



BioSim™ Infliximab (Remsima®)(Human) ELISA Kit

rev 11/17

(Catalog # E4378-100, 100 assays, Store at 4°C)

I. Introduction:

Infliximab (CT-P13)(Remsima®) is a chimeric monoclonal antibody and used to treat auto-immune disorders. Infliximab reduces the amount of active human tumor 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. Infliximab is used for the treatment of psoriasis, Crohn's disease, ankylosing spondylitis, psoriatic arthritis, rheumatoid arthritis, ulcerative colitis. In controlled trials, clinical response rates of 20-40% have been achieved with above-mentioned regimens in Crohn's disease and rheumatoid arthritis. However, the therapeutic benefits must be balanced against the risk of a variety of severe adverse events such as severe infections including tuberculosis, hepatotoxicity, infusion reactions, serum sickness-like disease and lymphoma. 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: 100 - 3000 ng/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: Except for the other therapeutic anti-TNF antibodies such as etanercept (Enbrel®) and/or adalimumab (Humira®) with which cross reaction might occur to some extends, there is no cross reaction with native serumimmunoglobulin.

III. Sample Type:

Human serum and plasma

IV. Kit Contents:

| Components | E4378-100 | Part No. |
|--------------------------------|------------|---------------|
| Micro ELISA Plate | 1 plate | E4378-100-1 |
| Infliximab Standards (S1 – S7) | 0.3 ml X 7 | E4378-100-2.x |
| Assay Buffer | 50 ml | E4378-100-3 |
| HRP-conjugate Probe | 12 ml | E4378-100-4 |
| TMB substrate (Avoid light) | 12 ml | E4378-100-5 |
| Stop Solution | 12 ml | E4378-100-6 |
| Wash buffer (20X) | 50 ml | E4378-100-7 |
| Plate sealers | 2 | E4378-100-8 |

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. Standard Preparation:

Ready to use.

| Name | S1 | S2 | S3 | S4 | S5 | S6 | S7 |
|---------------|------|------|-----|-----|----|--------------|-------------|
| Conc. (ng/ml) | 3000 | 1000 | 300 | 100 | 0 | High Control | Low Control |

3. Sample Dilution:

- **Serum/Plasma:** Initially dilute samples 1:20 (10 µl Serum/Plasma+ 190 µl Assay Buffer) or 1:100 (5 µl Sample + 495 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.



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 100 μ l of **Assay Buffer** non-exceptionally into each of the wells to be used
3. Add 10 μ l 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 μ l of diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
5. Add 100 μ l 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 μ l of 1X **TMB substrate** solution and incubate the plate in dark at RT for 10 minutes
8. Add 100 μ l 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 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.

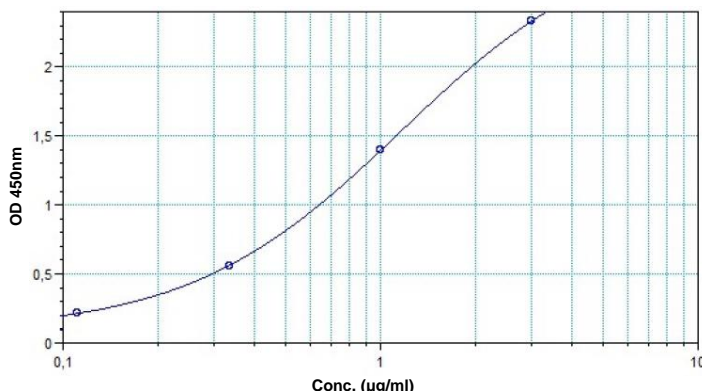


Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

X. 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)