



BioSim™ anti- Aflibercept (Eylea®) (Human) ELISA Kit

07/20

(Catalog # E4855-100, 96 assays, Store at 4°C)

I. Introduction:

Aflibercept is a recombinant protein composed of the binding domains of two human vascular endothelial growth factor (VEGF) receptors fused with the Fc region of human immunoglobulin gamma 1 (IgG1). Aflibercept, as an ophthalmic agent, is used in the treatment of macular edema following Central Retinal Vein Occlusion (CRVO) and neovascular Age-Related Macular Degeneration (AMD). Compared to other anti-VEGF drugs like bevacizumab and ranibizumab, aflibercept has a higher binding affinity to VEGF-A. BioVision's BioSim™ anti-Aflibercept ELISA kit is designed to detect antibody against Aflibercept with high specificity and sensitivity in serum and plasma samples. anti-Aflibercept ELISA is based on the sandwich ELISA principle. Controls and samples are incubated in the microtiter plate coated with the drug aflibercept. After incubation, the wells are washed. Then, HRP conjugated probe is added and binds to aflibercept antibodies captured by the drug aflibercept on the surface of the wells. Following incubation wells are washed and the bound enzymatic activity is detected by addition of chromogen-substrate. Finally, the reaction is terminated with an acidic stop solution. The color developed is proportional to the amount of aflibercept antibodies in the sample or controls. The results can be evaluated with using cut-off value.

II. Application:

This ELISA kit is used for *in vitro* qualitative determination of antibody against Aflibercept in serum and plasma

Cross Reactivity: Aflibercept (Eylea®) infusion camouflages/masks the presence of antibody to Aflibercept in serum/plasma samples. Therefore, blood sampling time is critical for detection of anti-drug-antibodies. It is convenient to obtain blood sample just before the infusion or at least 2 weeks after the infusion of Aflibercept.

III. Sample Type:

Human serum and plasma

IV. Kit Contents:

Components	E4855-100	Part No.
Micro ELISA Plate	8 x 12 strips	E4855-100-1
Positive Control	0.3 ml	E4855-100-2
Negative Control	1 ml	E4855-100-3
Assay Buffer	12 ml	E4855-100-4
Peroxidase Conjugate	12 ml	E4855-100-5
TMB substrate (Avoid light)	12 ml	E4855-100-6
Stop Solution	12 ml	E4855-100-7
Wash buffer (20X)	50 ml	E4855-100-8
Plate sealers	2	E4855-100-9

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- 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: 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. **Samples preparation:** The usual precautions for venipuncture should be observed. Samples are stable at 4°C for 7 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 100 µl of **Assay Buffer** into each of the wells to be used.
3. Add 10 µl of **negative control** (2 wells), **positive control** (1 well), and **samples** into appropriate wells. Cover wells and incubate for 60 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 **Peroxidase Conjugate** into each well. Cover wells with adhesive plate sealer and incubate at RT for 60 minutes.
6. Discard the solution and wash the wells as step 3.

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



7. Add 100 µl of **TMB substrate** solution and incubate the plate in dark at RT for 20 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. QUALITATIVE INTERPRETATION:

- For the run to be valid, the OD_{450/650} nm of Positive Control (Standard A) should be ≥ 1.500 and the OD_{450/650} nm of each Negative Control should be <0.150 , if not, improper technique or reagent deterioration may be suspected and the run should be repeated.

The results are evaluated by a cut-off value which is estimated by multiplying the mean OD 450/650 nm of the negative controls by 3

- If "Sample OD_{450/650} / the mean Negative Control OD_{450/650}" is < 3 , the sample is **NEGATIVE** for Antibody to Aflibercept.
- If "Sample OD_{450/650} / the mean Negative Control OD_{450/650}" is ≥ 3 , the sample is **POSITIVE** for Antibody to Aflibercept.

Note: The cut-off information provided with this kit can only be considered as a recommendation. Cut-off values must be calculated/set or verified according to scientific standards by the users.

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™ Infliximab (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™ Avelumab (Bavencio®) (Human) ELISA Kit (Cat. No. E4384-100)
- BioSim™ anti-Rituximab (Mabthera®) (Human) ELISA Kit (Cat. No. E4385-100)
- BioSim™ anti-Trastuzumab (Herceptin®) (Human) ELISA Kit (Cat. No. E4386-100)
- BioSim™ anti-Infliximab (Remicade®) (Human) ELISA Kit (Cat. No. E4387-100)
- BioSim™ anti-Adalimumab (Humira®) (Human) ELISA Kit (Cat. No. E4388-100)
- BioSim™ anti-Bevacizumab (Avastin®) (Human) ELISA Kit (Cat. No. E4389-100)
- BioSim™ anti-Infliximab (Remsima®) (Human) ELISA Kit (Cat. No. E4390-100)
- BioSim™ anti-Cetuximab (Erbix®) (Human) ELISA Kit (Cat. No. E4391-100)
- BioSim™ anti-Etanercept (Enbrel®) (Human) ELISA Kit (Cat. No. E4392-100)
- BioSim™ anti-Golimumab (Simponi®) (Human) ELISA Kit (Cat. No. E4393-100)
- BioSim™ anti-Denosumab (Prolia®) (Human) ELISA Kit (Cat. No. E4394-100)
- BioSim™ anti-Omalizumab (Xolair®) (Human) ELISA Kit (Cat. No. E4395-100)
- BioSim™ anti-Nivolumab (Opdivo®) (Human) ELISA Kit (Cat. No. E4396-100)
- BioSim™ anti-Pembrolizumab (Keytruda®) (Human) ELISA Kit (Cat. No. E4397-100)
- BioSim™ Avelumab (Bavencio®) (Human) ELISA Kit (Cat. No. E4556-100)
- BioSim™ anti-Filgrastim (Herceptin®) (Human) ELISA Kit (Cat. No. E4399-100)
- BioSim™ anti-Ipilimumab (Yervoy®) (Human) ELISA Kit (Cat. No. E4398-100)
- BioSim™ Avelumab (Bavencio®) (Human) ELISA Kit (Cat. No. E4556-100)