

BioSim™ anti-VEGF-A (Human) ELISA Kit

rev 06/20

(Catalog # E4312-100, 100 assays, Store at -20°C)

I. Introduction:

Anti-VEGF-A is a recombinant human IgG1 monoclonal antibody fragment (Fab) that blocks angiogenesis by inhibiting vascular endothelial growth factor-A (VEGF-A) isoforms. The humanized anti-VEGF monoclonal antibody has been approved by the FDA for treatment of patients with wet age-related macular degeneration. Age-related macular degeneration (AMD) is the leading cause of irreversible blindness in people over the age of 50 in the developed world. Although an estimated 80% of patients with AMD have the non-neo vascular form, the neo vascular (wet or exudative) form is responsible for almost 90% of severe visual loss resulting from AMD. BioVision's anti- VEGF-A ELISA kit is developed for the quantification of anti-VEGF-A antibody concentration in human serum or plasma with high sensitivity and reproducibility.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of anti-VEGF-A antibody.
 Detection Range: 1.22 – 625 ng/ml

III. Sample Type:

Human serum and plasma

IV. Kit Contents:

Components	E4312-100	Part No.	Storage
Micro ELISA Plate	1	E4312-100-1	-20°C
anti-VEGF-A Standard (10 mg/ml)	10 µl	E4312-100-2	2 - 8°C
Detection Antibody (500X)	40 µl	E4312-100-3	-20°C
Assay Diluent	100 ml	E4312-100-4	2 - 8°C
TMB substrate (20X) (Avoid light)	1 ml	E4312-100-5	2 - 8°C
Wash buffer-A (20X)	70 ml	E4312-100-6	2 - 8°C
Plate sealers	2	E4312-100-7	RT

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- Normal human serum or plasma
- **Stop Solution:** (2N) H₂SO₄
- Precision pipettes with disposable tips
- Distilled or deionized water
- Clean eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

VI. Storage and Handling:

The entire unused kit may be stored at -20°C for up to 12 months from the date of shipment. Once opened, components should be stored as specified in section IV.

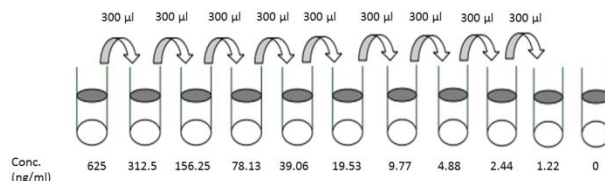
VII. Reagent and Sample Preparation:

Note: Prepare reagents within 30 min before the experiment. Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Wash Buffer-A:** Thaw the wash buffer at room temperature (RT) until it is a clear solution. Prepare 1X Wash buffer A as needed (For e.g: Dilute 50 ml of 20X Wash buffer-A with ddH₂O to a total volume of 1000 ml). Do not store and reuse unused 1 X Wash buffer A solution.
2. **Assay Matrix:** Dilute healthy human serum or plasma at minimum dilution of 1:300 in assay diluent. Use this assay matrix for standards and quality control preparation.
3. **TMB Substrate:** Prepare 1X as needed. (For e.g: Dilute the 20X TMB substrate to 1X solution with ddH₂O by adding 600 µl of TMB substrate to 11.4 ml of ddH₂O) Mix the 1X solution thoroughly by vortex manually. Do not store and reuse unused 1 X TMB substrate.
4. **Detection Antibody:** Dilute the Detection Antibody in assay diluent at 1:500 as needed (For e.g: Dilute 24 µl of 500X detection antibody to 12 ml of assay diluent). Gently mix the detection antibody before use. Do not store and reuse unused antibody.

5. Standard Preparation:

- Prepare a **main stock** of 2000 µg/ml by diluting the anti-VEGF-A Standard (10 mg/ml) in normal human serum or plasma (5 µl of standard in 20 µl of normal human serum or plasma).
- Prepare a **sub stock** of 10 µg/ml by diluting 5 µl of main stock into 995 µl of Assay Diluent.
- Prepare a Standard #1 of 625 ng/ml by diluting 60 µl of sub stock into 900 µl of **Assay Matrix**
- Perform 2-fold serial dilutions of the standards (300 µl standards + 300 µl **assay matrix**) to make the standard curve within the range of this assay. Use 0.3 ml **Assay Matrix** as blank control or 0 ng/ml.
- Suggested standard points are: 625, 312.5, 156.25, 78.13, 39.06, 19.53, 9.77, 4.88, 2.44, 1.22, 0 ng/ml



6. Sample Preparation:

FOR RESEARCH USE ONLY! Not to be used on humans.

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- **Serum:** Use serum clot tube and allow the blood sample to coagulate at room temperature (RT) for 30 minutes. Centrifuge at 5000 rpm for 10 minutes at RT. Aliquot the clear serum and store at -20°C. Avoid repeated freeze/ thaw cycles.
- **Plasma:** Use K₂ EDTA as anticoagulant for blood collection and allow at RT for 30 minutes. Centrifuge the sample at 5000 rpm for 10 minutes at RT. Aliquot the clear plasma and store at -20°C. Avoid repeated freeze/ thaw cycles.

7. Sample Preparation:

- Prepare minimum of three QC samples in assay matrix
- Keep the diluent buffer control in two replicates.

VIII. Assay Protocol:

Notes: a. Bring all reagents, microplate and samples to RT 15 min prior to the assay.
b. It is recommended that all standards and samples be run at least in duplicate.
c. A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in section VII.
2. Add 100 µl of **standards, QC samples and test samples** into appropriate wells. Cover wells and incubate for 1 hr at RT.
3. Discard the contents of each well and wash 4 times with **1X Wash Buffer-A**, allowing 2 min for incubation between each wash step. Blot the microtiter plate on absorbent paper to remove any residual reagent.
4. Add 100 µl of **Detection Antibody** solution to each well of the microtiter plate. Cover wells with adhesive plate sealer and incubate at RT for 30 min.
5. Discard the solution and wash the wells as step 3.
6. Add 100 µl of **1X TMB substrate** solution and incubate the plate in dark at RT for 15 min.
7. Add 50 µl of **Stop solution** (2N H₂SO₄) to stop the reaction.
8. Read the absorbance in micro plate reader set to 450 nm, set the reference wavelength to 600 nm.

IX. Calculation:

After the absorbance is read at 450 nm and 600 nm as reference wave length, construct a standard curve of difference data using software capable of generating four or five parameter logistic (4PL or 5PL) curve fit. Absorbance of the test/specimen and the QC samples are interpolated from the standard curve. Report the values of test/specimen samples within the assay range.

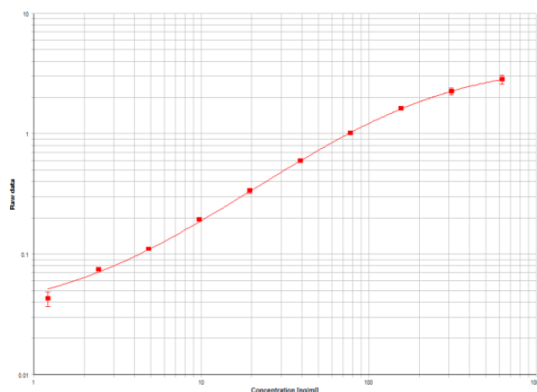


Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

X. Related Products:

- BioSim™ Rituximab (Human) ELISA Kit (Cat. No. E4385-100)
- BioSim™ Infliximab (Human) ELISA Kit (Cat. No. E4387-100)
- BioSim™ Adalimumab (Human) ELISA Kit (Cat. No. E4388-100)
- BioSim™ Bevacizumab (Human) ELISA Kit (Cat. No. E4389-100)
- BioSim™ Infliximab (Human) ELISA Kit (Cat. No. E4390-100)
- BioSim™ Cetuximab (Human) ELISA Kit (Cat. No. E4391-100)
- BioSim™ Etanercept (Human) ELISA Kit (Cat. No. E4392-100)
- BioSim™ Golimumab (Human) ELISA Kit (Cat. No. E4393-100)
- BioSim™ Denosumab (Human) ELISA Kit (Cat. No. E4394-100)
- BioSim™ Omalizumab (Human) ELISA Kit (Cat. No. E4395-100)
- BioSim™ Nivolumab (Human) ELISA Kit (Cat. No. E4396-100)
- BioSim™ Pembrolizumab (Human) ELISA Kit (Cat. No. E4397-100)
- BioSim™ Ipilimumab (Human) ELISA Kit (Cat. No. E4398-100)
- BioSim™ Filgrastim (Human) ELISA Kit (Cat. No. E4399-100)

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