



# BioSim™ Ranibizumab (Human) ELISA Kit

(Catalog # E4871-100, 96 assays, Store at 4°C)

09/20

## I. Introduction:

Ranibizumab is a recombinant humanized IgG1 monoclonal antibody fragment that targets human vascular endothelial growth factor A (VEGF-A). The antibody lacks an Fc-region and is smaller than the full-sized antibody, thus allowing efficient penetration into the retina and choroid. It inhibits the interaction of VEGF-A with its ligands on the surface of endothelial cells and thereby prevents neovascularization. The antibody has been approved to treat wet age-related macular degeneration, retinal vein occlusion, and diabetic retinopathy. BioSim™ Ranibizumab ELISA kit has been developed for specific quantification of Ranibizumab concentration in human aqueous humor samples with high sensitivity and reproducibility. The kit is based on the sandwich ELISA principle. Standards and samples (aqueous humor) are added in the microtiter plate coated with the reactant for Ranibizumab. After incubation, the wells are washed. The HRP conjugated probe is added and binds to Ranibizumab captured by the reactant on the surface of the wells. Following incubation, wells are washed and the bound enzymatic activity is detected by the addition of TMB chromogen substrate. Finally, the reaction is terminated with an acidic stop solution. The color developed is proportional to the amount of Ranibizumab in the sample or standard. The results of samples can be determined directly using the standard curve.

## II. Features and Benefits:

For *in vitro* quantitative determination of Ranibizumab in human aqueous humor samples

Detection Range: 0.3-30 ng/ml

Sensitivity: 0.3 ng/ml

Assay Precision: Intra-Assay and Inter-Assay CV < 30%

Recovery rate: < 100 ± 30% with known concentrations of normal human aqueous humor samples

Cross Reactivity: Except for Ranibizumab, there is no cross reaction with other therapeutic antibodies and native aqueous humor immunoglobins

## III. Sample Type:

Human aqueous humor

## IV. Kit Contents:

Components	E4871-100	Part Number
Microtiter Plate	8 x 12 strips	E4871-100-1
Ranibizumab Standards (S1 – S7)	0.3 ml	E4871-100-2
Assay Buffer	50 ml x 2	E4871-100-3
HRP-conjugate Probe	12 ml	E4871-100-4
TMB substrate (Avoid light)	12 ml	E4871-100-5
Stop Solution	12 ml	E4871-100-6
Wash buffer (20X)	50 ml	E4871-100-7
Plate sealers	2	E4871-100-8

## V. User Supplied Reagents and Equipment:

- Micropipettes and tips
- Eppendorf tubes
- Absorbent paper
- Microtiter plate reader capable of measuring absorbance at 450 nm

## VI. Storage Conditions 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:** Samples and reagents must be prepared freshly before the start of the experiment. Allow all reagents and samples to reach room temperature (RT). Gently swirl each sample and reagent, without foaming, prior to use.

- 1. Wash Buffer:** Dilute 20X Wash Buffer to 1X solution in ddH<sub>2</sub>O (10 ml of 20X Wash Buffer + 190 ml ddH<sub>2</sub>O). To dissolve the crystals, warm the Wash Buffer at 37°C. Mix vigorously. The working stock is stable for 2 weeks after preparation at 4°C.
- 2. Standard and Controls Dilution:** Dilute Standards and Controls 1:10 in Assay Buffer (20 µl Standard or Control + 180 µl Assay Buffer).

Name	S1	S2	S3	S4	S5	S6	S7
<b>Conc. (ng/ml)</b>	300	100	30	10	0	High Control	Low Control
<b>Working Conc. (ng/ml)</b>	30	10	3	1	0	-	-



- Sample Dilution:** Dilute Serum/Plasma samples 1:1000 in Assay Buffer. First, make 1:10 dilution (10 µl sample + 90 µl Assay Buffer). Next, prepare 1:100 dilution (5 µl previously diluted sample + 495 µl Assay Buffer).

#### VIII. Assay Protocol:

**Note:** Bring all reagents, samples and microtiter plate to room temperature (RT)

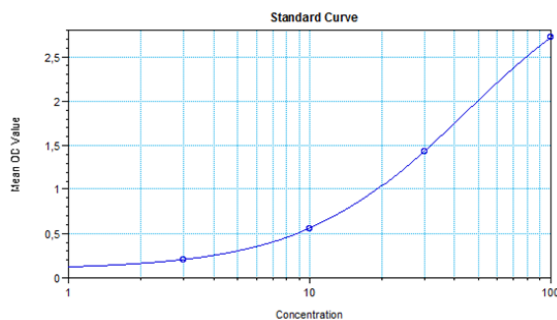
It is recommended that all standards and samples be run at least in duplicates

A standard curve must be run with each assay

- Prepare standards, controls and samples (aqueous humor) as instructed in **Section VII**.
- Add 100 µl of **standards, controls** and **samples** into appropriate wells. Cover the plate with Plate sealer, gently mix the contents in the plate, and incubate at RT for 30 mins.
- Remove the sealer and discard the incubation solution. Wash the plate 3 times with 300 µl of 1X **Wash Buffer**. Remove excess solution by tapping the inverted plate on an absorbent paper.
- Add 100 µl of **HRP-conjugate Probe** into each well. Cover the plate and incubate at RT for 30 mins.
- Discard the incubation solution and wash wells as mentioned in Step 3.
- Add 100 µl of **TMB Substrate** into each well. Incubate the plate without plate sealer in the dark at RT for 10 mins.
- Add 100 µl of **Stop Solution** to stop the reaction. Gently mix the plate. The color changes from blue to yellow.
- Measure the absorbance using microplate reader at 450 nm within 30 minutes of adding **Stop Solution**. (Use reference wavelength as 650 nm).

#### IX. Calculation:

Prepare a standard curve using the standards (disregard standard zero). Plot OD (450/650 nm) values for each standard on the vertical (Y-axis) axis versus the corresponding Ranibizumab concentration on the horizontal (X-axis) 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 must be multiplied by the dilution factor (1000x).



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

#### X. Related Products:

- BioSim™ Pembrolizumab (Human) ELISA Kit (E4383)
- BioSim™ Tocilizumab (Human) ELISA Kit (E4858)
- BioSim™ Natalizumab (Human) ELISA Kit (E4856)
- BioSim™ Denosumab (Human) ELISA Kit (E4394)
- BioSim™ Etanercept (Human) ELISA Kit (E4392)
- BioSim™ Bevacizumab (Human) ELISA Kit (E4373)
- BioSim™ Golimumab (Human) ELISA Kit (E4377)
- BioSim™ Omalizumab (Human) ELISA Kit (E4381)

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