated samples.

- **Serum**: collect blood with non-pyrogenic and endotoxin tubes to avoid any cell stimulation. Centrifuge 3000 rpm for 10 minutes and separate the serum and red blood cells as quickly as possible. If precipitation appears, centrifuge again.

FOR RESEARCH USE ONLY! Not to be used on humans.

- **Plasma**: Collect plasma with heparin or EDTA as the anticoagulant. Mix for 20 minutes and centrifuge for 30 min at 2-8°C at 3000 rpm. For eliminating the platelet effect, suggesting that further centrifugation for 10 min at 2-8°C at 10000xg. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.
- **Homogenate:** Homogenize the sample with saline buffer and centrifuge for 10 minutes at the speed of 3000 rpm, collect supernatant for detection.
- **Urine:** Collect urine in sterile container, centrifuge for 20 minutes at 2000-3000 rpm. Collect supernatant. If precipitation appears, centrifuge again. This method can also be used to process hydrothorax and cerebrospinal fluid.
- **Cell culture supernatant**: Centrifuge supernatant for 20 minutes at 2000 – 3000 rpm to remove insoluble impurity and cell debris. Collect the clear supernatant and carry out the assay immediately or aliquot and store at -20°C.
- **Note:** End user should estimate the concentration of the target protein in the test sample first, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit.

VIII. Assay Protocol:

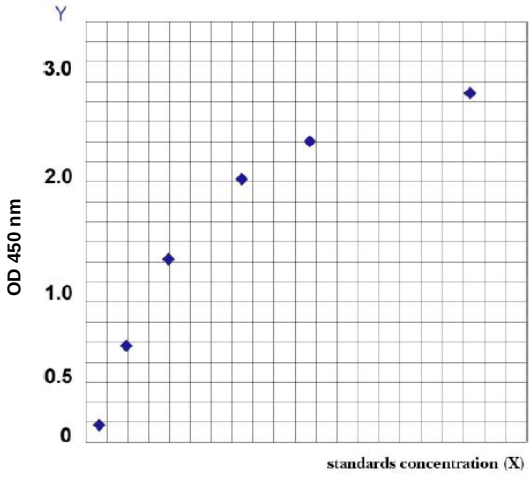
Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay.
It is recommended that all standards and samples be run at least in duplicate.
A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in section VII.
2. Set blank wells, standard wells, and test sample wells respectively:
   (1) Blank well: do not add samples and horseradish peroxidase (HRP), other operations are the same.
   (2) Standard wells: Add standard 50µl to Standard wells.
   (3) Test sample wells: Add 40µl of Special diluent and then add 10µl of sample. (The final sample dilution is five times and the final result calculation should be multiplied by five times).
   (4) Add 50µl of horseradish peroxidase (HRP) into each well, except blank well. Then seal the plate, and gently shake, then incubate 60 minutes at 37°C.
3. Discard the solution and wash 5 times with **1X Wash Solution**. Wash by filling each well with Wash Buffer (350 µl) using a multi-channel pip ette or autowasher. Let it soak for 1-2 minutes, and then remove all residual wash-liquid from the wells by aspiration. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Clap the plate on absorbent filter papers or other absorbent materials.
4. Add 50µl of **Chromogen solution A** to each well, and then add 50µl of **Chromogen solution B** to each well. Gently shake and incubate for 10 minutes at 37°C away from light.
5. Add **Stop Solution** 50 µl into each well to stop the reaction.
6. Measure the optical density (OD) at 450 nm wavelength within 15 minutes after adding the stop solution.

IX. CALCULATION:

Set blank well zero, measure the optical density (OD) at 450 nm. The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Human PDL1 concentration of the samples can be interpolated from the standard curve. If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.



X. RELATED PRODUCTS:

- PD-L1/CD274 (Human) ELISA Kit (Cat. No. K4155-100)
- Human CellExp™ PD-L1 /CD274 /B7-H1, human recombinant (Cat. No. 7429-10, 50)
- RBP4 (human) ELISA Kit Kit (Cat. No. K4912-100)
- PD-1 (human) ELISA Kit (Cat. No. K4153-100)