



[Go to Product page](#)

Datasheet for ABIN2862661

Bevacizumab Antibody ELISA Kit

3 Images

Overview

Quantity:	96 tests
Target:	Bevacizumab Antibody
Binding Specificity:	Free Chain
Reactivity:	Chemical, Human
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	Enzyme immunoassay for the semi-quantitative determination of free antibodies to Bevacizumab in human serum and plasma samples.
Brand:	ImmunoGuide®
Sample Type:	Serum, Plasma (EDTA - heparin)
Analytical Method:	Semi-Quantitative
Detection Method:	Colorimetric
Specificity:	Free antibodies against Bevacizumab (Avastin®)S creening test was performed with 80 different native and RF-negative human sera. All produced OD450nm values (ranged from 0.053 to 0.094) less than the cut-off value (3x0.068).
Sensitivity:	10 ng/mL
Characteristics:	This test does not measure the antibodies if they already are bound to the drug Bevacizumab
Components:	<ul style="list-style-type: none">1 x 12 x 8 Microtiter Plate Break apart strips pre-coated with the drug Bevacizumab.

Product Details

- 1 x 2 mL Positive Control Ready to use. Contains reactive antibody, proteins, stabilizer and <15 mM NaN₃.
- 1 x 2 mL Negative Control Ready to use. Contains human serum, stabilizer and <15 mM NaN₃.
- 1 x 60 mL Assay Buffer Blue colored. Ready to use. Contains proteins and <15 mM NaN₃.
- 1 x 12 mL Enzyme Conjugate Red colored. Ready to use. Contains horseradish peroxidase(HRP)-conjugated Bevacizumab, 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 Kathon™.
- 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 Antibody

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. As with all therapeutic proteins, there is a potential for immunogenicity. According to the manufacturer's product insert, the incidence of antibody development in patients receiving Bevacizumab has not been adequately determined because the assay sensitivity was inadequate to reliably detect lower titers. The Antibody to Bevacizumab ELISA Kit can be used for monitoring anti-Bevacizumab antibodies during therapy and offers the clinician a tool for decision on possible preventive measures.

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: Antibody to Bevacizumab ELISA is suitable also for using by an automated ELISA processor.

Sample Volume: 5 μ L

Assay Time: 2.5 h

Plate: Pre-coated

Protocol: The Antibody to Bevacizumab ELISA is a sandwich type ELISA for the determination of free antibodies against Bevacizumab in serum and plasma samples. During the first incubation period, the drug Bevacizumab, coated on the wall of the microtiter wells, captures the antibodies to Bevacizumab in patient serum and plasma samples. After washing away the unbound components from samples, a Peroxidase-labelled Bevacizumab conjugate is added to each well and then incubated. Antibody to Bevacizumab, if present in sample, will make a bridge, with its two identical Fab arms, between the Bevacizumab coated on the well and the other Bevacizumab labeled with peroxidase. After a second washing step, the bound enzymatic activity is detected by addition of tetramethylbenzidine (TMB) chromogen-substrate. Finally, the reaction is terminated with an acidic stop solution. The intensity of the reaction color is related to the presence of antibodies to Bevacizumab in the sample.

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: Dilute Serum/ Plasma (Sample) at the ratio of 1:101 with Assay Buffer.
For dilution at 1:101, 5 μ L Sample + 500 μ L Assay Buffer
Negative and Positive Controls are ready-to-use and should NOT be diluted with the assay buffer.

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 ≤ -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 each Ready-to Use Negative Control, Positive Control, and 1:101 Diluted Samples (as described in section 10.2) into the respective wells of microtiter plate. Wells A1: Negative Control B1: Negative Control C1: Positive Control D1 and so on: Diluted samples (Serum/Plasma)
2. Cover the plate with adhesive seal. Shake plate carefully. Incubate 60 min at room temperature (RT) (18-25 °C).
3. 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.
4. Pipette 100 µL of Enzyme Conjugate (HRP-Bevacizumab) into each well.
5. Cover plate with adhesive seal. Shake plate carefully. Incubate 60 min at RT.
6. 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.
7. Pipette 100 µL of Ready-to-Use TMB Substrate Solution into each well.
8. Incubate 20 min at RT. Avoid exposure to direct sunlight..
9. 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.
10. Measure optical density (OD) with a photometer at 450 nm (Reference at OD_{620nm} is optional) within 15 min after pipetting the Stop Solution.

Calculation of Results:

For the run to be valid, the OD_{450 nm} of the Positive Control should be ≥ 1.000 and the OD_{450 nm} of each Negative Control should be ≤ 0.150 . If not, improper technique or reagent deterioration may be suspected and the run should be repeated. The results are evaluated by dividing all individual results by the mean OD_{450nm} of the Negative Controls. The results are expressed in arbitrary units (AU/mL).

Range: > 3 AU/mL (Positive)

Range: ≤ 3 AU/mL (Negative)

Application Details

The results themselves should not be the only reason for any therapeutical consequences. They have to be correlated to other clinical observations.

Sample calculation for a positive sample:

OD of sample = 0.740 Cut-off = 3 x

Average OD of Negative Controls = 3 x 0.100 = 0.300 = 3 AU/mL

Concentration of sample = 0.740/0.100 = 7.4 AU/mL (positive)

Assay Precision: Intra-assay CV: <10%.
Inter-assay CV: <10%

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

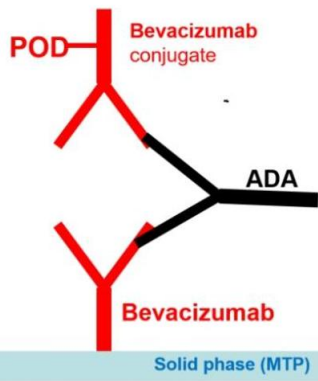


Image 1.

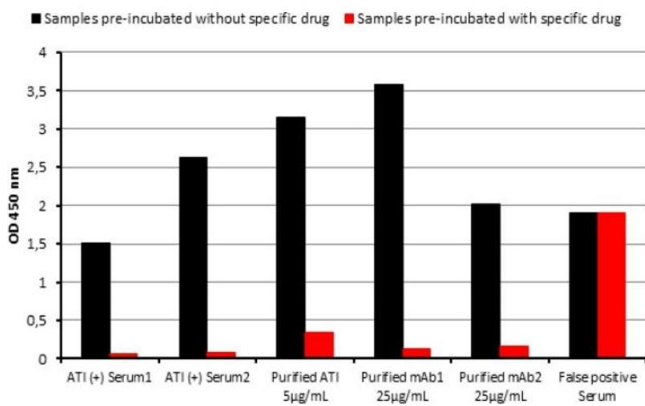


Image 2.

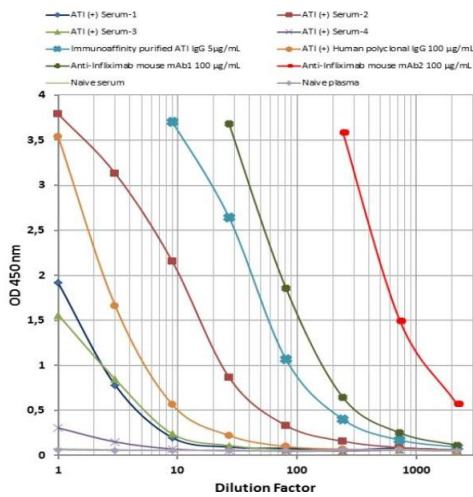


Image 3.