

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 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 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.

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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 is 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 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.

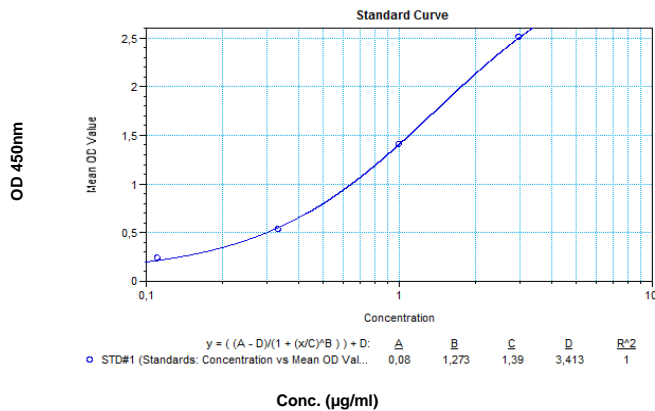


Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

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)

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