Instructions for Use

Antibody to Bevacizumab ELISA

Enzyme immunoassay for the semi-quantitative determination of free antibodies to Bevacizumab in serum and plasma

REF:MBS495308

12X8  2-8°C

For Research Use Only - Not for Use in Diagnostic Procedures
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1. INTENDED USE
Enzyme immunoassay for the semi-quantitative determination of free antibodies to Bevacizumab in serum and plasma samples.

2. SUMMARY AND EXPLANATION
The drug Bevacizumab (trade name Avastin®) is a recombinant human IgG1:κ monoclonal antibody specific for all human vascular endothelial growth factor-A (VEGF-A) isoforms. 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. The ImmunoGuide Antibody to Bevacizumab ELISA Kit can be used for monitoring anti-Bevacizumab antibodies against this drug. It does not detect such antibodies which already are bound to the drug.

3. PRINCIPLE OF THE TEST
The ImmunoGuide 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 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 with the presence of antibodies to Bevacizumab in the sample.

4. WARNINGS AND PRECAUTIONS
1. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood. For further information (clinical background, test performance, automation protocols, alternative applications, literature, etc.) please refer to the local distributor.
2. In case of severe damage of the kit package, please contact your local supplier in writing, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
3. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
4. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
5. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details.
6. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guidelines or regulations.
7. Avoid contact with Stop solution. It may cause skin irritations and burns.
8. If any component of this kit contains human serum or plasma it is indicated and if so, it has been tested and found to be negative for HIV I/II, HBsAg and HCV. However, the presence of these or other infectious agents cannot be excluded absolutely and therefore reagents should be treated as potential biohazards in use and for disposal.
9. Some reagents contain sodium azide (NaN₃) as preservatives. In case of contact with eyes or skin, flush immediately with water. NaN₃ may react with lead and copper plumbing to form explosive metal azides. When disposing reagents, flush with large volume of water to avoid azide build-up.

5. STORAGE AND STABILITY OF THE KIT
The kit is shipped at ambient temperature and should be stored at 2-8°C. Keep away from heat or direct sun light. The 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.

6. SPECIMEN COLLECTION, HANDLING AND STORAGE
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 (Aliquots)
≤-20°C (Cubes)
Stability: 3 d
6 mon
Keep away from heat or direct sun light
Avoid repeated freeze-thaw cycles

14. REFERENCES

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES.
11. 4. CALCULATION OF RESULTS

For the run to be valid, the OD450 nm of the Positive Control should be \( \geq 1.00 \) and the OD450 nm of each Negative Control should be \( \leq 0.150 \). 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 OD450nm of the Negative Controls. The results are expressed in arbitrary units (AU/mL).

<table>
<thead>
<tr>
<th>Range</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 3 AU/mL</td>
<td>POSITIVE</td>
</tr>
<tr>
<td>( \leq 3 ) AU/mL</td>
<td>NEGATIVE</td>
</tr>
</tbody>
</table>

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

12. ASSAY CHARACTERISTICS

12.1. SPECIFICITY

Screening test was performed with 80 different native and RF-negative serum samples. All samples showed OD values (ranged from 0.053 to 0.094) less than the cut-off value (3x 0.068).

This test system measures the concentration of free antibodies directed against Bevacizumab. It cannot detect these antibodies if the drug already is bound to it.

12.2. SENSITIVITY

When the Bevacizumab-reactive antibody prepared at a concentration of 1µg/mL and then diluted to 1:101 with the Assay Buffer, it showed an OD value higher than the cut-off value (3x mean OD of Negative Controls). It was observed that the lowest detectable level that can be clearly distinguished from the cut-off value is less than 10 ng/mL with the *ImmunoGuide* bridging ELISA format.

12.3. PRECISION OF THE KIT

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

13. AUTOMATION

Experiments have shown that the ImmunoGuide Antibody to Bevacizumab ELISA is suitable also for using by an automated ELISA processor.

8. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (< 3% CV) and tips to deliver 5-1000 µL.
2. Bidistilled or deionised water and calibrated glasswares (e.g. flasks or cylinders).
3. Wash bottle, automated or semi-automated microtiter plate washing system.
4. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength at 600-650 nm is optional).
5. Absorbent paper towels, standard laboratory glass or plastic vials, and a timer.

9. PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared readily at the appropriate
time. Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.

3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each reagent, standard or specimen. Do not interchange the caps of vials. Always cap not used vials. Do not reuse wells or reagents.

4. Use a pipetting scheme to verify an appropriate plate layout.

5. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.

6. Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.

7. Humidity affects the coated wells. Do not open the pouch until it reaches room temperature. Unused wells should be returned immediately to the resealed pouch including the desiccant.

10. PRE-TEST SETUP INSTRUCTIONS

10.1. Preparation of Components*

<table>
<thead>
<tr>
<th>Dilute/ dissolve</th>
<th>Component</th>
<th>Diluent</th>
<th>Relation</th>
<th>Remarks</th>
<th>Storage</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mL</td>
<td>Wash Buffer</td>
<td>up to 200 mL</td>
<td>D1:</td>
<td>Warm up at 37°C to dissolve crystals; Mix vigorously.</td>
<td>2-8 °C</td>
<td>4 w</td>
</tr>
</tbody>
</table>

*, Prepare Wash Buffer before starting the assay procedure.

10.2. Dilution of Samples*

<table>
<thead>
<tr>
<th>Sample</th>
<th>To be diluted</th>
<th>With</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum/Plasma</td>
<td>1:101</td>
<td>Assay Buffer</td>
<td>For dilution at 1:101; 5μl Sample + 500μl Assay Buffer</td>
</tr>
</tbody>
</table>

*, Negative and Positive Controls are ready-to-use and should NOT be diluted with the assay buffer.

11. TEST PROCEDURE

11.1. GENERAL REMARKS

11.1.1. 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.

11.1.2. All Standards should be run with each series of unknown samples.

11.1.3. Standards should be subject to the same manipulations and incubation times as the samples being tested.

11.1.4. All steps of the test should be completed without interruption.

11.1.5. Use new disposable plastic pipette tips for each reagent, standard or specimen in order to avoid cross contamination.

11.2. 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.

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 OD620nm is optional) within 15 min after pipetting the Stop Solution.

11. 3. QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. All standards/controls must be found within the acceptable ranges as stated above and/or label. If the criteria are not met, the run is not valid and should be repeated. In case of any deviation, the following technical issues should be reviewed: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.