



# BioSim™ Infliximab (Remicade®) (Human) ELISA Kit Rev 06/21

(Catalog # E4375-100, 100 assays, Store at 4°C)

## I. Introduction:

Infliximab (Remicade®) is a chimeric monoclonal antibody and used to treat auto-immune disorders. Infliximab reduces the amount of active human tumour necrosis factor alpha (hTNFα) in the body by binding to it and preventing it from signaling the receptors for TNFα on the surface of various cell types. TNFα is one of the key cytokines that triggers and sustains the inflammatory reactions. Infliximab is used for the treatment of psoriasis, Crohn's disease, ankylosing spondylitis, psoriatic arthritis, rheumatoid arthritis, ulcerative colitis. BioVision's BioSim™ Infliximab ELISA kit has been developed for specific quantification of Infliximab concentration in human serum or plasma with high sensitivity and reproducibility.

## II. Application:

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

Detection Range: 0.1 - 3 µg/ml

Sensitivity: 30 ng/ml

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

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

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

## III. Sample Type:

Human serum and plasma

## IV. Kit Contents:

Components	E4375-100	Part No.
Micro ELISA Plate	1 plate	E4375-100-1
Infliximab Standards (S1 – S7)	0.3 ml X 7	E4375-100-2.x
Assay Buffer	50 ml	E4375-100-3
HRP-conjugate Probe	12 ml	E4375-100-4
TMB substrate (Avoid light)	12 ml	E4375-100-5
Stop Solution	12 ml	E4375-100-6
Wash buffer (20X)	50 ml	E4375-100-7
Plate sealers	2	E4375-100-8

## 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 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 2 weeks after preparation at 4°C.

### 2. Standard Preparation:

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

Name	S1	S2	S3	S4	S5	S6	S7
Conc. (µg/ml)	3	1	0.3	0.1	0	High Standard	Low Standard

### 3. Sample Dilution:

- **Serum/Plasma:** Dilute samples at 1:20 (10 µl Serum/Plasma+190 µl Assay Buffer) or 1:100 (5 µl diluted sample + 495 µl Assay Buffer) (Dilution factor : 20 - 100)
- Diluted samples should further be diluted if the concentration of Infliximab 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.



## VIII. Assay Protocol:

**Note:** Bring all reagents, microplate and samples to room temperature 15 minutes prior to the assay.

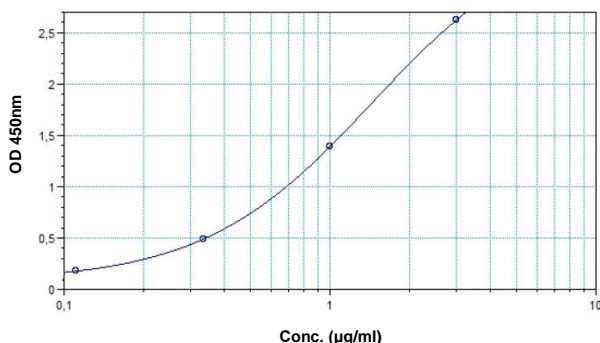
It is recommended that all standards and samples be run at least in duplicate.

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 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 to 650 nm)

## IX. CALCULATION:

Using the standards disregarding zero standard, construct a standard curve by plotting the OD<sub>450/650 nm</sub> for each of 4 standards on the Y-axis versus the corresponding Infiximab 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™ Ipilimumab (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™ 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)