

BioSim™ anti-Adalimumab (Humira®) (Human) ELISA Kit rev11/17

(Catalog # E4388-100, 100 assays, Store at 4°C)

I. Introduction:

Adalimumab (Humira®) 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 the inflammation and subsequently improves the patient's health. Drug level quantification can be important to adapt patient prescription or to switch to an alternative TNF inhibitor drug. However, some patients develop unwanted immunogenicity, which leads to production of anti-drug-antibodies (ADAs) inactivating the therapeutic effects of the treatment and, in rare cases, inducing adverse effects. BioVision's BioSim™ anti-Adalimumab ELISA kit is designed to quantify/measure the antibody against Adalimumab with high specificity and sensitivity in biological matrices.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of antibody against Adalimumab in serum and plasma

Detection Range: 62 - 500 ng/ml

Sensitivity: 30 ng/ml

Assay Precision: Intra-Assay: CV < 15%; Inter-Assay: CV < 15% (CV (%) = SD/mean X 100)

Cross Reactivity: Adalimumab infusion camouflages/masks the presence of antibody to adalimumab (ATA) in serum/plasma samples. Therefore, blood sampling time is critical for detection of ATA. It is convenient to obtain blood sample just before the infusion of adalimumab or at least 2 weeks after the infusion of adalimumab.

Recovery rate: 85 – 115% with normal human serum samples with known concentrations

III. Sample Type:

Human serum and plasma

IV. Kit Contents:

Components	E4388-100	Part No.
Micro ELISA Plate	1 plate	E4388-100-1
Adalimumab Standards (S1 – S7)	1 ml X 7	E4388-100-2.x
Assay Buffer	50 ml	E4388-100-3
Confirmation Reagent	12 ml	E4388-100-4
Peroxidase Conjugate	12 ml	E4388-100-5
TMB substrate (Avoid light)	12 ml	E4388-100-6
Stop Solution	12 ml	E4388-100-7
Wash buffer (20X)	50 ml	E4388-100-8
Plate sealers	2	E4388-100-9

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)	500	250	125	62	0	High Control	Low Control

3. **Sample Dilution:**

- **Serum/Plasma:** First dilute samples at 1:10 (20 µl Serum/Plasma + 180 µl ddH₂O) or 1:100 (5 µl diluted sample + 495 µl ddH₂O)
- Diluted samples should further be diluted if the concentration of Adalimumab is higher than the measuring range.

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- The usual precautions for venipuncture should be observed. Samples are stable at 4°C for 7 days and -20°C for 6 months. Avoid freeze-and-thaw cycle.
4. **Confirmation Test Mixture:** Mix 20 µl undiluted (positive) serum/plasma sample with 180 µl confirmation reagent for 60 minutes in a microtube prior to the test.

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 µl of **standards, controls, diluted-samples, and confirmation test mixture** (if applicable) 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 µl of diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
4. Add 100 µl of **Peroxidase Conjugate** into each well. Cover wells with adhesive plate sealer and incubate at RT for 60 minutes.
5. Discard the solution and wash the wells as step 3.
6. Add 100 µl of 1X **TMB substrate** solution and incubate the plate in dark at RT for 20 minutes
7. Add 100 µl of **Stop solution** to stop the reaction
8. Read the absorbance in micro plate reader set to 450 nm within 20 minutes. (reference wavelength to 650 nm)

IX. QUANTITATIVE CALCULATION:

Using the standards disregarding zero standard, construct a standard curve by plotting the OD_{450/650 nm} for each standard 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.

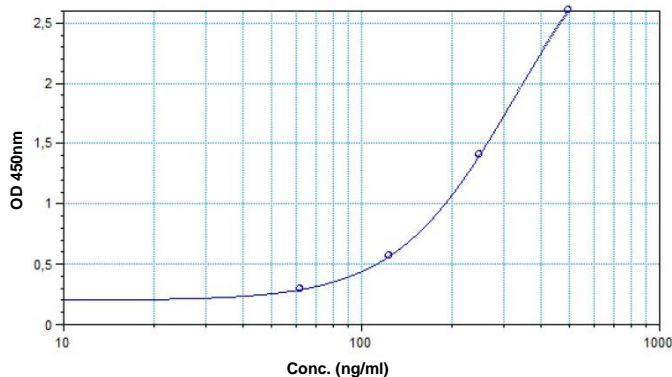


Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

X. QUALITATIVE INTERPRETATION:

- If “Sample OD_{450/650} / Zero Standard (S5) OD_{450/650}” is < 3, the sample is **NEGATIVE** for Antibody to Adalimumab (ATR)
- If “Sample OD_{450/650} /Zero Standard (S5) OD_{450/650}” is ≥ 3, the sample is **POSITIVE** for ATR and if required samples may be extrapolated for quantitative analysis and confirmation.
- For the run to be valid, the OD_{450/650 nm} of Positive Control (Standard A) should be ≥ 1.000 and the OD_{450/650 nm} of each Negative Control should be <0.200, if not, improper technique or reagent deterioration may be suspected and the run should be repeated.
- Interpretation of true and false positive: For true positive sample, inhibition should be equal or greater than 25%

$$\frac{\text{OD}_{(450/650)} \text{ sample} - \text{OD}_{(450/650)} \text{ sample w/confirmation reagent}}{\text{OD}_{(450/650)} \text{ sample}} \times 100 = \text{inhibition \%}$$

XI. 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™ Infliximab (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)

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- 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™ Nivolumab (Opdivo®)(Human) ELISA Kit (Cat. No. E4382-100)
- BioSim™ Pembrolizumab (Keytruda®)(Human) ELISA Kit (Cat. No. E4383-100)
- BioSim™ Ipilimumab (Yervoy®)(Human) ELISA Kit (Cat. No. E4384-100)
- BioSim™ Rituximab (Mabthera®) (Human) ELISA Kit (Cat. No. E4385-100)
- BioSim™ Trastuzumab (Herceptin®) (Human) ELISA Kit (Cat. No. E4386-100)
- BioSim™ Infliximab (Remicade®) (Human) ELISA Kit (Cat. No. E4387-100)
- BioSim™ Adalimumab (Humira®) (Human) ELISA Kit (Cat. No. E4388-100)
- BioSim™ Bevacizumab (Avastin®) (Human) ELISA Kit (Cat. No. E4389-100)
- BioSim™ Infliximab (Remsima®) (Human) ELISA Kit (Cat. No. E4390-100)
- BioSim™ Cetuximab (Erbix®) (Human) ELISA Kit (Cat. No. E4391-100)
- BioSim™ Etanercept (Enbrel®) (Human) ELISA Kit (Cat. No. E4392-100)
- BioSim™ Golimumab (Simponi®) (Human) ELISA Kit (Cat. No. E4393-100)
- BioSim™ Denosumab (Prolia®) (Human) ELISA Kit (Cat. No. E4394-100)
- BioSim™ Omalizumab (Xolair®) (Human) ELISA Kit (Cat. No. E4395-100)
- BioSim™ Nivolumab (Opdivo®) (Human) ELISA Kit (Cat. No. E4396-100)
- BioSim™ Pembrolizumab (Keytruda®) (Human) ELISA Kit (Cat. No. E4397-100)
- BioSim™ Ipilimumab (Yervoy®) (Human) ELISA Kit (Cat. No. E4398-100)
- BioSim™ Filgrastim (Herceptin®) (Human) ELISA Kit (Cat. No. E4399-100)

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