Datasheet for ABIN2862655

Infliximab specific ELISA Kit

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Overview

Quantity:	96 tests
Target:	Infliximab specific
Reactivity:	Chemical, Human
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 specific and quantitative determination of free Infliximab in human serum and plasma.
Brand:	ImmunoGuide®
Sample Type:	Serum, Plasma (EDTA - heparin)
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	Infliximab (Remicade®, Remsima®), no cross reaction to any other TNF-a catchersT. Here is no cross reaction with any other proteins present in native human serum and no cross reaction was observed with the other therapeutic antibodies including Etanercept, Adalimumab and Golimumab tested at concentrations up to 500 µg/mL.
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 without any cross reaction to other TNF-a catcher.
Components:	• 1 x 12 x 8 Microtiter Plate Break apart strips coated with Peri-1 mAb specific for Infliximab only. • 5 x 0.5 mL Infliximab Standards A-E 300, 100, 30, 10, and 0 ng/mL Ready to use. Used for construction of the standard curve. Contains Infliximab, 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 mAb, 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. • 2 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:	Infliximab specific
Background:	Infliximab is a chimeric monoclonal antibody and used to treat auto-immune disorders. Infliximab reduces the amount of active human tumour necrosis factor alpha (hTNFα) in the patient by binding to it and preventing it from signaling the receptors for TNFα on the surface of various cell types. TNFα is one of the key cytokines that triggers and sustains the inflammatory reactions. Infliximab is used for the treatment of psoriasis, Crohn's disease, ankylosing spondylitis, psoriatic arthritis, rheumatoid arthritis, ulcerative colitis. This drug is approved by the FDA. When relating serum concentrations to the clinical response in patients, it can be assumed that trough concentrations above 1 µg/mL could be used as a kind of therapeutic target. The rate of clinical remission was higher for patients with a detectable trough serum Infliximab compared with patients in whom serum Infliximab was undetectable, including those without antibodies. A detectable trough serum Infliximab was also associated with a lower C-

Target Details

	reactive protein and a higher rate of endoscopic improvement. For Crohn's disease patients treated with scheduled maintenance infusions of Infliximab, the serum concentration of Infliximab seemed to predict clinical outcome. It was also proposed that, the surveillance of circulating Infliximab concentration during maintenance therapy represents an indirect but reliable method to monitor anti-Infliximab immunization. In this context, identification of biomarkers for (non-)response and risk factors for adverse drug reactions relating to serum concentrations and therapeutic drug monitoring of Infliximab would be very helpful.
Molecular Weight:	144 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:	Infliximab ELISA (mAb-based) is suitable also for using by an automated ELISA processor. The reliability of the data regarding the pharmacokinetics of Infliximab is expected to be highly dependent on the specificity of the assay used. The data reported in the literature were almost totally obtained using ELISA in which the capture ligand, coated either directly or indirectly using anti-TNF antibody, was human tumor necrosis factor alpha (hTNFα). However, it is well known that human serum contain soluble TNF receptors (TNF-RI (p55/60 kDa) and TNF-RII (p75/80 kDa)). In addition, serum sample might contain other anti-TNF therapeutic immunoglobulins such as Etanercept, Adalimumab and/or Golimumab. ImmunoGuide Infliximab ELISA (mAb-based) is developed for the specific measurement of Infliximab in sera, plasma and other biological fluids by the advantage of using a site-directed Peri-1 monoclonal antibody (mAb) specific for Infliximab only. Binding of Infliximab to the solid phase, pre-coated with Peri-1, is inhibited by TNFα in a concentration dependent manner. In addition, when the antibody to Infliximab (ATI), immunoaffinity purified from various ATI-positive human sera, was pre-incubated with Infliximab the reaction was inhibited in a concentration dependent manner. Therefore, the ImmunoGuide Infliximab (mAb-Based) ELISA measures the free form of Infliximab (i.e. Infliximab molecules not pre-occupied either with TNFα or ATI). The choice of specifically measuring the free form allows investigators to analyze the concentration-effect

Application Details

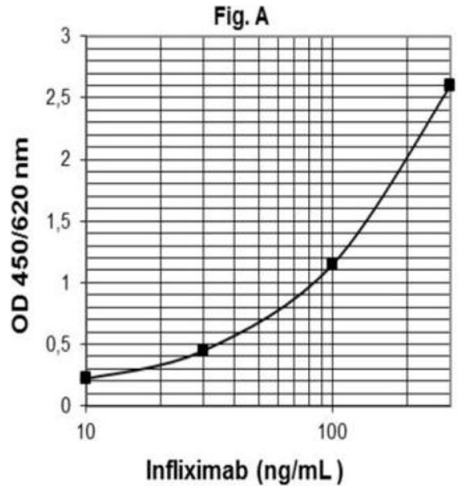
	relationship.
Sample Volume:	20 µL
Assay Time:	1.5 h
Plate:	Pre-coated
Protocol:	This ELISA is based on Infliximab-specific Peri-1 monoclonal antibody (catcher Ab). Standards and diluted samples are incubated in the microtitre plate coated with Peri-1 mAb. After incubation, the wells are washed. Anti-human IgG mAb (clone 1B5) conjugated to horse radish peroxidase (HRP) is added and binds to the Fc region of Infliximab. Following incubation, wells are washed and the bound enzymatic activity is detected by addition of chromogen-substrate. The colour developed is proportional to the amount of Infliximab 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 Samples with a concentration of Infliximab above the measuring range 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 d 6 mon
Assay Procedure:	1. Pipette 100 µL of Assay Buffer 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 the microtiter plate. Wells A1: Standard A B1: Standard B C1: Standard C D1: Standard D E1: Standard E F1 and so 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 IgGmAb) 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. Avoid exposure to direct sunlight. 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 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 Infliximab 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

Images



ELISA

Image 1.