



# BioSim™ Aflibercept (Eylea®) (Human) ELISA Kit

07/20

(Catalog # E4854-100, 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. BioSim™ Aflibercept ELISA kit has been developed for specific quantification of Aflibercept concentration in human serum or plasma with high sensitivity and reproducibility. Aflibercept ELISA kit is based on the sandwich ELISA principle. Standards and samples (serum or plasma) are added in the microtiter plate coated with the reactant for aflibercept. After incubation, the wells are washed. The HRP conjugated probe is added and binds to aflibercept captured by the reactant on the surface of the wells. Following incubation wells are washed and the bound enzymatic activity is detected by addition of TMB chromogen substrate. Finally, the reaction is terminated with an acidic stop solution. The color developed is proportional to the amount of aflibercept in the sample or standard. Results of samples can be determined directly using the standard curve.

## II. Application:

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

Detection Range: 30 - 1000 ng/ml

Sensitivity: 30 ng/ml

Assay Precision: Intra-Assay: CV < 30%; Inter-Assay: CV < 30% (CV (%) = SD/mean X 100)

Recovery rate: <100±30% with normal human serum samples

Cross Reactivity: Except for Aflibercept, there is no cross reaction with other therapeutic antibodies and native serum immunoglobins.

## III. Sample Type:

Human serum and plasma

## IV. Kit Contents:

Components	E4854-100	Part No.
Micro ELISA Plate	8 X 12 Strips	E4854-100-1
Aflibercept Standards (S1 – S7)	0.3 ml X 7	E4854-100-2.x
Assay Buffer	50 ml	E4854-100-3
HRP-conjugate Probe	12 ml	E4854-100-4
TMB substrate (Avoid light)	12 ml	E4854-100-5
Stop Solution	12 ml	E4854-100-6
Wash buffer (20X)	50 ml	E4854-100-7
Plate sealers	2	E4854-100-8

## V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- 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 ddH<sub>2</sub>O (10 ml of Wash Buffer stock to 190 ml of ddH<sub>2</sub>O). Mix the 1X solution thoroughly by vortex manually. The working stock can be stable for 4 weeks after preparation at 4°C.

### 2. Standard Preparation:

Ready to use standards:

Name	S1	S2	S3	S4	S5	S6	S7
Conc. (ng/ml)	1000	300	100	30	0	High Control	Low Control

### 3. Sample Dilution:

- **Serum/Plasma:** Dilute samples 1:100 (5 µl Sample + 495 µl Assay Buffer).
- Diluted samples should further be diluted if the concentration of Aflibercept 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 cycles.



### VIII. Assay Protocol:

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

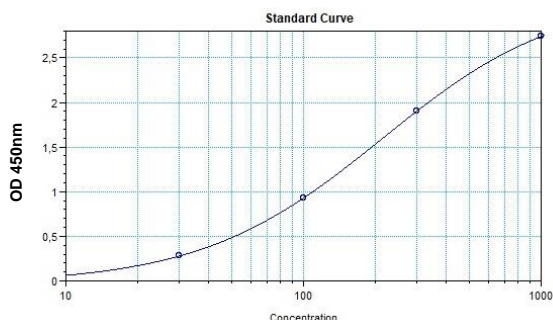
It is recommended that all standards and samples be run at least in duplicate.

A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in section VII.
2. Pipette 100  $\mu$ l of **Assay Buffer** non-exceptionally into each of the wells to be used
3. Add 20  $\mu$ l of **standards** and **diluted-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  $\mu$ l of diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
5. Add 100  $\mu$ l 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  $\mu$ l of **TMB substrate** solution and incubate the plate in dark at RT for 15 minutes
8. Add 100  $\mu$ l of **Stop solution** to stop the reaction
9. Read the absorbance in micro plate reader set to 450 nm within 30 minutes. (reference wavelength is 650 nm)

### IX. CALCULATION:

Using the standards disregarding zero standard, construct a standard curve by plotting the OD<sub>450/650 nm</sub> for each of 5 standards on the Y-axis versus the corresponding Aflibercept 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™ Avelumab (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™ Avelumab (Bavencio®) (Human) ELISA Kit (Cat. No. E4854-100)
- BioSim™ Pembrolizumab (Keytruda®) (Human) ELISA Kit (Cat. No. E4383-100)
- BioSim™ Ipilimumab (Yervoy®) (Human) ELISA Kit (Cat. No. E4384-100)
- BioSim™ Avelumab (Bavencio®) (Human) ELISA Kit (Cat. No. E4854-100)