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BioSim™ Nivolumab (Human) ELISA Kit

rev 12/20

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

I. Introduction:

Nivolumab 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 signaling through this pathway can contribute to inhibition of active T-cell immune surveillance of tumor 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 tumor growth in mouse tumor 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. Applications:

This ELISA kit is used for *in vitro* quantitative determination of Nivolumab
Detection Range: 3 - 100 ng/ml
Sensitivity: 30 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: Except for Nivolumab, there is no cross reaction with other therapeutic antibodies and native serum immunoglobins.

III. Sample Type:

Human serum and plasma (EDTA, Heparin)

IV. Kit Contents:

Components	E4382-100	Part No.
Micro ELISA Plate	1 plate	E4382-100-1
Nivolumab Standards (S1 – S7)	0.3 ml X 7	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:

Notes:
a) Prepare reagents within 30 min before the experiment.
b) 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 ddH_2O (10 ml of Wash Buffer stock to 190 ml of ddH_2O). 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 and Controls 1:10 with Assay Buffer (10 µl Standard/Control + 90 µl Assay Buffer)

Name	S1	S2	S3	S4	S5	S6	S7
Conc. (µg/ml)	10	3	1	0.3	0	High Control	Low Control
Working Con. (ng/ml)	100	30	10	3	0	-	-

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 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 cycles.

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

VIII. Assay Protocol:

Notes:

a) Bring all reagents, microplate and samples to room temperature (RT) 15 min prior to the assay.
b) It is recommended that all Standards and samples be run at least in duplicates.
c) A Standard Curve must be run with each assay.

1. Prepare all reagents, samples and Standards as instructed in section VII.
2. Pipette 100 µl of **Assay Buffer** non-exceptionally into each of the wells to be used
3. Add 10 µl of each **Standards, Low-Control, High-Control** and **diluted-samples** into appropriate wells. Cover wells and incubate for 30 min at RT.
4. Discard incubation solution. Wash plate 3 times each with 300 µl of diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
5. Add 100 µl of **HRP-conjugate** into each well. Cover wells with adhesive plate sealer and incubate at RT for 30 min.
6. Discard the solution and wash the wells as step 4.
7. Add 100 µl of 1X **TMB substrate** solution and incubate the plate in dark at RT for 10 min
8. Add 100 µl of **Stop Solution** to stop the reaction. Briefly mix contents by gently shaking the plate. Color changes from blue to yellow.
9. Read the absorbance in micro plate reader set to 450 nm within 30 min (with reference wavelength 650 nm)

IX. CALCULATION:

Using the Standards disregarding zero standard, construct a Standard Curve by plotting the OD 450/650 nm for each Standard 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. The concentration of the samples can be read directly from this Standard Curve. For diluted samples, the concentration determined from the Standard Curve should be multiplied by the sampe dilution factor.

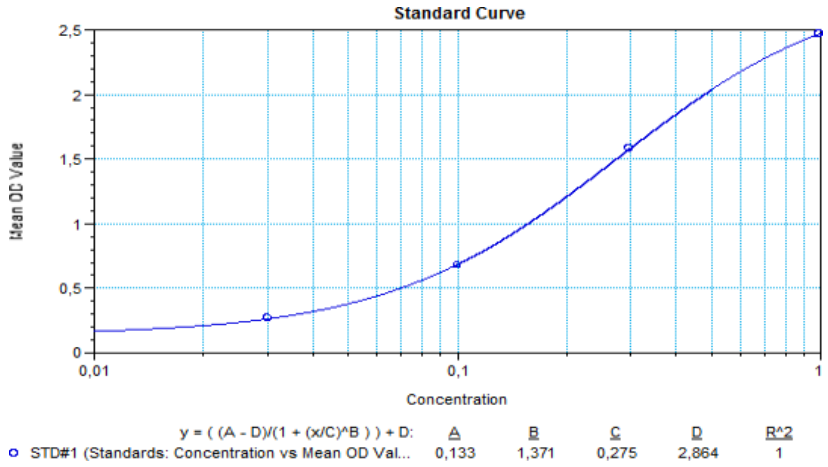

Figure: Typical Standard Curve: These Standard Curves are for demonstration only. A Standard Curve must be run with each assay.

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)