BioSim™ anti-Etanercept (Human) ELISA Kit - II

rev 01/21

(Catalog # E4392-100, 100 assays, Store at 4°C)

I. Introduction:

Etanercept is a therapeutic fusion protein specific for Tumor Necrosis Factor-Alpha (TNF-α) and is used to treat rheumatic arthritis, intestinal disorders, dermatological diseases and cancer. Etanercept specifically binds to TNF alpha and blocks its interaction with cell surface TNF receptors and reduces the inflammation and subsequently improves the patient's health. 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-Etanercept ELISA kit is designed to detect the antibody against Etanercept with high specificity and sensitivity in biological matrices.

II. Application:

This ELISA kit is used for *in vitro* qualitative determination of antibody against Etanercept in serum and plasma
Cross Reactivity: Etanercept infusion camouflages/masks the presence of antibody to Etanercept (ATC) in serum/plasma samples. Therefore, blood sampling time is critical for detection of ATC. It is convenient to obtain blood sample just before the infusion or at least 2 weeks after the infusion of Etanercept.

III. Sample Type:

Human serum and plasma

IV. Kit Contents:

Components	E4392-100	Part No.
Micro ELISA Plate	1 plate	E4392-100-1
Positive Control	0.3 ml	E4392-100-2
Negative Control	1 ml	E4392-100-3
Assay Buffer	12 ml	E4392-100-4
Peroxidase Conjugate	12 ml	E4392-100-5
TMB substrate (Avoid light)	12 ml	E4392-100-6
Stop Solution	12 ml	E4392-100-7
Wash buffer (20X)	50 ml	E4392-100-8
Plate sealers	2	E4392-100-9

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- 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: 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. **Sample preparation:** 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 µl of **Assay Buffer** into each of the wells to be used.
3. Add 10 µl of **negative control** (2 wells), **positive control**, and **samples** into appropriate wells. Cover wells and incubate for 60 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 **Peroxidase Conjugate** into each well. Cover wells with adhesive plate sealer and incubate at RT for 60 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 20 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)

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

IX. QUALITATIVE INTERPRETATION:

- For the run to be valid, the OD 450/650 nm of positive control should be >1.500 and the OD 450/650 nm of each negative control should be <0.150. if not, improper technique or reagent deterioration may be suspected and the run should be repeated.
- The results are evaluated by a cut-off value which is estimated by multiplying the mean OD 450/650 nm of the negative controls by 3.

  e.g.
- If "Sample OD 450/650 / the mean negative control OD 450/650 ≥3" then the sample is POSITIVE
- If "Sample OD 450/650 / the mean negative control OD 450/650 <3" then the sample is NEGATIVE

Note: The cut-off information provided with this kit can only be considered as a recommendation. Cut-off values must be calculated/set or verified according to scientific standards by the users/laboratories.

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™ Infliximab (Human) ELISA Kit-1 (Cat. No. E4375-100)
- BioSim™ anti-HER2 (Human) ELISA Kit (Cat. No. E4376-100)
- BioSim™ Golimumab (Human) ELISA Kit (Cat. No. E4377-100)
- BioSim™ Infliximab (Human) ELISA Kit-2 (Cat. No. E4378-100)
- 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)
- BioSim™ Rituximab (Human) ELISA Kit (Cat. No. E4385-100)
- BioSim™ anti-HER2 mab (Human) ELISA Kit (Cat. No. E4386-100)
- BioSim™ Filgrastim (Human) ELISA Kit (Cat. No. E4399-100)