Datasheet for ABIN3219142

Certolizumab Pegol specific ELISA Kit

2 Images

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Overview

Quantity:	96 tests
Target:	Certolizumab Pegol specific
Reactivity:	Chemical, Human
Method Type:	Sandwich ELISA
Detection Range:	0-2000 ng/mL
Minimum Detection Limit:	0 ng/mL
Application:	ELISA

Product Details

Purpose:	Enzyme immunoassay for the specific and quantitative determination of free Certolizumab pegol (Cimzia®) in serum and plasma.
Brand:	ImmunoGuide®
Sample Type:	Serum, Plasma
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	There is no cross reaction with any other proteins present in native human serum. A screening test was performed with 48 different native human sera at a dilution of 1:500. All produced OD450/620 nm values (ranged from 0.018 to 0.022) less than the mean OD (0.151) of the diluted standard D (6 ng/mL). In addition, binding of Certolizumab pegol is inhibited by recombinant human tumor necrosis factor alpha (rhTNFα) in a concentration dependent manner. Therefore, the ImmunoGuide Certolizumab pegol ELISA (mAb-based) measures the

Product Details

	biologically active free form of Certolizumab pegol, i.e. not pre-occuppied by TNFα. No cross reaction was observed with sera spiked with the other therapeutic antibodies including Infliximab, Etanercept, Adalimumab, Golimumab, Remsima, Tocillizumab, Trastuzumab and Rituximab tested at concentration of 100 µg/mL. All produced mean OD450/620 nm values less than 0.040.
Sensitivity:	2 ng/mL
Components:	• 1 x 12 x 8 Microtiter Plate Break apart strips coated with anti-Cerolizumab pegol monoclonal antibody. • 5 x 0.3 mL Certolizumab pegol Standards A-E, Concentrate (500x), 100, 30, 10, 3, and 0 µg/mL. Used for construction of the standard curve. Contains Certolizumab pegol, proteins, stabilizer and <15mM NaN3. • 1 x 12 mL Assay Buffer Blue colored. Ready to use. Contains proteins and <15mM NaN3. • 1 x 60 mL Dilution Buffer, Concentrate (5X), Contains proteins and <15mM NaN3. • 1 x 12 mL Biotinylated TNFα Green colored. Ready to use. Contains biotinylated recombinant human tumor necrosis factor alpha (rhTNFα), proteins, stabilizers and <15 mM NaN3. • 1 x 12 mL Enzyme Conjugate Red colored. Ready to use. Contains horseradish peroxidase(HRP)-conjugated streptavidin (HRP-Streptavidin), 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:	Certolizumab Pegol specific
Background:	Certolizumab pegol is a tumor necrosis factor alpha (TNFα) blocker and binds to human TNFα with a KD of 90pM. Certolizumab pegol is a recombinant, humanized antibody Fab' fragment, with specificity for TNFα, conjugated to an approximately 40kDa polyethylene glycol (PEG2MAL40K). The Fab' fragment is manufactured in E. coli and is subsequently subjected to purification and conjugation to PEG2MAL40K, to generate Certolizumab pegol. Steady-state concentrations range from 0.5 to 90 µg/mL for a fixed dose of 400 mg of certolizumab pegol.

Target Details

	For patients developing anti-certolizumab pegol antibodies, the steady state concentrations range from 0.5 to 75 µg/mL. Patients were tested at multiple times for antibodies to Certolizumab pegol during studies CD1 and CD2. The overall percentage of antibody positive patients was reported to be 8% in patients continuously exposed to Certolizumab pegol, of which approximately 80% were neutralizing in vitro. 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 Certolizumab pegol in order to potentially avoid some side effects with a reliable method might be beneficial.

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:	ELISA Kits are suitable also for using by an automated ELISA processor.
Assay Time:	2.5 h
Plate:	Pre-coated
Protocol:	This ELISA is based on Certolizumab pegol-specific mouse monoclonal antibody (catcher Ab, clone CY). Standards and diluted samples are incubated in the microtiter plate coated with IG-CY mAb. After incubation, the wells are washed. A biotinylated recombinant human tumor necrosis factor alpha (rhTNFα) is added and binds to the Fab part of Certolizumab pegol. Following incubation, wells are washed and the horseradish peroxidase (HRP)-conjugated streptavidin is added and binds to the biotinylated rhTNFα. 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 Certolizumab pegol in the sample or standard. Results of samples can be determined by using the standard curve. Preincubation of Certolizumab pegol with rhTNFα inhibited the reaction in a concentration dependent manner. Therefore, the Certolizumab pegol ELISA (mAb-based) measures the free form of Certolizumab pegol.
Reagent Preparation:	Dilution of Standards and Samples The dilutions depicted below are examples of how to obtain final 1:500 dilution. Standards and

Application Details

	samples should be properly diluted as homogenous mixture before starting the assay procedure. It is recommended mixing the standards and samples well to homogenous solution at each dilution step. 1. 10 µL of standard or sample added to 90 µL of 1X dilution buffer, giving the total volume of 100 µL and a dilution of 1:10. 2. 10 µL of 1:10 diluted standard or sample added to 490 µL of 1X dilution buffer, giving the total volume of 500 µL and a dilution of 1:500. 3. Samples with a drug concentration 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 1X Dilution Buffer and then retested.
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, it is recommended 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. 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 individual dilution factor, which usually will be 500. If any diluted sample is reading greater than the highest standard, it should be further diluted appropriately with 1X Dilution 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 >95% with native human serum and plasma samples when spiked with exogenous Certolizumab pegol at 100, 30, 10 and 3 µg/mL.
Restrictions:	For Research Use only

Handling

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.

Handling

	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

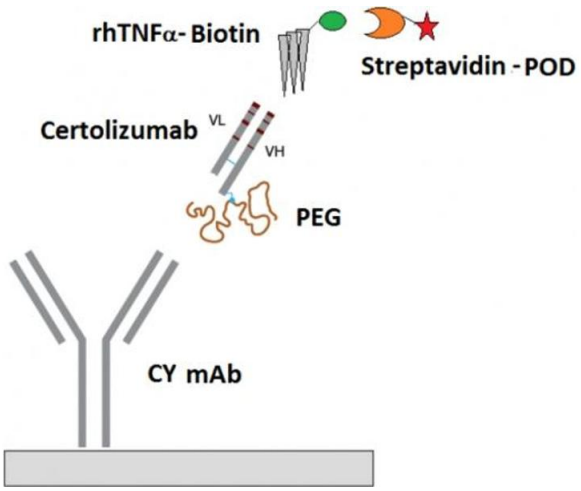

Image 1.

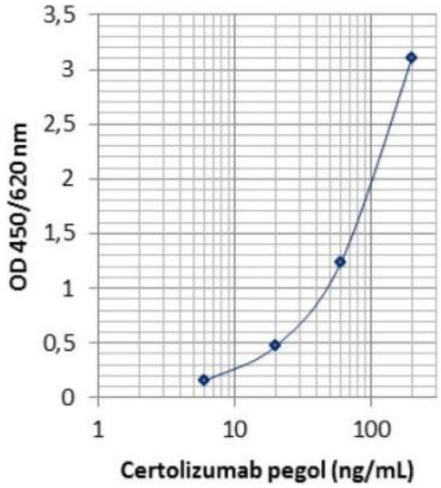

Image 2.