INTENDED USE

The Human Anti-Avastin (Bivacucizumab) ELISA Kit is an immunosay assay suitable for detecting and quantifying human antibody (IgG) activity specific for Avastin, or any isoform in serum or plasma of human or other species, including monkey, rat, rabbit and pig. For research use only.

GENERAL INFORMATION

VEGF (Vascular Epidermal Growth Factor) is a dimer (Kda 42) signal glycoprotein that stimulates endothelial cell proliferation and new blood vessel formation. VEGF is the target of the monocular antibody bevacizumab (Avastin; by Roche). Avastin is a recombinant, humanized monoclonal antibody (IgG kappa) containing human framework regions and CDR regions from a mouse antibody that binds to VEGF. In humans, Avastin is used for the treatment of metastatic colorectal cancer and renal cell carcinoma, non-squamous non-small cell lung cancer and multiple myeloma.

Avastin is a fully humanized antibody without significant 'mouse' derived sequences sequences that would be recognized by the injected patient as a foreign antigen. The human framework regions expressed on the Avastin molecule may induce an immune response to human framework regions expressed on the Avastin molecule. However, when large amounts of a monocular antibody are continually encountered in circulation, the host may mount similar responses to the natural human anti-avastin antibodies. Such antibodies might be expected to diminish the effectiveness of avastin as a drug, and perhaps have other metabolic consequences. Like many humanized antibodies, avastin can induce antibodies (human anti-avastin or anti-drug antibodies, HAH/AADA) in patients receiving Avastin. Lucentis also induced anti-drug antibodies in 1-9% patients.

The avastin antigen coating level and HRP conjugate are applied to the plate. Anti-avastin antibodies from any species may be detected. ADI has developed ELISA kits to detect antibodies to Avastin. Avastin is a recombinant, humanized monoclonal antibody (IgG) activity specific for Avastin, of any isotype, in serum or plasma of human or other species, including monkey, rat, rabbit and pig. For research use only.

ASSAY DESIGN AND SET-UP

Sample Collection and Handling

Serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference. For serum, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. For other samples, clarify the sample by centrifugation and/or filtration prior to dilution in Sample Diluent. If samples will not be assayed immediately, store at −20°C or −80°C for long-term storage.

Antibody Stability

Initial dilution of serum into Working Sample Diluent (WSD) is recommended to stabilize antibody activity. This enhances reproducible sampling, and stabilizes the antibody activity for years, stored refrigerated or frozen.

Assay Design

Review Calculation of Results (pS-7) and Limits of the Assay (above) before proceeding:

- Select the proper sample dilutions accounting for expected potency of positive results and minimizing non-specific binding (NSB) and other matrix effects for example, high signal for sample, net signal for normal serum samples should be lower than the 3 Ulm Calibrator. This is usually 1/10 or greater dilution for human sera with normal levels of IgG and IgM.

- Run a Sample Diluent Blank. This signal is an indicator of proper assay performance, especially of washing efficacy, and is used for NSB OD calculations, if required. Blank OD should be <0.3.

- Run a set of Calibrators. Calibrators validate that the assay was performed to specifications; results can be used to normalize between-assay variation for enhanced precision. Reading values off a Calibrator curve, Method A, has no limitations. See Limits of the Assay (above).

Plate Setup

Bring all reagents to room temperature (18-30°C) equilibration (at least 30 minutes).

- Determine the number of wells for the assay run. Duplicate samples are recommended, including 1 Calibrator well and 2 wells for each sample and internal control to be assayed.

- Remove the appropriate number of microwell strips from the pouch and reinstate unused strips to the pouch. Reseal the pouch and store refrigerated.

ASSAY DESIGN AND SET-UP (continued)

- Add 200-300ul Working Wash Solution to each well and let stand for about 5 minutes. Aspirate and dispose of liquid and paper towel on a paper towel before sample addition.

Sample Preparation Instructions

Component

Conjugate Cat. No. WB-100, 10ml

Prepare the microtiter plate and all other reagents, if unopened, are stable at 2-8°C until the expiration date printed on the box label. Stop Solution is prepared by dissolving 1g/L H2SO4 in distilled water into a clean stock bottle. Label 190ml with distilled or deionized water into a clean stock bottle. Label 990ml with distilled or deionized water. All Microwell plate and all other reagents, if unopened, are stable at 2-8°C until the expiration date printed on the box label.

Wash Solution Component

Conjugate Cat. No. WB-100, 10ml

Prepare the microtiter plate and all other reagents, if unopened, are stable at 2-8°C until the expiration date printed on the box label. Stop Solution is prepared by dissolving 1g/L H2SO4 in distilled water into a clean stock bottle. Label 190ml with distilled or deionized water into a clean stock bottle. Label 990ml with distilled or deionized water.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipettor is recommended.

- Disposable polystyrene or polypropylene microtubes for diluting samples and Avastin HRP Concentrate.

- Graduated cylinder to dilute Wash Concentrate: 0.2 to 1L. Stock bottle to store diluted Wash Solution: 200 to 1L.

- Distilled or deionized water to dilute reagent concentrates.

- Microplate reader at 450 nm wavelength.

Carcinoma, non-squamous non-small cell lung cancer and multiple myeloma.

Principle of the Test

The human anti-Avastin ELISA is an antigen sandwich ELISA based on the binding of anti-avastin antibodies (any isotype or species) in samples to avastin immobilized on the plate; bound anti-avastin antibody is detected by simultaneous binding of HRP-conjugated anti-avastin antibody to avastin and a stable color reaction is developed.

Product Specifications

Specificity

Purified avastin (Bivacucizumab) is used to coat the microwells; thus the assay is specific for antibodies directed to avastin or other similar human IgG. The avastin-HRP conjugate reacts with divalent or multivalent antibodies of any isotype (IgG, IgM, IgA, IgE) that are specific to avastin and bind to the avastin antigen coated on the plate. Anti-avastin antibodies from any species may be detected in the assay.

Assay Sensitivity

The avastin antigen coating level and HRP conjugate are optimized to differentiate anti-avastin from background (non-antibody) signal with serum samples diluted 1/20 or more.

Quantitation of Antibody in a Sample

The ELISA measures anti-avastin activity, a combination of antibody concentration and avidity for the Avastin antigen. Antibody levels in different samples may display similar anti-avastin activities, due to differences in avidity. The quantitation or activity of the samples is, therefore, appropriately expressed in activity Units (IU), rather than mass units of Ig (e.g., ug/ml).

Abbreviations

(OD) above 2.0, incubate for less time, or read OD at 405- 410 nm.; LLOQ = lower limit of quantitation.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

Stop Step

- Add 100ul of Stop Solution to each well.

- Incubate for 30 minutes.

- Wash wells 5 times as in step 2.

- Add 100ul of diluted Avastin HRP to each well.

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This graph shows dilution curves of affinity-purified antibodies reactive with Avastin as antigen, as follows:

- GAHA-Fc – goat polyclonal antibodies specific for the Fc region of Avastin; affinity-purified.
- MAHA-Fc – mouse monoclonal antibody specific for the Fc region of Avastin; affinity-purified.
- GAHA-IgA – goat polyclonal antibodies specific for the kappa light chain of Avastin; affinity-purified.
- GAHA-IgM – goat polyclonal antibodies specific for human lambda light chain; Avastin has no lambda light chain.

This calculation also quantifies the positive Antibody Activity level, assigning a higher value to samples with higher Antibody Activity, and vice versa.

Precautions and Safety Instructions

Controls, Sample Diluent, and Antibody HRP contain bromonitrobenzene (BND): 0.05%, w/v). Stop Solution contains dilute sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with dilute sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with dilute sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with dilute sulfuric acid.

Calibration of Results

Consider several data reduction methods to best represent the relationships among experimental and control groups, to determine Positive Immunee and Negative Non-immune or Pre-immune, and to Quantitate positive antibody levels.

Method A. Use of a Calibrator Curve

When the dilution curves of samples are parallel to the Calibrator curve (see Limits of the Assay, page 3, and Assay Performance, page 5), the anti-Avastin activity units may be determined by interpolation from the Calibrator curve.

Sample values = curve value, 1/10 sample dilution

Method B. Antibody Activity Threshold Index

Compare Samples to 2 U/ml Calibrator or Internal Control

Example: 2 U/ml Calibrator (Bevacizumab) antibody detected by an electrochemiluminescent (ECL) based assay. Among these 14 patients, three tested positive for neutralizing antibodies against bevacizumab using an enzyme-linked immunosorbent assay (ELISA). The clinical significance of these anti-product antibodies responses to bevacizumab is unknown. Immunogenicity assay results are highly dependent on the sensitivity specificity of the test method and may be influenced by several factors, including sample handling, timing of sample collection, concomitant medications, and underlying disease. For these reasons, comparison of the incidence of antibodies to Avastin with the incidence of antibodies to other products may be misleading.

Result of Positive/Negative, as described in the previous section. Values above 1.0 are Positive for antibody.

Immunogenicity of Avastin

As with all therapeutic proteins, there is a potential for an immune response to Avastin. In clinical trials of adjuvant colon carcinoma, 14 of 2233 evaluable patients (0.63%) tested positive for treatment-emergent anti-bevacizumab antibodies detected by an electrochemiluminescent (ECL) based assay. Among these 14 patients, three tested positive for neutralizing antibodies against bevacizumab using an enzyme-linked immunosorbent assay (ELISA). The clinical significance of these anti-product antibodies responses to bevacizumab is unknown. Immunogenicity assay results are highly dependent on the sensitivity specificity of the test method and may be influenced by several factors, including sample handling, timing of sample collection, concomitant medications, and underlying disease. For these reasons, comparison of the incidence of antibodies to Avastin with the incidence of antibodies to other products may be misleading.

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