Datasheet for ABIN2862651

Bevacizumab ELISA Kit

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Overview

Quantity:	96 tests
Target:	Bevacizumab
Reactivity:	Human, Chemical
Method Type:	Sandwich ELISA
Detection Range:	0.6-20 µg/mL
Minimum Detection Limit:	0.6 µg/mL
Application:	ELISA

Product Details

Purpose:	Enzyme immunoassay for the quantitative determination of free Bevacizumab in human serum and plasma.
Brand:	ImmunoGuide®
Sample Type:	Serum, Plasma (EDTA - heparin)
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	There is no cross reaction with native serum immunoglobulins. Thirty seven native human sera were screened and all produced OD450/620 nm lower than 0.112. Other therapeutic antibodies (Omalizumab, Golimumab, Infliximab, Trastuzumab, Rituximab, Etanercept, Adalimmab and Tocilizumab) are also tested at the concentrations up to 400 µg/mL and observed that there are no cross reactions (OD 450/620nm values were less than 0,112). In addition, interference of Ranibizumab, binds to the same antigen VEGF-A, was tested at 25 ng/mL (nearly ten times of

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	the serum Cmax of Ranibizumab) and no measurable inhibition was observed.
Sensitivity:	2 ng/mL
Characteristics:	0.6 - 20 µg/mL (in case of the recommended sample dilution factor) 30 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 vascular endothelial growth factor-A (rhVEGF-A). • 5 x 0.3 mL Bevacizumab Standards A-E, Concentrate (1000x), 200, 60, 20, 6, and 0 µg/mL Ready to use. Used for construction of the standard curve. Contains Bevacizumab, human serum, 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 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:	Bevacizumab
Abstract:	Bevacizumab Products
Target Type:	Antibody
Background:	Bevacizumab is a recombinant human IgG1:κ, monoclonal antibody specific for all human vascular endothelial growth factor-A (VEGF-A) isoforms and it has been approved by the FDA as a first-line treatment for metastatic colorectal cancer in combination with chemotherapy. Furthermore, VEGF is implicated in intraocular neovascularization associated with diabetic retinopathy and age-related macular degeneration. Serum through levels might be related to

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	predict some clinical outcome during maintenance therapy. It was also possible that the surveillance of circulating concentration during maintenance therapy represents a direct and/or indirect factor for 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 drug levels and maintaining the effective concentration in order to potentially avoid some side effects with a reliable method might be beneficial.
Molecular Weight:	150 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. 6 • Use new disposable plastic pipette tips for each reagent, standard or specimen in order to avoid cross contamination.
Comment:	Bevacizumab ELISA is suitable also for using by an automated ELISA processor.
Sample Volume:	20 µL
Assay Time:	1.5 h
Plate:	Pre-coated
Protocol:	This ELISA is based on sandwich type ELISA. Diluted standards and samples (serum or plasma) are incubated in the microtiter plate coated with recombinant human vascular endothelial growth factor-A (rhVEGF-A). 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 Bevacizumab pre-captured by the rhVEGF-A 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 Bevacizumab in the sample or standard. Results of samples can be determined 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

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Sample Preparation:	Dilution of Standards and Samples The dilutions depicted below are examples of how to obtain final 1:1000 dilution. Standards and 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 990 µL of 1X dilution buffer, giving the total volume of 1000 µL and a dilution of 1:1000. 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 Dilution Buffer and then retested.
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 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. 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. The concentration of the samples can be read from this standard curve as follows. Using the absorbance value for each

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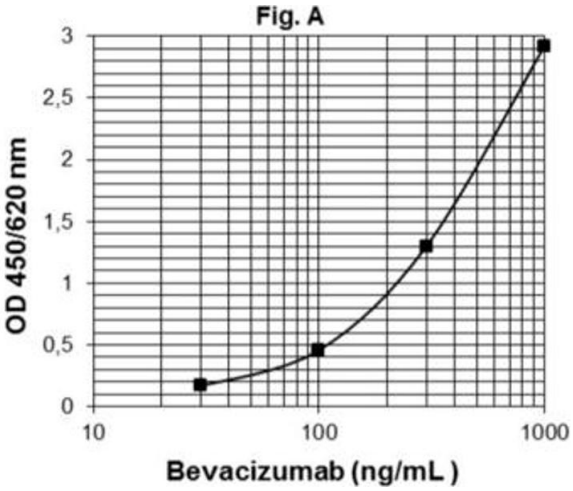
	sample, determine the corresponding concentration of the drug from the standard curve. This value always has to be multiplied by the dilution factor. If any diluted sample is reading greater than the highest standard, it should be further diluted appropriately with Assay Buffer and retested. Also this 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 >95% with native human serum and plasma samples when spiked with exogenous Bevacizumab at 200 µg/mL, 60 µg/mL, 20 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.