

BioSim™ Nivolumab (Opdivo®)(Human) ELISA Kit

rev 11/17

(Catalog # E4382-100, 100 assays, Store at 4°C)

I. Introduction:

Nivolumab (Opdivo®) is a human immunoglobulin G4 (IgG4) monoclonal antibody that binds to the programmed cell death 1 (PD-1) receptor and selectively blocks interaction with its programmed death ligands PD-L1 and PD-L2. Upregulation of PD-1 ligands occurs in some tumors and signalling through this pathway can contribute to inhibition of active T-cell immune surveillance of tumour tissue. The inhibitory effect of PD-1 and its ligands occurs through the promotion of apoptosis in antigen specific T cells while simultaneously blocking apoptosis in suppressor T cells. Blocking PD-1 activity has been shown to lead to decreased tumour growth in mouse tumour models. BioSim™ Nivolumab ELISA kit has been developed for specific quantification of Nivolumab concentration in human serum or plasma with high sensitivity and reproducibility.

II. Application:

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

Detection Range: 30 - 3000 ng/ml

Sensitivity: 10 ng/ml

Assay Precision: Intra-Assay: CV < 15%; Inter-Assay: CV < 15% (CV (%) = SD/mean X 100)

Recovery rate: 85 – 115% with normal human serum samples with known concentrations

Cross Reactivity: Except for Nivolumab, there is no cross reaction with other therapeutic antibodies and native serum immunoglobulins.

III. Sample Type:

Human serum and plasma

IV. Kit Contents:

Components	E4382-100	Part No.
Micro ELISA Plate	1 plate	E4382-100-1
Nivolumab Standards (S1 – S8)	0.3 ml X 8	E4382-100-2.x
Assay Buffer	50 ml X 2	E4382-100-3
HRP-conjugate Probe	12 ml	E4382-100-4
TMB substrate (Avoid light)	12 ml	E4382-100-5
Stop Solution	12 ml	E4382-100-6
Wash buffer (20X)	50 ml	E4382-100-7
Plate sealers	2	E4382-100-8

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- 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:**

Dilute standards 1:100 with Assay Buffer (5 µl Sample + 495 µl Assay Buffer)

Name	S1	S2	S3	S4	S5	S6	S7	S8
Conc. (µg/ml)	300	100	30	10	3	0	High Control	Low Control
Working Con. (ng/ml)	3000	1000	300	100	30	0	-	-

3. **Sample Dilution:**

- **Serum/Plasma:** Dilute samples 1:100 (5 µl Sample + 495 µl Assay Buffer).
- Diluted samples should further be diluted if the concentration of Nivolumab 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.

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

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** and **diluted-samples** into appropriate wells. Cover wells and incubate for 30 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 30 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 10 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 5 standards on the Y-axis versus the corresponding Nivolumab 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.

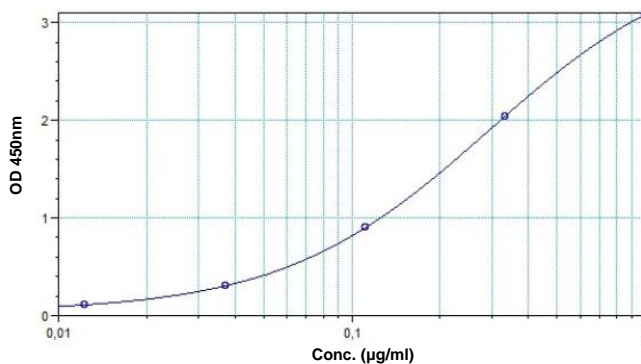


Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

X. RELATED PRODUCTS:

- BioSim™ Rituximab (Mabthera®) (Human) ELISA Kit (Cat. No. E4371-100)
- BioSim™ Adalimumab (Humira®) (Human) ELISA Kit (Cat. No. E4372-100)
- BioSim™ Bevacizumab (Avastin®) (Human) ELISA Kit (Cat. No. E4373-100)
- BioSim™ Etanercept (Enbrel®) (Human) ELISA Kit (Cat. No. E4374-100)
- BioSim™ Nivolumab (Remicade®) (Human) ELISA Kit (Cat. No. E4375-100)
- BioSim™ Trastuzumab(Herceptin®)(Human) ELISA Kit (Cat. No. E4376-100)
- BioSim™ Golimumab (Simponi®)(Human) ELISA Kit (Cat. No. E4377-100)
- BioSim™ Infliximab (Remsima®)(Human) ELISA Kit (Cat. No. E4378-100)
- BioSim™ Cetuximab (Erbix®)(Human) ELISA Kit (Cat. No. E4379-100)
- BioSim™ Denosumab (Prolia®)(Human) ELISA Kit (Cat. No. E4380-100)
- BioSim™ Omalizumab (Xolair®)(Human) ELISA Kit (Cat. No. E4381-100)
- BioSim™ Nivolumab (Opdivo®)(Human) ELISA Kit (Cat. No. E4382-100)
- BioSim™ Pembrolizumab (Keytruda®)(Human) ELISA Kit (Cat. No. E4383-100)
- BioSim™ Ipilimumab (Yervoy®)(Human) ELISA Kit (Cat. No. E4384-100)

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