

BioSim™ Durvalumab (Human) ELISA Kit

(Catalog # E4925-100, 96 assays, Store at 4°C)

I. Introduction:

Durvalumab is a human IgG1 monoclonal antibody that binds to and inhibits programmed cell death ligand 1 (PD-L1). PD-L1 is an important immune checkpoint molecule expressed on tumor cells and tumor-infiltrating immune cells. PD-L1 binds to its receptors PD-1 and B7.1 located on the surface of activated T cells and antigen-presenting cells and in turn suppresses cytotoxic T-cell activity, the proliferation of T-cells, and production of cytokines. The monoclonal antibody binds to PD-L1 and inhibits its anti-tumor immune response, and thus reduces tumor growth without causing antibody-dependent cellular cytotoxicity. The original antibody is approved to treat locally advanced or metastatic urothelial carcinoma and locally advanced unresectable non-small cell lung cancer (NSCLC). BioSim™ Durvalumab ELISA kit measures free Durvalumab concentration in human serum or plasma with high sensitivity and reproducibility. The kit is based on the Sandwich ELISA principle. Standards and samples (serum or plasma) are added to the microtiter plate coated with the reactant for Durvalumab. After incubation, the wells are washed. The HRP conjugated probe is added and binds to Durvalumab captured by the reactant on the surface of the wells. Following incubation, wells are washed and the enzymatic activity is detected by the addition of TMB chromogen substrate. Finally, the reaction is terminated with an acidic stop solution. The color developed is proportional to the amount of Durvalumab in the sample or standard. The sample results can be determined directly using the standard curve.

II. Features and Benefits:

For *in vitro* quantitative determination of free Durvalumab in human serum and plasma samples

Detection Range: 10 - 300 ng/ml

Sensitivity: 10 ng/ml

Assay Precision: Intra-Assay and Inter-Assay CV < 30%

Recovery rate: < 100 ± 30% with known concentrations of normal human serum samples

Cross Reactivity: Except for Durvalumab, there is no cross reaction with other therapeutic antibodies and native serum immunoglobins

III. Sample Type:

Human Plasma and Serum

IV. Kit Contents:

| Components | E4925-100 | Part Number |
|--------------------------------|---------------|-------------|
| Microtiter Plate | 8 x 12 strips | E4925-100-1 |
| Durvalumab Standards (S1 – S7) | 1 ml | E4925-100-2 |
| Assay Buffer | 50 ml x 2 | E4925-100-3 |
| HRP-conjugate Probe | 12 ml | E4925-100-4 |
| TMB substrate (Avoid light) | 12 ml | E4925-100-5 |
| Stop Solution | 12 ml | E4925-100-6 |
| Wash buffer (20X) | 50 ml | E4925-100-7 |
| Plate sealers | 2 | E4925-100-8 |

V. User Supplied Reagents and Equipment:

- Micropipettes and tips
- Eppendorf tubes
- Absorbent paper
- Microtiter plate reader capable of measuring absorbance at 450 nm

VI. Storage Conditions and Handling:

The entire kit may be stored at 4°C for up to 12 months from the date of shipment

VII. Reagent and Sample Preparation:

Note: Samples and reagents must be prepared freshly before the start of the experiment. Allow all reagents and samples to reach room temperature (RT). Gently swirl each sample and reagent, without foaming, prior to use.

1. **Wash Buffer:** Dilute 20X Wash Buffer to 1X solution in ddH₂O (10 ml of 20X Wash Buffer + 190 ml ddH₂O). To dissolve the crystals, warm the Wash Buffer at 37°C. Mix vigorously. The working stock is stable for 2 weeks after preparation at 4°C.

2. **Standard and Controls Dilution:** Standards provided are ready to use.

| Name | S1 | S2 | S3 | S4 | S5 | S6 | S7 |
|---------------|-----|-----|----|----|----|--------------|-------------|
| Conc. (ng/ml) | 300 | 100 | 30 | 10 | 0 | High Control | Low Control |

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3. **Sample Dilution:** Dilute Serum/Plasma samples 1:1000 in Assay Buffer. First, make 1:10 dilution (10 µl sample + 90 µl Assay Buffer). Next, prepare 1:100 dilution (5 µl previously diluted sample + 495 µl Assay Buffer).

VIII. Assay Protocol:

Note: Bring all reagents, samples and microtiter plate to room temperature (RT)

It is recommended that all standards and samples be run at least in duplicates

A standard curve must be run with each assay

1. Prepare samples (serum/plasma) and reagents as instructed in **Section VII**.
2. Add 100 µl of **standards, controls** and **samples** into appropriate wells. Cover the plate with Plate sealer, gently mix the contents in the plate, and incubate at RT for 30 mins.
3. Remove the sealer and discard the incubation solution. Wash the plate 3 times with 300 µl of 1X **Wash Buffer**. Remove excess solution by tapping the inverted plate on an absorbent paper.
4. Add 100 µl of **HRP-conjugate Probe** into each well. Cover the plate and incubate at RT for 30 mins.
5. Discard the incubation solution and wash wells as mentioned in Step 3.
6. Add 100 µl of **TMB Substrate** into each well. Incubate the plate without plate sealer in the dark at RT for 10 mins.
7. Add 100 µl of **Stop Solution** to stop the reaction. Gently mix the plate. The color changes from blue to yellow.
8. Measure the absorbance using microplate reader at 450 nm within 30 minutes of adding **Stop Solution**. (Use reference wavelength as 650 nm).

IX. Calculation:

Prepare a standard curve using the standards (disregard standard zero). Plot OD (450/650 nm) values for each standard on the vertical (Y-axis) axis versus the corresponding Durvalumab concentration on the horizontal (X-axis) axis. Construct a standard curve of difference data using software capable of generating four-parameter logistic (4PL) or point-to-point calculation curve fit. To obtain the exact values of the samples, the concentration determined from the standard-curve must be multiplied by the dilution factor (1000x).

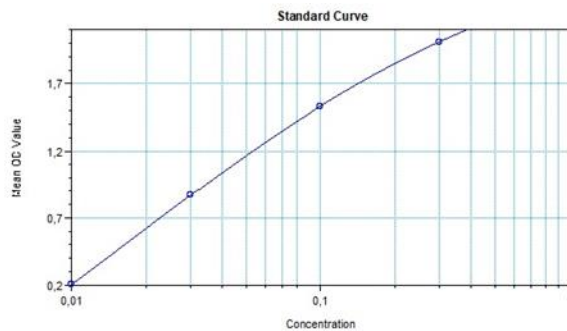


Figure: Typical Standard Curve. These standard curves are for demonstration only. A standard curve must be run with each assay.

X. Related Products:

- BioSim™ Nivolumab (Human) ELISA Kit (E4382)
- BioSim™ Rituximab (Human) ELISA Kit (E4371)
- BioSim™ anti-PD-L1 (Human) ELISA Kit (E4921)
- BioSim™ anti-Rituximab (Human) ELISA Kit - I (E4385)
- BioSim™ anti-HER2 mab (Human) ELISA Kit (E4386)
- BioSim™ anti-HER2 (Human) ELISA Kit (E4376)

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