



# BioSim™ Vedolizumab (Human) ELISA Kit

rev 02/21

(Catalog # E4697-100, 96 assays, Store at 4°C)

## I. Introduction:

Vedolizumab is a recombinant humanized IgG1 monoclonal antibody directed against the human lymphocyte  $\alpha 4\beta 7$  integrin, a key mediator of gastrointestinal inflammation. It is used in the treatment of moderate to severe active ulcerative colitis and Crohn's disease for patients who have had an inadequate response with, lost response to, or were intolerant to inhibitors of tumor necrosis factor-alpha (TNF-alpha) or other conventional therapies. BioSim™ Vedolizumab ELISA kit is a Solid-phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. Standards and samples (serum or plasma) are incubated in the microtitre plate coated with the reactant for Vedolizumab. After incubation, the wells are washed. A horseradish peroxidase (HRP) conjugated probe is added and binds to Vedolizumab captured by the reactant on the surface of the wells. Following incubation, wells are washed and the bound enzymatic activity is detected by the addition of chromogen-substrate. The color developed is proportional to the amount of Vedolizumab in the sample or standard. The test results can be deduced directly from the standard curve.

## II. Application:

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

Detection Range: 3 - 100 ng/ml

Sensitivity: 3 ng/ml

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

## III. Sample Type:

Human serum and plasma

## IV. Kit Contents:

Components	E4697-100	Part No.
Micro ELISA Plate	1 plate	E4697-100-1
Vedolizumab Standards (S1 – S7)	0.3 ml X 7	E4697-100-2.x
Assay Buffer	50 ml X 2	E4697-100-3
HRP-conjugate Probe	12 ml	E4697-100-4
TMB substrate (Avoid light)	12 ml	E4697-100-5
Stop Solution	12 ml	E4697-100-6
Wash buffer (20X)	50 ml	E4697-100-7
Plate sealers	2	E4697-100-8

## 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 and Controls (10X) with assay buffer (1: 10 dilution: 20  $\mu$ L standard/control + 180  $\mu$ L assay buffer)

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

### 3. Sample Dilution:

- **Serum/Plasma:** First dilute samples at 1:20 (10  $\mu$ L Serum/Plasma + 190  $\mu$ L ddH<sub>2</sub>O) then 1:100 (5  $\mu$ L diluted sample + 495  $\mu$ L ddH<sub>2</sub>O) to get the final samples with dilution factor 1:2000.
- Diluted samples should further be diluted if the concentration of Vedolizumab 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.

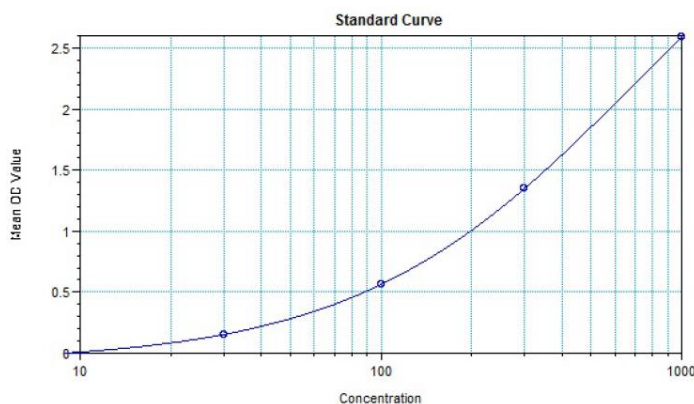
It is recommended that all standards and samples be run at least in duplicate.

A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in **section VII**.
2. Add 100  $\mu$ l of **diluted standards, controls, and diluted samples** into appropriate wells. Cover wells and incubate for 60 minutes at room temperature (RT).
3. Discard incubation solution. Wash plate 3 times each with 300  $\mu$ l of **1X Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
4. Add 100  $\mu$ l of **HRP-conjugate** into each well. Cover wells with adhesive plate sealer and incubate at RT for 30 minutes.
5. Discard the solution and wash the wells as **step 3**.
6. Add 100  $\mu$ l of **1X TMB substrate** solution and incubate the plate in dark at RT for 10 minutes
7. Add 100  $\mu$ l of **Stop solution** to stop the reaction
8. Read the absorbance in micro plate reader set to 450 nm within 30 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 Vedolizumab 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™ Infliximab (Human) ELISA Kit - 1 (Cat. No. E4375-100)
- BioSim™ anti-HER2 (Human) ELISA Kit (Cat. No. E4376-100)
- BioSim™ Golimumab (Human) ELISA Kit (Cat. No. E4377-100)
- BioSim™ Infliximab (Human) ELISA Kit - 2 (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™ Vedolizumab (Human) ELISA Kit (Cat. No. E4697-100)
- BioSim™ Pembrolizumab (Human) ELISA Kit (Cat. No. E4383-100)
- BioSim™ Ustekinumab (Human) ELISA Kit (Cat. No. E4695-100)