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# **Etanercept ELISA Kit**





#### Overview

Quantity:	96 tests
Target:	Etanercept
Reactivity:	Human, Mouse, Rat
Method Type:	Sandwich ELISA
Detection Range:	1.56-50 ng/mL
Minimum Detection Limit:	1.56 ng/mL
Application:	ELISA

# **Product Details**

Purpose:	Quantification of Etanercept in biological matrices
Sample Type:	Plasma, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	Etanercept (Enbrel)
Cross-Reactivity (Details):	sTNFa, TNFb, sTNFRI, sTNFRII prepared at 250 ng/mL were assayed and exhibited no crossreactivity or interference.
Sensitivity:	1.5 ng/mL
Components:	Coated microtiter plate, 96 wells
	Calibrator diluent 1.8ml
	Calibrator 12ul

10X wash buffer - 25ml

Assay buffer - 50ml

1000X detection reagent - 17ul

TMB - 12ml

TMB stop solution - 12ml

Plate sealers - 3

Material not included:

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 subtrac

### Target Details

Target:	Etanercept
Abstract:	Etanercept Products
Background:	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 (lgG1). 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.
Gene ID:	7124

### **Application Details**

Application Notes:	Optimal working dilution should be determined by the investigator.
Sample Volume:	15 μL
Assay Time:	2.5 h

Plate:	Pre-coated
Protocol:	The Etanercept ELISA kit is designed to measure free Etanercept with high specificity and sensitivity. This assay employs the sandwich enzyme immunoassay technique. A precoated anti-Etanercept 96 well plate is provided. Calibrator, quality control samples and test samples are pipetted into the appropriate wells. Etanercept present in biological matrices is bound by the immobilized capture antibody. After washing away any unbound substances, enzyme linked detection 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 and the concentration is calculated from the standard series.
Reagent Preparation:	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 20 mL concentrate to 180 mL ultra-pure water). Mix well. 2. Detection Reagent (1X) Preparation: Dilute detection reagent with assay buffer 1/1000 before use (for example add 11 µL concentrate to 11 mL 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.
Sample Collection:	This kit is compatible with EDTA-plasma, heparinplasma and serum samples. Samples can be stored at or below -20 °C for up to 1 year.
Sample Preparation:	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.
Assay Procedure:	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.
Calculation of 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 calibrator diluent and retested. If the

## **Application Details**

samples have been diluted, the concentration determined from the standard curve must be	
multiplied by the dilution factor.	

#### Assay Precision:

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) < 10%. Inter-assay CV < 10%.

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.

Restrictions:

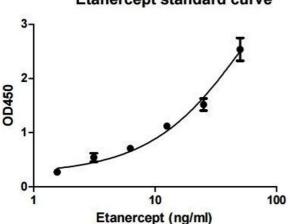
For Research Use only

## Handling

Preservative:	Without preservative
Precaution of Use:	Read manual completely before beginning
Storage:	-20 °C
Storage Comment:	Store kit components at -20°C unless specified otherwise. DO NOT USE past kit expiration date. Some vials contain a small amount of reagents. Spin tubes on pulse setting prior to opening.
Expiry Date:	12 months

#### **Images**

# Etanercept standard curve



#### **ELISA**

Image 1.