# BioSim™ anti-Infliximab (Remicade®) (Human) ELISA Kit

*08/21*

**(Catalog # E4387-100, 100 assays, Store at 4°C)**

## I. Introduction:

Infliximab (Remicade®) is a therapeutic chimeric monoclonal antibody against tumor necrosis factor (TNF) and is used to treat rheumatic arthritis, intestinal disorders, dermatological diseases and cancer. Infliximab drug inhibits the action of TNF 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. Infliximab is used for the treatment of psoriasis, Crohn's disease, ankylosing spondylitis, psoriatic arthritis, rheumatoid arthritis, ulcerative colitis. 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-Infliximab ELISA kit is designed to quantify/measure the antibody against Infliximab with high specificity and sensitivity in biological matrices.

## II. Application:

This ELISA kit is used for *in vitro* quantitative determination of antibody against Infliximab in serum and plasma
Detection Range: 62 - 500 ng/ml
Sensitivity: 15 ng/ml
Assay Precision: Intra-Assay: CV < 15%; Inter-Assay: CV < 15% (CV (%) = SD/mean X 100)
Cross Reactivity: Infliximab (Remicade®) infusion camouflages/masks the presence of antibody to infliximab (ATI) in serum/plasma samples. Therefore, blood sampling time is critical for detection of ATI. It is convenient to obtain blood sample just before the infusion of infliximab or at least 2 weeks after the infusion of infliximab.
Recovery rate: 85 – 115% with normal human serum samples with known concentrations

## III. Sample Type:

Human serum and plasma

## IV. Kit Contents:

| Components | E4387-100 | Part No. |
|---|---|---|
| Micro ELISA Plate | 1 plate | E4387-100-1 |
| Infliximab Standards (S1 – S7) | 1 ml X 7 | E4387-100-2.x |
| Assay Buffer | 50 ml | E4387-100-3 |
| Confirmation Reagent | 12 ml | E4387-100-4 |
| Peroxidase Conjugate | 12 ml | E4387-100-5 |
| TMB substrate (Avoid light) | 12 ml | E4387-100-6 |
| Stop Solution | 12 ml | E4387-100-7 |
| Wash buffer (20X) | 50 ml | E4387-100-8 |
| Plate sealers | 2 | E4387-100-9 |

## 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 $ddH_2O$ (10 ml of Wash Buffer stock to 190 ml of $ddH_2O$). 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 | 62.5 | 0 | High standard | Low Standard |

3. **Sample Dilution:**
   - **Serum/Plasma**: First dilute samples at 1:10 (20 µL sample + 180 µL assay buffer)
   - 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 7 days and -20°C for 6 months. Avoid freeze-and-thaw cycle.

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

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, 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 30 minutes after pipetting the Stop Solution. (reference wavelength to 650 nm)

## IX. QUANTITATIVE CALCULATION:

Using the standards disregarding zero standard, construct a standard curve by plotting the OD450/650 nm for each standard on the Y-axis versus the corresponding Infliximab 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.

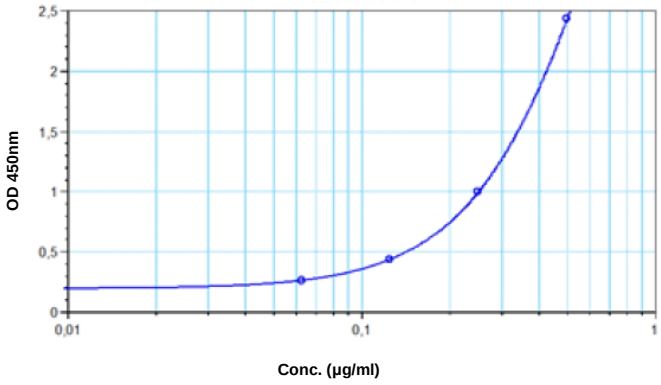


**Figure**: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

## X. QUALITATIVE INTERPRETATION:

- If "Sample $OD_{450/650}$ / Zero Standard (S5) $OD_{450/650}$" is < 3, the sample is NEGATIVE for Antibody to Infliximab (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 OD450/650 nm of Positive Control (Standard A) should be ≥ 1.000 and the OD450/650 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%

$$\frac{OD_{(450/650)}\text{ sample - }OD_{(450/650)}\text{ sample w/confirmation reagent}}{OD_{(450/650)}\text{ sample}} \text{ x100 = inhibition \%}$$

## 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)
- BioSim™ Cetuximab (Erbitux®)(Human) ELISA Kit (Cat. No. E4379-100)

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- 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 (Erbitux®) (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)