

BioSim™ Bevacizumab (Avastin®) (Human) ELISA Kit

10/17

(Catalog # E4373-100, 100 assays, Store at 4°C)

I. Introduction:

Bevacizumab (Avastin®) is a recombinant human IgG1 monoclonal antibody specific for all human vascular endothelial growth factor-A (VEGF-A) isoforms. The humanized anti-VEGF monoclonal antibody, bevacizumab, has been approved by the FDA as a first-line treatment for metastatic colorectal cancer in combination with chemotherapy. The pharmacokinetic properties of bevacizumab in several species have been previously described and are consistent with a typical humanized monoclonal antibody. It was shown in the literature that the surveillance of circulating concentration during maintenance therapy represents a direct and/or indirect factor for some other side effects. Identification of biomarkers for (non-)response and risk factors for adverse drug reactions that might be related to serum concentrations and maintaining the effective concentration of Bevacizumab in order to potentially avoid some side effects with a reliable method might be beneficial. BioVision's BioSim™ Bevacizumab ELISA kit has been developed for specific quantification of Bevacizumab concentration in human serum or plasma with high sensitivity and reproducibility.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Bevacizumab.

Detection Range: 30 - 1000 ng/ml

Sensitivity: 30 ng/ml

Assay Precision: Intra-Assay: CV < 15%; Inter-Assay: CV < 15% (CV (%) = SD/mean X 100)

Recovery rate: 85 – 115% with normal human serum samples with known concentrations

Cross Reactivity: No significant cross-reactivity or interference with other proteins present in native human serum or other therapeutic immunoglobulins.

III. Sample Type:

Human serum and plasma

IV. Kit Contents:

Components	E4373-100	Part No.
Micro ELISA Plate	1 plate	E4373-100-1
Bevacizumab Standards (S1 – S7)	0.1 ml X 7	E4373-100-2.x
Assay Buffer	50 ml X 2	E4373-100-3
HRP-conjugate Probe	12 ml	E4373-100-4
TMB substrate (Avoid light)	12 ml	E4373-100-5
Stop Solution	12 ml	E4373-100-6
Wash buffer (20X)	50 ml	E4373-100-7
Plate sealers	2	E4373-100-8

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- Calibrated measures
- Precision pipettes with disposable tips
- Clean eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

VI. Storage and Handling:

The entire kit may be stored at 4°C for up to 12 months from the date of shipment.

VII. Reagent and Sample Preparation:

Note: Prepare reagents within 30 minutes before the experiment.

Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Wash Buffer:** Dilute the 20X Wash Buffer to 1X solution in ddH₂O (10 ml of Wash Buffer stock to 190 ml of ddH₂O). Mix the 1X solution thoroughly by vortex manually. The working stock can be stable for 2 weeks after preparation at 4°C.

2. Standard Preparation:

Dilute 100X stock with Assay Buffer. (5 µl Standards + 495 µl Assay Buffer)

Name	S1	S2	S3	S4	S5	S6	S7
Conc. (µg/ml)	100	30	10	3	0	High Control	Low Control
Working conc. (ng/ml)	1000	300	100	30	0	-	-

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3. Sample Dilution:

- **Serum/Plasma:** First dilute samples at 1:100 (10 µl Serum/Plasma+90 µl Assay Buffer). (Dilution factor : 100)
- Diluted samples should further be diluted if the concentration of Bevacizumab is higher than the measuring range.
- The usual precautions for venipuncture should be observed. Samples are stable at 4°C for 2 days and -20°C for 6 months. Avoid freeze-and-thaw cycle.

VIII. Assay Protocol:

Note: Bring all reagents, microplate and samples to room temperature 15 minutes prior to the assay.

It is recommended that all standards and samples be run at least in duplicate.

A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in section VII.
2. Pipette 100µl of **Assay Buffer** non-exceptionally into each of the wells to be used.
3. Add 25 µl of **standards** and **diluted-samples** into appropriate wells. Cover wells and incubate for 30 minutes at room temperature (RT).
4. Discard incubation solution. Wash plate 3 times each with 300 µl of diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
5. Add 100 µl of **HRP-conjugate** into each well. Cover wells with adhesive plate sealer and incubate at RT for 30 minutes.
6. Discard the solution and wash the wells as step 4.
7. Add 100 µl of 1X **TMB substrate** solution and incubate the plate in dark at RT for 10 minutes
8. Add 100 µl of **Stop solution** to stop the reaction
9. Read the absorbance in micro plate reader set to 450 nm within 20 minutes. (reference wavelength to 650 nm)

IX. CALCULATION:

Using the standards disregarding zero standard, construct a standard curve by plotting the OD_{450/650 nm} for each of 4 standards on the Y-axis versus the corresponding Bevacizumab concentration on the X-axis. Construct a standard curve of difference data using software capable of generating four parameter logistic (4PL) or point-to-point calculation curve fit. To obtain the exact values of the samples, the concentration determined from the standard-curve should be multiplied by the dilution factor.

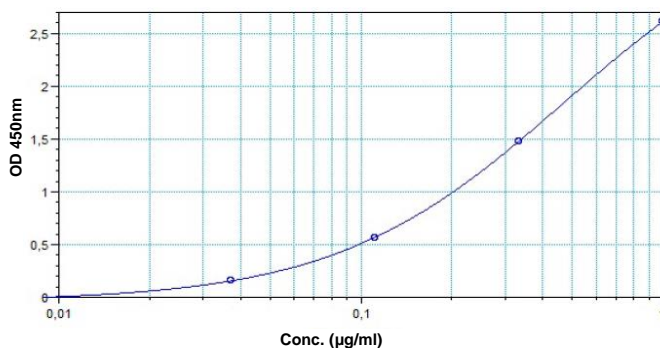


Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

X. RELATED PRODUCTS:

- BioSim™ Rituximab (Mabthera®) (Human) ELISA Kit (Cat. No. E4371-100)
- BioSim™ Adalimumab (Humira®) (Human) ELISA Kit (Cat. No. E4372-100)
- BioSim™ Bevacizumab (Avastin®) (Human) ELISA Kit (Cat. No. E4373-100)
- BioSim™ Etanercept (Enbrel®) (Human) ELISA Kit (Cat. No. E4374-100)
- BioSim™ Ipilimumab (Remicade®) (Human) ELISA Kit (Cat. No. E4375-100)
- BioSim™ Trastuzumab(Herceptin®)(Human) ELISA Kit (Cat. No. E4376-100)
- BioSim™ Golimumab (Simponi®)(Human) ELISA Kit (Cat. No. E4377-100)
- BioSim™ Infliximab (Remsima®)(Human) ELISA Kit (Cat. No. E4378-100)
- BioSim™ Cetuximab (Erbix®)(Human) ELISA Kit (Cat. No. E4379-100)
- BioSim™ Denosumab (Prolia®)(Human) ELISA Kit (Cat. No. E4380-100)
- BioSim™ Omalizumab (Xolair®)(Human) ELISA Kit (Cat. No. E4381-100)
- BioSim™ Nivolumab (Opdivo®)(Human) ELISA Kit (Cat. No. E4382-100)
- BioSim™ Pembrolizumab (Keytruda®)(Human) ELISA Kit (Cat. No. E4383-100)
- BioSim™ Ipilimumab (Yervoy®)(Human) ELISA Kit (Cat. No. E4384-100)

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