Datasheet for ABIN2862653

Golimumab ELISA Kit

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Overview

Quantity:	96 tests
Target:	Golimumab
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 quantitative determination of free Golimumab in human serum and plasma samples.
Brand:	ImmunoGuide®
Sample Type:	Serum, Plasma (EDTA - heparin)
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	Golimumab (Simponi®)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 Golimumab Standards A-E 300, 100, 30, 10, and 0 ng/mL Ready to use. Used for construction of the standard curve. Contains Golimumab, 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. • 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:	Golimumab
Target Type:	Antibody
Background:	Golimumab is a human monoclonal antibody that binds to both the soluble and transmembrane bioactive forms of human TNFα. This interaction prevents the binding of TNFα to its receptors, thereby inhibiting the biological activity of TNF. Golimumab has been proven effective in the treatment of Rheumatoid Arthritis (RA), Ankylosing Spondylitis (AS), Psoriatic Arthritis (PsA) or Ulcerative Colitis (UC). Antibodies to Golimumab were detected in 57 (4 %) of Golimumab -treated patients across the Phase 3 RA, PsA, and AS trials through Week 24. Similar rates were observed in each of the three indications. Patients who received Golimumab with concomitant Methotrexate (MTX) had a lower proportion of antibodies to Golimumab than patients who received Golimumab without MTX (approximately 2 % versus 7 %, respectively). Of the patients with a positive antibody response to Golimumab in the Phase 2 and 3 trials, most

Target Details

	were determined to have neutralizing antibodies to Golimumab as measured by a cell- based functional assay. The data from the literature demonstrated that Anti-Drug Antibody positivity was significantly associated with low Golimumab levels and poor therapeutic response. The positive correlation between serum drug trough levels and therapeutic response indicates that drug monitoring could be useful for optimising the dosing of biologics in a personalised therapy strategy. 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 Golimumab in order to potentially avoid some side effects with the reliable Golimumab ELISA might be beneficial.
Molecular Weight:	147 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:	Golimumab 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 Golimumab ELISA is a sandwich-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 Golimumab 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 Golimumab in the standards or samples. 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.

Application Details

	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 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 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 IgG 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.

Application Details

	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 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 Golimumab at 0,6 µg/mL or 6 µg/mL.
Restrictions:	For Research Use only

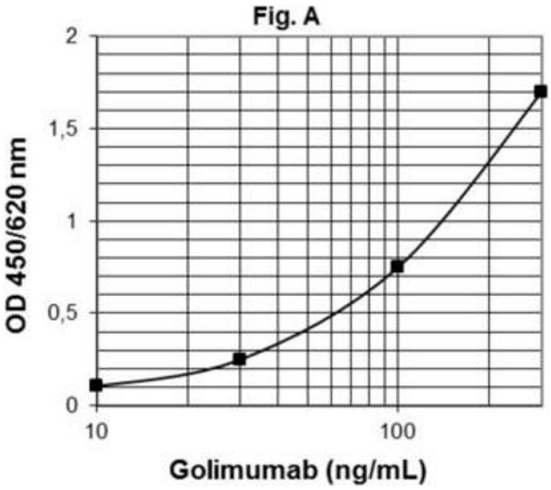
Handling

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.

Handling

	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.