

BioSim™ Adalimumab (Human) ELISA Kit

rev 12/20

(Catalog # E4372-100, 96 assays, Store at 4°C)

I. Introduction:

Adalimumab is a recombinant human IgG1 monoclonal antibody specific for Tumor Necrosis Factor-Alpha (TNF- α) and is used to treat rheumatic arthritis, intestinal disorders, dermatological diseases, and cancer. Adalimumab specifically binds to TNF alpha and blocks its interaction with p55 and p75 cell surface TNF receptors and reduces inflammation and subsequently improves the patient's health. Drug level quantification can be important to adapt to patient prescription or to switch to an alternative TNF inhibitor drug. This Adalimumab ELISA kit has been developed for specific quantification of Adalimumab concentration in human serum or plasma with high sensitivity and reproducibility. BioVision's BioSim™ Adalimumab ELISA kit is a sandwich ELISA assay for the quantitative measurement of Adalimumab in human serum, plasma. The density of color is proportional to the amount of human Adalimumab captured from the samples.

II. Application:

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

Detection Range: 30 - 1000 ng/ml

Sensitivity: 10 ng/ml

Assay Precision: Intra-Assay: CV < 30%; Inter-Assay: CV < 30% (CV (%) = SD/mean X 100)

Cross Reactivity: No significant cross-reactivity or interference with other proteins present in native human serum or other therapeutic immunoglobulins.

Recovery rate: < 100 \pm 30% with normal human serum samples with known concentrations

III. Sample Type:

Human serum and plasma

IV. Kit Contents:

Components	E4372-100	Part No.
Micro ELISA Plate	1 plate	E4372-100-1
Adalimumab Standards (S1 – S7)	1 ml X 7	E4372-100-2.x
Assay Buffer	50 ml	E4372-100-3
HRP-conjugate Probe	12 ml	E4372-100-4
TMB substrate (Avoid light)	12 ml	E4372-100-5
Stop Solution	12 ml	E4372-100-6
Wash buffer (20X)	50 ml	E4372-100-7
Plate sealers	2	E4372-100-8

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. Standards:

Ready to use, Concentration for High and low standards are indicated on vials.

Name	S1	S2	S3	S4	S5	S6	S7
Conc. (ng/ml)	1000	300	100	30	0	High Standard	Low Standard

3. Sample Dilution:

- **Serum/Plasma:** Dilute samples at 1:10 (20 μ l Serum/Plasma + 180 Assay Buffer)
- Diluted samples should further be diluted if the concentration of Adalimumab 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.

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

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. Pipette 100µl of **Assay Buffer** non-exceptionally into each of the wells to be used.
3. Add 20 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 3.
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 Adalimumab 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.

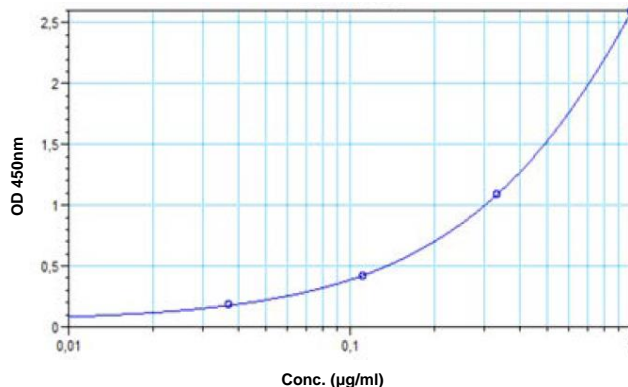


Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

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™ 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)

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