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Bevacizumab ELISA Kit





Overview

Quantity:	96 tests
Target:	Bevacizumab
Reactivity:	Human, Mouse, Rat
Method Type:	Sandwich ELISA
Detection Range:	1.56-50 ng/mL
Minimum Detection Limit:	1.56 ng/mL
Application:	ELISA

Product Details

Purpose:	Quantification of Bevacizumab in biological matrices
Sample Type:	Plasma, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	Bevacizumab (Avastin)
Cross-Reactivity (Details):	hlgG1, rituximab, and infliximab prepared at 250 ng/mL were assayed and exhibited no crossreactivity or interference.
Sensitivity:	1.5 ng/mL
Components:	Coated microtiter plate, 96 wells Calibrator diluent 1.8ml
	Calibrator 12ul

10X wash buffer - 25ml

Assay buffer - 50ml

1000X detection reagent - 17ul

TMB - 12ml

TMB stop solution - 12ml

Plate sealers - 3

Material not included:

Precision pipettes calibrated to deliver 5-1000µL

Multi-channel pipette calibrated to deliver $50-200\mu L$

Plate shaker

Disposable tips

Vortex-Mixer

Distilled or de-ionized water

Microplate reader capable of reading 450nm with background subtrac

Target Details

Target:	Bevacizumab
Abstract:	Bevacizumab Products
Target Type:	Antibody
Background:	Bevacizumab (Trade name 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. Bevacizumab was approved by
	the U.S. Food and Drug Administration (FDA) for certain metastatic cancers. It received its first
	approval in 2004, for combined use with standard chemotherapy for metastatic colon cancer. It
	has since been approved for use in certain lung cancers, renal cancers, ovarian cancers, and
	glioblastoma multiforme of the brain. It had been approved for breast cancer, but that approval
	was withdrawn when later studies showed no evidence of effectiveness
Gene ID:	7422

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Sample Volume:	15 μL

Application Details

Assay Time:	2.5 h
Plate:	Pre-coated
Protocol:	The Bevacizumab ELISA kit is designed to measure free Bevacizumab with high specificity and sensitivity. This assay employs the sandwich enzyme immunoassay technique. A precoated anti-Bevacizumab 96 well plate is provided. 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 Bevaciuzumab present in test samples and the concentration is calculated from the standard series.
Reagent Preparation:	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 20 mL concentrate to 180 mL ultra-pure water). Mix well. 2. Detection Reagent (1X) Preparation: Dilute detection reagent with assay buffer 1/1000 before use (for example add 11 µL concentrate to 11 mL 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.
Sample Collection:	This kit is compatible with EDTA-plasma, heparinplasma and serum samples. Samples can be stored at or below -20 °C for up to 1 year.
Sample Preparation:	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.
Assay Procedure:	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.
Calculation of 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

Application Details

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.

Assay Precision:

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%.

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%.

Restrictions:

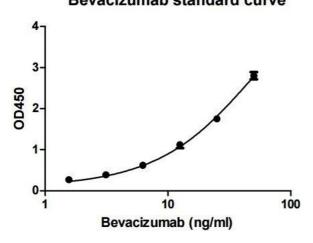
For Research Use only

Handling

Preservative:	Without preservative
Precaution of Use:	Read manual completely before beginning
Storage:	-20 °C
Storage Comment:	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.
Expiry Date:	12 months

Images

Bevacizumab standard curve



ELISA

Image 1.