



# BioSim™ Avelumab (Bavencio®)(Human) ELISA Kit

rev 04/20

(Catalog # E4556-100, 100 assays, Store at 4°C)

## I. Introduction:

Avelumab (Bavencio®) is a fully human anti-PD-L1 IgG1 lambda monoclonal antibody that has a molecular weight of approximately 147 kDa. Avelumab binds PD-L1 and blocks the interaction between PD-L1 and its receptors PD-1 and B7-1. By inhibiting PD-L1 interactions, avelumab is thought to enable the activation of T-cells and the adaptive immune system. By retaining an active Fc-region, avelumab is thought to engage the innate immune system and may induce antibody-dependent cell-mediated cytotoxicity. Importantly, avelumab has not shown antibody-dependent cell mediated cytotoxicity against immune cell subsets in humans. BioSim™ Avelumab ELISA kit has been developed for specific quantification of Avelumab concentration in human serum or plasma with high sensitivity and reproducibility.

## II. Application:

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

Detection Range: 100 - 3000 ng/ml

Sensitivity: 100 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 Avelumab, there is no cross reaction with other therapeutic antibodies and native serum immunoglobins.

## III. Sample Type:

Human serum and plasma

## IV. Kit Contents:

Components	E4556-100	Part No.
Micro ELISA Plate	1 plate	E4556-100-1
Avelumab Standards (S1 – S7)	0.3 ml X 7	E4556-100-2.x
Assay Buffer	50 ml X 2	E4556-100-3
HRP-conjugate Probe	12 ml	E4556-100-4
TMB substrate (Avoid light)	12 ml	E4556-100-5
Stop Solution	12 ml	E4556-100-6
Wash buffer (20X)	50 ml	E4556-100-7
Plate sealers	2	E4556-100-8

## V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- 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 ddH<sub>2</sub>O (10 ml of Wash Buffer stock to 190 ml of ddH<sub>2</sub>O). Mix the 1X solution thoroughly by vortex manually. The working stock can be stable for 4 weeks after preparation at 4°C.

2. **Standard Preparation:**

Dilute standards 1:100 with Assay Buffer (10 µl Sample + 990 µl Assay Buffer)

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

## 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.

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

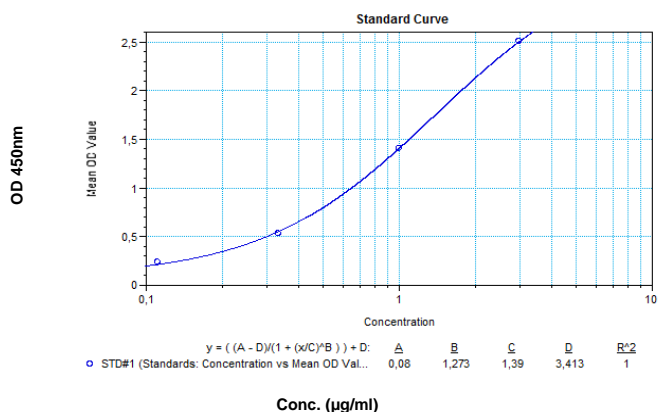


A standard curve must be run with each assay.

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** 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 is 650 nm)

#### IX. CALCULATION:

Using the standards disregarding zero standard, construct a standard curve by plotting the OD<sub>450/650</sub> nm for each of 5 standards on the Y-axis versus the corresponding Avelumab 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™ Avelumab (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™ Avelumab (Bavencio®) (Human) ELISA Kit (Cat. No. E4556-100)
- BioSim™ Pembrolizumab (Keytruda®) (Human) ELISA Kit (Cat. No. E4383-100)
- BioSim™ Ipilimumab (Yervoy®) (Human) ELISA Kit (Cat. No. E4384-100)
- BioSim™ Avelumab (Bavencio®) (Human) ELISA Kit (Cat. No. E4556-100)