

Introduction

Bevacizumab (Avastin®) is a recombinant humanized monoclonal antibody that blocks angiogenesis by inhibiting vascular endothelial growth factor A (VEGF-A). VEGF-A stimulates angiogenesis in a variety of diseases, including cancer. Bevacizumab was the first clinically available angiogenesis inhibitor in the United States.

The Bevacizumab ELISA kit is designed to measure free Bevacizumab with high specificity and sensitivity. The assay design uses a pair of antibodies allowing detection of whole Bevacizumab molecules in biological matrices.

Principle of the assay

This assay employs the sandwich enzyme immunoassay technique. Anti- Bevacizumab is coated onto a 96 well microplate. Calibrator, quality control samples and test samples are pipetted into the appropriate wells. Bevacizumab present in biological matrices is bound by the immobilized capture antibody. After washing away any unbound substances, enzyme linked detection antibody is added to the wells. The plate is washed to remove any unbound antibody-enzyme reagent and a substrate solution is added to the wells for color development. The color development is proportional to the amount of Bevacizumab present in test samples.

Materials and storage

Store kit components at -20°C unless specified otherwise. DO NOT USE past kit expiration date. Some vials contain a small amount of reagents. Spin tubes on pulse setting prior to opening.

Each kit includes:	Units
Coated microtiter plate, 96 wells (1x8 strips)	1
Calibrator diluent	1.8ml
Calibrator (250 µg/mL)	12µl
10X wash buffer	25ml
Assay buffer	50ml
1000X detection reagent	17µl
TMB	12ml
TMB stop solution	12ml
Plate sealers	3
Do not mix or substitute reagents with those from other lots.	

Materials and instruments required but not supplied

- Precision pipettes calibrated to deliver 5-1000µL
- Multi-channel pipette calibrated to deliver 50-200µL
- Plate shaker
- Disposable tips
- Vortex-Mixer
- Distilled or de-ionized water
- Microplate reader capable of reading 450nm with background subtraction at 620nm

Safety precautions

- The test protocol must be followed strictly.
- All reagents containing human material should be handled as if potentially infectious. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.
- The kit reagents contain antimicrobial agents, acid and 3,3',5,5'-tetramethylbenzidine. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
- Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Disposal must be performed in accordance with local regulations.
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- Only trained laboratory personnel should execute this test.

Preparation of reagents

Prepare only the appropriate amount of required reagent on the day of use. Store all reagents as per instructions stated on the label.

- 1. Wash Buffer (1X) Preparation:** Dilute wash buffer concentrate with ultra-pure water 1/10 before use (for example add 20mL concentrate to 180mL ultra-pure water). Mix well.
- 2. Detection Reagent (1X) Preparation:** Dilute detection reagent with assay buffer 1/1000 before use (for example add 11µl concentrate to 11ml of assay buffer). Mix well.
- 3. Preparation of Calibrators:** Prepare calibrators with concentrations ranging from 2,500 ng/mL to 78 ng/mL. The following is an example calibrator curve.

Sol'n ID	Source	Source Vol (µL)	Cal* Diluent (µL)	Final Vol (µL)	Final Concentration (ng/mL)
1*	Stock cal* (250 µg/ml)	5	495	500	2,500
2*	1*	50	50	100	1,250
3*	2*	50	50	100	625
4*	3*	50	50	100	313
5*	4*	50	50	100	156
6*	5*	50	50	100	78
Neg	-	-	100	100	0
*Calibrator					

Specimen storage

This kit is compatible with EDTA-plasma, heparin-plasma and serum samples. Samples can be stored at or below -20°C for up to 1 year.

Assay procedure

1. Remove coated microtiter plate from -20°C and allow it to acclimate to room temperature for 15-20 minutes.
2. Dilute calibrators and test samples 1/50 with assay buffer (for example add 5µL of prepared calibrator or sample to 245µL of assay buffer). Mix well. Do not store diluted samples.

3. Add 100µL diluted calibrators and samples to appropriate wells on the plate. Incubate for 1 hour at room temperature on a plate shaker at 300rpm.
4. Discard the content of the plate and wash the wells 3x with 200µL wash buffer per well.
5. Add 100µL detection reagent to appropriate wells on the plate. Incubate for 1 hour at room temperature on a plate shaker at approx 300rpm.
6. Discard the content of the plate and wash the wells 3x with 200µL wash buffer per well.
7. Add 100µL of TMB to each well on plate. Incubate for 5-10 minutes at room temperature protected from light.
8. Add 100µL of TMB stop solution to each well on plate. Mix by gently tapping the side of the plate.
9. Determine absorbance with a microplate reader at 450nm against 620nm.

Calculations and results

1. Construct a standard curve by plotting the absorbance obtained from each standard against concentration. Use a 4 or 5 parameter curve fit. Alternatively a log-log curve fit may be used.
2. The concentration of the unknowns can be read directly from this standard curve using the absorbance value for each sample.
3. Any sample undiluted or diluted still reading greater than the highest standard should be diluted appropriately with calibrator diluent and retested. If the samples have been diluted, the concentration determined from the standard curve must be multiplied by the dilution factor.

Performance characteristics

Precision: The precision was determined by analyzing samples prepared at 500 ng/mL in 6 replicates on 6 different occasions. Intra-assay coefficient of variation (CV) < 10%. Inter-assay CV < 10%.

Detection Limit: The detection limit is 1.5 ng/mL.

Recovery: 250 ng/mL of Bevacizumab was spiked in 10 lots of human serum. Recovery ranges are from 91-111% with an average recovery of 106%.

Specificity: hIgG1, rituximab, and infliximab prepared at 250 ng/mL were assayed and exhibited no cross-reactivity or interference.

Sample Standard Curve

The standard curve below was generated with the supplied calibrator using the recommended conditions and diluted 1 in 50 to give a final concentration range of 50ng/ml to 1.56ng/ml. Each sample was run with 6 replicates. Intra-assay coefficient of variance is <10%.

Ordering Information

Please visit www.affinityimmuno.com to order this product. Visa, Mastercard, AMEX and PayPal are accepted in our online store.

Your order will be processed immediately and you will be notified with a delivery timeframe.

