

# AffinityImmuno®

## Etanercept (Enbrel®) Pharmacokinetic ELISA Catalog EL-1611-051

For the quantitative determination of Etanercept in serum and plasma.

### Introduction

Etanercept (trade name Enbrel®) is a protein drug used to treat autoimmune diseases by adsorbing tumor necrosis factor (TNF; a soluble inflammatory cytokine). Etanercept is a fusion protein produced through expression of recombinant DNA by linking the extracellular ligand-binding portion of TNFRSF1B to the Fc component of human immunoglobulin G1 (IgG1). It reduces the effect of naturally present TNF, functioning as a decoy receptor that binds to TNF.

Etanercept is indicated for the treatment of moderate to severe rheumatoid arthritis (RA), psoriatic arthritis, ankylosing spondylitis, and moderately to severely active polyarticular juvenile idiopathic arthritis. Serum concentration of ENBREL® may predict some clinical outcome during maintenance therapy.

In clinical trials Etanercept has been reported to elicit an antibody response. Both binding and neutralizing antibodies against Etanercept have been reported. The formation of binding or neutralizing antibodies to therapeutic agent may decrease the efficacy of these agents leading to losses of clinical response over time. Anti-drug antibodies may cause infusion reactions and anaphylactic reactions. Detection, measurement, and characterization of anti-therapeutic antibody are critical in understanding the safety, exposure, and efficacy profile of the therapeutic.

The Etanercept ELISA kit is designed to measure free Etanercept with high specificity and sensitivity. The assay design uses a pair of antibodies that allows detection of whole Etanercept molecules in biological matrices.

### Principle of the assay

This assay employs the sandwich enzyme immunoassay technique. Capture antibody is coated onto a 96 well microplate. Calibrator and test samples are pipetted into the appropriate wells. Etanercept present in biological matrices is bound by the immobilized anti-Etanercept antibody. After washing away any unbound substances, enzyme linked anti-Etanercept antibody is added to the wells. The plate is washed to remove any unbound antibody-enzyme reagent and a substrate solution is added to the wells for color development. The color development is proportional to the amount of Etanercept present in test samples. The color development is stopped and the intensity of the color is measured.

### Materials and storage

Store kit components at -20 °C unless specified otherwise. DO NOT USE past kit expiration date. Some vials contain a small amount of reagents. Centrifuge tubes on pulse setting prior to opening.

Each kit includes:	Units
Coated microtiter plate, 96 wells (1x8 strips)	1
Calibrator diluent	1.8ml
Calibrator (500 µg/mL)	12µl
10X wash buffer	25ml
Assay buffer	50ml
1000X detection reagent	17µl
TMB	12ml
TMB stop solution	12ml
<b>Do not</b> mix or substitute reagents with those from other lots.	

### Materials and instruments required but not supplied

- Precision pipettes calibrated to deliver 5-1000µL
- Multi-channel pipette calibrated to deliver 50-200µL
- Plate shaker
- Disposable tips
- Vortex-Mixer
- Distilled or de-ionized water
- Microplate reader capable of reading 450nm with background subtraction at 620nm

### Safety precautions

- The test protocol must be followed strictly.
- All reagents containing human material should be handled as if potentially infectious. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.
- The kit reagents contain antimicrobial agents, acid and 3,3',5,5'-tetramethylbenzidine. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
- Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Disposal must be performed in accordance with local regulations.

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- Disposal must be performed in accordance with local regulations.
- Only trained laboratory personnel should execute this test.

## Preparation of reagents

Prepare only the appropriate amount of required reagent on the day of use. Store all reagents as per instructions stated on the label.

- 1. Wash Buffer (1X) Preparation:** Dilute wash buffer concentrate with ultra-pure water 1/10 before use (for example add 2mL concentrate to 18mL ultra-pure water). Mix well.
- 2. Detection Reagent (1X) Preparation:** Dilute detection reagent with assay buffer 1/1000 before use (for example add 10µl concentrate to 10ml of assay buffer). Mix well.
- 3. Preparation of Calibrators:** Prepare calibrators with concentrations ranging from 2500 ng/mL to 78 ng/mL. The following is an example calibrator curve.

Sol'n ID	Source	Source Vol (µL)	Cal* Diluent (µL)	Final Vol (µL)	Final Concentration (ng/mL)
1**	Stock Cal* (500µg/mL)	5	245	250	10,000
2**	Inter-mediate 1	50	50	100	5,000
1*	Inter-mediate 2	50	50	100	2,500
2*	1*	50	50	100	1,250
3*	2*	50	50	100	625
4*	3*	50	50	100	313
5*	4*	50	50	100	156
6*	5*	50	50	100	78
Neg	Cal* diluent	-	100	100	0

\*Calibrator \*\*Intermediate

## Specimen storage

This kit is compatible with EDTA-plasma, heparin-plasma and serum samples. Samples can be stored at or below -20°C for up to 1 year.

## Assay procedure

1. Remove coated microtiter plate from -20°C and allow it to acclimate to room temperature for 15-20 minutes.
2. Dilute calibrators and test samples 1/50 with assay buffer (for example add 5µL of prepared calibrator or sample to 245µL of assay buffer). Mix well. Do not store diluted samples.
3. Add 100µL diluted calibrators and samples to appropriate wells on the plate. Incubate for 1 hour at room temperature on a plate shaker at approx 300rpm.
4. Discard the content of the plate and wash the wells 3x with 200µL wash buffer per well.
5. Add 100µL detection reagent to appropriate wells on the plate. Incubate for 1 hour at room temperature on a plate shaker at approx 300rpm.
6. Discard the content of the plate and wash the wells 3x with 200µL wash buffer per well.
7. Add 100µL of TMB to each well on plate. Incubate for 3-5 minutes at room temperature protected from light.
8. Add 100µL of TMB stop solution to each well on plate. Mix by gently tapping the side of the plate.
9. Determine absorbance with a microplate reader at 450nm against 620nm.

## Calculations and results

1. Construct a standard curve by plotting the absorbance obtained from each standard against concentration. Use a 4 or 5 parameter curve fit. Alternatively a log-log curve fit may be used.
2. The concentration of the unknowns can be read directly from this standard curve using the absorbance value for each sample.
3. Any sample undiluted or diluted still reading greater than the highest standard should be diluted appropriately with assay buffer and retested. If the samples have been diluted, the concentration determined from the standard curve must be multiplied by the dilution factor.

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## Performance characteristics

**Precision:** The precision was determined by analyzing samples prepared at 500 ng/mL in 6 replicates on 6 different occasions. Intra-assay coefficient of variation (CV) ranges from 3% to 9%. Inter-assay CV was 13%.

**Detection Limit:** The detection limit is 78 ng/mL.

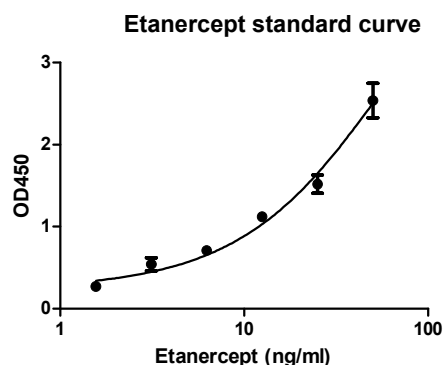
**Recovery:** 250 ng/mL of Etanercept was spiked in 10 lots of human serum. Recovery ranges are from 91-112% with an average recovery of 110%.

**Hook Effect:** No hook effect was observed up to 40000 ng/mL of Etanercept.

**Specificity:** sTNFa, TNFb, sTNFR1, sTNFR2 prepared at 250 ng/mL were assayed and exhibited no cross-reactivity or interference.

## Sample Standard Curve

The standard curve below was generated with the supplied calibrator using the recommended conditions and diluted 1 in 50 to give a final concentration range of 50ng/ml to 1.56ng/ml. Each sample was run with 6 replicates. Intra-assay coefficient of variance is <10%.



## Ordering Information

Please visit [www.affinityimmuno.com](http://www.affinityimmuno.com) to order this product. Visa, Mastercard, AMEX and PayPal are accepted in our online store.

Your order will be processed immediately and you will be notified with a delivery timeframe.

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