



BioSim™ Ipilimumab (Human) ELISA Kit

rev 01/21

(Catalog # E4384-100, 100 assays, Store at 4°C)

I. Introduction:

Ipilimumab is a fully human IgG1κ antibody that binds to CTLA-4 (cytotoxic T lymphocyte-associated antigen 4), a molecule on T-cells that is indicated for unresectable or metastatic melanoma. The absence or presence of CTLA-4 can augment or suppress the immune system's T-cell response in fighting disease. Ipilimumab is designed to block the activity of CTLA-4, thereby sustaining an active immune response in its attack on cancer cells. The proposed mechanism of action is indirect and maybe through T-cell - mediated anti-tumor immune responses. BioSim™ Ipilimumab ELISA kit has been developed for specific quantification of Ipilimumab concentration in human serum or plasma with high sensitivity and reproducibility.

II. Application:

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

Detection Range: 30 - 1000 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: There is no cross reaction with native serum immunoglobulins and tested monoclonal antibodies such as infliximab (Remicade®), adalimumab, etanercept, bevacizumab, and trastuzumab

III. Sample Type:

Human serum and plasma

IV. Kit Contents:

Components	E4384-100	Part No.
Micro ELISA Plate	1 plate	E4384-100-1
Ipilimumab Standards (S1 – S7)	0.3 ml X 7	E4384-100-2.x
Assay Buffer	50 ml x 2	E4384-100-3
HRP-conjugate Probe	12 ml	E4384-100-4
TMB substrate (Avoid light)	12 ml	E4384-100-5
Stop Solution	12 ml	E4384-100-6
Wash buffer (20X)	50 ml	E4384-100-7
Plate sealers	2	E4384-100-8

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 Control	Low Control

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 Ipilimumab 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.

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

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 µl of **Assay Buffer** non-exceptionally into each of the wells to be used
3. Add 10 µl of **standards, controls** 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 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 minutes.
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 minutes
8. Add 100 µl 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 Ipilimumab 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.

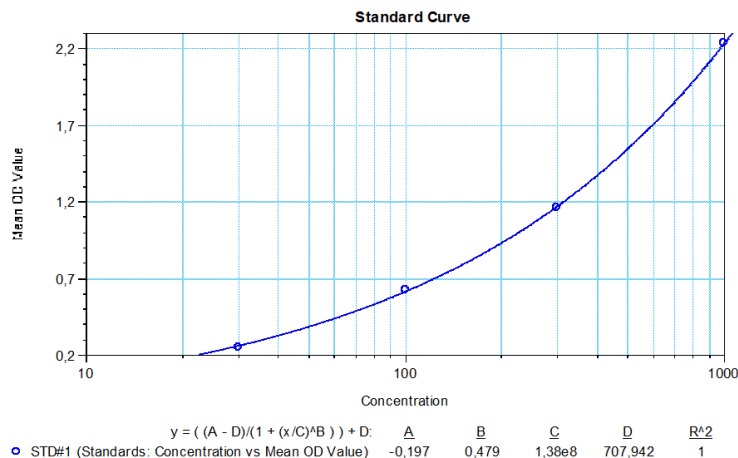


Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

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™ Infliximab (Human) ELISA Kit – 1 (Cat. No. E4378-100)
- 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)