



BioSim™ Pembrolizumab (Human) ELISA Kit

rev 12/20

(Catalog # E4383-100, 100 assays, Store at 4°C)

I. Introduction:

Pembrolizumab is an antibody-drug that targets the cell surface receptor programmed cell death protein 1 (PD-1) found on T cells. By preventing the binding of its ligands (PD-L1 and PD-L2), pembrolizumab induces an antitumor immune response. Upregulation of PD-1 ligands is a mechanism for tumors to evade antitumor immune response; when PD-1 binds its ligand, the T cell receives an inhibitory signal leading to T cell anergy and blockade of the anti-tumor immune response. Instead of directly targeting tumor tissue to induce tumor cell death, pembrolizumab acts as a checkpoint inhibitor to stimulate immune responses to eliminate cancer cells. BioSim™ Pembrolizumab ELISA kit has been developed for specific quantification of Pembrolizumab concentration in human serum or plasma with high sensitivity and reproducibility.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Pembrolizumab

Detection Range: 30 - 1000 ng/ml

Sensitivity: 10 ng/ml

Assay Precision: Intra-Assay: CV < 30%; Inter-Assay: CV < 30% (CV (%) = SD/mean X 100)

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

Cross Reactivity: There is no cross reaction with native serum immunoglobulins and tested monoclonal antibodies such as infliximab (Remicade®), adalimumab (Humira®), etanercept (Enbrel®), bevacizumab (Avastin®) and trastuzumab (Herceptin®)

III. Sample Type:

Human serum and plasma

IV. Kit Contents:

Components	E4383-100	Part No.
Micro ELISA Plate	1 plate	E4383-100-1
Pembrolizumab Standards (S1 – S7)	0.3 ml X 7	E4383-100-2.x
Assay Buffer	2 X 50 ml	E4383-100-3
HRP-conjugate Probe	11 ml	E4383-100-4
TMB substrate (Avoid light)	12 ml	E4383-100-5
Stop Solution	12 ml	E4383-100-6
Wash buffer (20X)	50 ml	E4383-100-7
Plate sealers	2	E4383-100-8

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- Calibrated measures
- Precision pipettes with disposable tips
- 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 12 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 the 20X Wash Buffer to 1X solution in ddH₂O (10 ml of Wash Buffer stock to 190 ml of ddH₂O). Mix the 1X solution thoroughly by vortex manually. The working stock can be stable for 2 weeks after preparation at 4°C.

2. **Standard Preparation:**

Ready to use.

Name	S1	S2	S3	S4	S5	S6	S7
Conc. (ng/ml)	1000	300	100	30	0	High Controls	Low Controls

3. **Sample Dilution:**

- **Serum/Plasma:** Dilute samples 1:100 (10 µl Sample + 990 µl Assay Buffer).
- Diluted samples should further be diluted if the concentration of Pembrolizumab is higher than the measuring range.
- The usual precautions for venipuncture should be observed. Samples are stable at 4°C for 2 days and -20°C for 6 months. Avoid freeze-and-thaw cycle.



VIII. Assay Protocol:

Note: Bring all reagents, microplate and samples to room temperature 15 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. Pipette 100 μ l of **Assay Buffer** non-exceptionally into each of the wells to be used
3. Add 10 μ l of **standards, controls** and **diluted-samples** into appropriate wells. Cover wells and incubate for 60 minutes at room temperature (RT).
4. Discard incubation solution. Wash plate 3 times each with 300 μ l of diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
5. Add 100 μ l of **HRP-conjugate** into each well. Cover wells with adhesive plate sealer and incubate at RT for 60 minutes.
6. Discard the solution and wash the wells as step 4.
7. Add 100 μ l of 1X **TMB substrate** solution and incubate the plate in dark at RT for 20 minutes
8. Add 100 μ l of **Stop solution** to stop the reaction
9. Read the absorbance in micro plate reader set to 450 nm within 20 minutes. (reference wavelength to 650 nm)

IX. CALCULATION:

Using the standards disregarding zero standard, construct a standard curve by plotting the OD_{450/650 nm} for each of 4 standards on the Y-axis versus the corresponding Pembrolizumab concentration on the X-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 should be multiplied by the dilution factor.

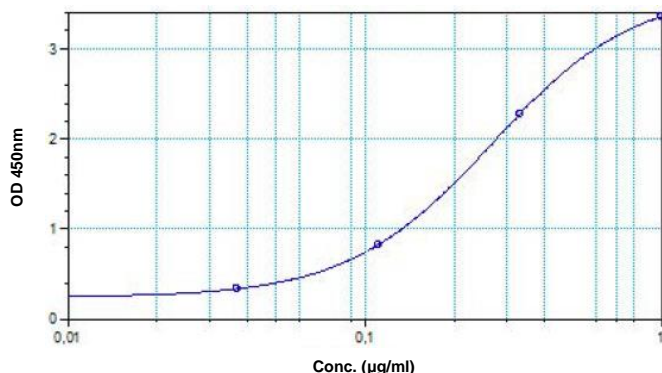


Figure: Typical Standard Curve:
These standard curves are for demonstration only. A standard curve must be run with each assay.

X. RELATED PRODUCTS:

- BioSim™ Rituximab (Human) ELISA Kit (Cat. No. E4371-100)
- BioSim™ Adalimumab (Human) ELISA Kit (Cat. No. E4372-100)
- BioSim™ Bevacizumab (Human) ELISA Kit (Cat. No. E4373-100)
- BioSim™ Etanercept (Human) ELISA Kit (Cat. No. E4374-100)
- BioSim™ anti-HER2 (Human) ELISA Kit (Cat. No. E4376-100)
- BioSim™ Golimumab (Human) ELISA Kit (Cat. No. E4377-100)
- BioSim™ Cetuximab (Human) ELISA Kit (Cat. No. E4379-100)
- BioSim™ Denosumab (Human) ELISA Kit (Cat. No. E4380-100)
- BioSim™ Omalizumab (Human) ELISA Kit (Cat. No. E4381-100)
- BioSim™ Nivolumab (Human) ELISA Kit (Cat. No. E4382-100)
- BioSim™ Pembrolizumab (Human) ELISA Kit (Cat. No. E4383-100)
- BioSim™ Ipilimumab (Human) ELISA Kit (Cat. No. E4384-100)