Datasheet for ABIN2862649

Etanercept ELISA Kit

1 Image

Go to Product page

Overview

Quantity:	96 tests
Target:	Etanercept
Reactivity:	Human, Chemical
Method Type:	Sandwich ELISA
Detection Range:	0.2-6 µg/mL
Minimum Detection Limit:	0.2 µg/mL
Application:	ELISA

Product Details

Purpose:	Enzyme immunoassay for the quantitative determination of free Etanercept in human serum and plasma.
Brand:	ImmunoGuide®
Sample Type:	Serum, Plasma (EDTA - heparin)
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	Etanercept (Enbrel®)T here is no cross-reaction with native serum immunoglobulin of human and other species and any of therapeutic antibodies other than anti-TNF ones. Because the solid phase is coated with rhTNF-α, other therapeutic anti-TNF antibodies cause full cross reaction. However, a quantification of other therapeutic antibodies is possible only by using the drug-specific standards.
Sensitivity:	10 ng/mL

Product Details

Characteristics:	0.2 - 6 µg/mL (in case of the recommended sample dilution factor) 10 ng/ml (sensitivity in the well) This kit measures the free drug concentration.
Components:	• 1 x 12 x 8 Microtiter Plate Break apart strips coated with recombinant human TNF-α (rhTNF-α). • 5 x 0.5 mL Etanercept Standards A-E 300, 100, 30, 10 and 0 ng/mL Ready to use. Used for construction of the standard curve. Contains Etanercept, human serum, proteins, stabilizer and <15mM NaN3. • 1 x 50 mL Assay Buffer Blue colored. Ready to use. Contains proteins and <15mM NaN3. • 1 x 12 mL Enzyme Conjugate Red colored. Ready to use. Contains horseradish peroxidase(HRP)-conjugated anti-human IgG mouse monoclonal antibody, Proclin® and stabilizers. • 1 x 12 mL TMB Substrate Solution Ready to use. Contains 3,3',5,5'-Tetramethylbenzidine (TMB). • 1 x 12 mL Stop Solution Ready to use. 1 N Hydrochloric acid (HCl). • 1 x 50 mL Wash Buffer, Concentrate (20x) Contains buffer, Tween® 20 and KathonTM. • 3 x 1 Adhesive Seal For sealing microtiter plate during incubation.
Material not included:	• Micropipettes (< 3 % CV) and tips to deliver 5-1000 µL. • Bidistilled or deionised water and calibrated glasswares (e.g. flasks or cylinders). • Wash bottle, automated or semi-automated microtiter plate washing system. • Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength at 600-650 nm is optional). • Absorbent paper towels, standard laboratory glass or plastic vials, and a timer.

Target Details

Target:	Etanercept
Abstract:	Etanercept Products
Background:	Etanercept is a dimeric fusion protein consisting of the extracellular ligand-binding portion of the human 75 kilodalton (p75) tumor necrosis factor receptor (TNFR) linked to the Fc portion of human IgG1. Etanercept binds specifically to human tumor necrosis factor alpha (TNF-α) and blocks its interaction with cell surface TNF receptors. Serum concentration of Etanercept might be related to predict some clinical outcome during maintenance therapy. It was also possible that the surveillance of circulating Etanercept concentration during maintenence therapy represents a direct and/or indirect factor for immunogenicity and some other side effects. In this context, identification of biomarkers for (non-)response and risk factors for adverse drug reactions that might be related to serum concentrations and maintaining the effective concentration of Etanercept in order to potentially avoid some side effects with a reliable

Target Details

	method might be beneficial.
Molecular Weight:	51 kDa

Application Details

Application Notes:	• Before performing the assay, samples and assay kit should be brought to room temperature (about 30 minutes beforehand) and ensure the homogeneity of the solution. • All Standards should be run with each series of unknown samples. • Standards should be subject to the same manipulations and incubation times as the samples being tested. • All steps of the test should be completed without interruption. • Use new disposable plastic pipette tips for each reagent, standard or specimen in order to avoid cross contamination.
Comment:	Etanercept ELISA is suitable also for using by an automated ELISA processor.
Sample Volume:	20 µL
Assay Time:	1.5 h
Plate:	Pre-coated
Protocol:	The Etanercept ELISA is a sandwitch-type ELISA. Standards and diluted samples (serum or plasma) are incubated in the microtitre plate coated with recombinant human TNF-α (rh TNF-α). After incubation, the wells are washed. A horseradish peroxidase (HRP)-conjugated anti-human IgG monoclonal antibody is added and binds to the Fc part of Etanercept pre-captured by the rh TNF-α on the surface of the wells. Following incubation, the wells are washed and the bound enzymatic activity is detected by addition of chromogen-substrate. The colour developed is proportional to the amount of Etanercept in the sample or standard. Results of samples can be determined directly by using the standard curve.
Reagent Preparation:	Wash Buffer: Dilute 10 mL Wash Buffer (up to 200 mL) at the ratio of 1:20 with distilled water. Warm up at 37 °C to dissolve crystals. Mix vigorously. Store at 2-8 °C for up to 4 weeks. Prepare Wash Buffer before starting the assay procedure.
Sample Collection:	Normal serum or plasma collection
Sample Preparation:	Serum/ Plasma: Initially dilute the Serum/ Plasma (Sample) at the ratio of 1:20 with Assay Buffer. Sample : Assay Buffer Relation can be 1:20-1:100. For dilution at 1:20, 10 µL Sample + 190 µL Assay Buffer

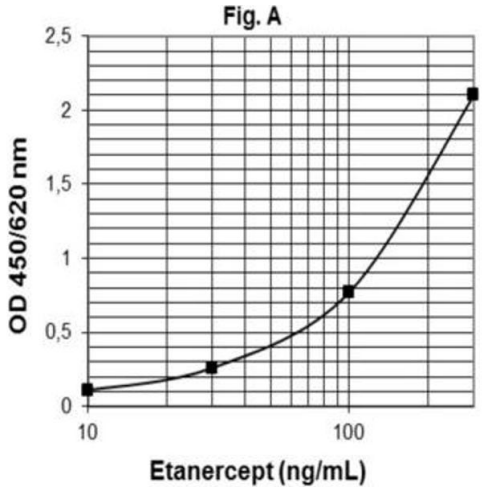
	For dilution at 1:100, 5 µL Sample + 495 µL Assay Buffer If any sample, initially diluted as indicated above, produces an OD value above the measuring range it should be rated as "> highest standard". The result should not be extrapolated. The sample in question should be further diluted with Assay Buffer and then retested. Serum, Plasma (EDTA, Heparin): The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material. Storage: 2-8 °C &leq,-20 °C (Aliquots) Keep away from heat or direct sun light. Avoid repeated freeze-thaw cycles. Stability: 3 days at 2-8 °C, 6 months at -20 °C
Assay Procedure:	1. Pipette 100 µL of Assay Buffer non-exceptionally into each of the wells to be used. 2. Pipette 20 µL of each Ready-to Use Standard, and Diluted Samples into the respective wells of microtiter plate. Wells A1: Standard A B1: Standard B C1: Standard C D1: Standard D E1: Standard E F1 and on: Diluted Samples (Serum/Plasma) 3. Cover the plate with adhesive seal. Shake plate carefully. Incubate 30 min at room temperature (RT) (18-25 °C). 4. Remove adhesive seal. Aspirate or decant the incubation solution. Wash the plate 3 X 300 µL of Diluted Wash Buffer per well. Remove excess solution by tapping the inverted plate on a paper towel. 5. Pipette 100 µL of Enzyme Conjugate (HRP-anti human IgGFc 1B5 mAb) into each well. 6. Cover plate with adhesive seal. Shake plate carefully. Incubate 30 min at RT. 7. Remove adhesive seal. Aspirate or decant the incubation solution. Wash the plate 3 X 300 µL of Diluted Wash Buffer per well. Remove excess solution by tapping the inverted plate on a paper towel. 8. Pipette 100 µL of Ready-to-Use TMB Substrate Solution into each well. 9. Incubate 15 min at RT in the dark (Without adhesive seal). 10. Stop the substrate reaction by adding 100 µL of Stop Solution into each well. Briefly mix contents by gently shaking the plate. Color changes from blue to yellow. 11. Measure optical density (OD) with a photometer at 450 nm (Reference at OD620 nm is Optional) within 15 min after pipetting of the Stop Solution.
Calculation of Results:	A standard curve should be calculated using the standard concentration (X-axis) versus the OD450 (or OD450/620) values (Y-axis). This can be done manually using graph paper or with a computer program. Concerning the data regression by computer we are recommending to

Application Details

	primarily use the "4 Parameter Logistic (4PL)" or alternatively the "point-to-point calculation". In case of manual plot there are 2 options: Semilog graph or linear graph . Semilog graph paper is available at http://www.papersnake.com/logarithmic/semilogarithmic/. The concentration of the samples can be read from this standard curve as follows. Using the absorbance value for each sample, determine the corresponding concentration of the drug from the standard curve. This value always has to be multiplied by the dilution factor. If any diluted sample is reading greater than the highest standard, it should be further diluted appropriately with Assay Buffer and retested. Also this second dilution has to be used for calculation the final result.
Assay Precision:	Intra-assay CV: <10%. Inter-assay CV: <10%. Recovery rate was found to be 98-102% with native human serum and plasma samples when spiked with exogenous Etanercept at 0,6 µg/mL or 6 µg/mL.
Restrictions:	For Research Use only

Handling

Buffer:	< 15mM NaN3
Preservative:	Sodium azide
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Storage:	4 °C
Storage Comment:	The kit is shipped at ambient temperature and should be stored at 2-8°C. Keep away from heat or direct sun light. The storage and stability of specimen and prepared reagents is stated in the corresponding chapters. The microtiter strips are stable up to the expiry date of the kit in the broken, but tightly closed bag when stored at 2-8°C.
Expiry Date:	24 months



ELISA

Image 1.