



BioSim™ Ramucirumab (Human) ELISA Kit

rev 09/20

(Catalog # E4698-100, 96 assays, Store at 4°C)

I. Introduction:

Ramucirumab is a human monoclonal antibody (IgG1) against vascular endothelial growth factor receptor 2 (VEGFR2), a type II transmembrane tyrosine kinase receptor expressed on endothelial cells. By binding to VEGFR2, Ramucirumab prevents binding of its ligands (VEGF-A, VEGF-C, and VEGF-D), thereby preventing VEGF-stimulated receptor phosphorylation and downstream ligand-induced proliferation, permeability, and migration of human endothelial cells. BioSim™ Ramucirumab ELISA kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. Standards and samples (serum or plasma) are incubated in the microtitre plate coated with the reactant for Ramucirumab. After incubation, the wells are washed. A horse radish peroxidase (HRP) conjugated probe is added and binds to Ramucirumab 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 chromogen-substrate. The color developed is proportional to the amount of Ramucirumab in the sample or standard.

II. Application:

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

Detection Range: 20 - 600 ng/ml

Sensitivity: 20 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 with known concentrations

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

III. Sample Type:

Human serum and plasma

IV. Kit Contents:

Components	E4698-100	Part No.
Micro ELISA Plate	1 plate	E4698-100-1
Ramucirumab Standards (S1 – S7)	0.3 ml X 7	E4698-100-2.x
Assay Buffer	50 ml x 2	E4698-100-3
HRP-conjugate Probe	12 ml	E4698-100-4
TMB substrate (Avoid light)	12 ml	E4698-100-5
Stop Solution	12 ml	E4698-100-6
Wash buffer (20X)	50 ml	E4698-100-7
Plate sealers	2	E4698-100-8

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 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 at 4°C after preparation. If there are crystals in 20X Wash Buffer, warm the Buffer at 37°C to dissolve the crystals.

2. Standard Preparation:

Ready to use

Name	S1	S2	S3	S4	S5	S6	S7
Conc. (ng/ml)	600	200	60	20	0	High Control	Low Control

3. Sample Dilution:

- **Serum/Plasma:** Dilute samples 1:1000. First, dilute 10-fold by adding 10 µl sample to 90 µl Assay Buffer. Second, dilute 100-fold by adding 5 µl sample (10-fold diluted) to 495 µl Assay Buffer.
- Diluted samples should further be diluted if the concentration of Ramucirumab 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. Add 50 μ l of **Assay Buffer** to each well. Then add 50 μ l **standards** and **diluted-samples** into appropriate wells. Cover wells and incubate for 30 minutes at room temperature (RT).
3. Discard incubation solution. Wash plate 3 times each with 300 μ l of diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
4. Add 100 μ l of **HRP-conjugate** into each well. Cover wells with adhesive plate sealer and incubate at RT for 30 minutes.
5. Discard the solution and wash the wells as step 3.
6. Add 100 μ l of 1X **TMB substrate** solution and incubate the plate in dark at RT for 10 minutes
7. Add 100 μ l of **Stop solution** to stop the reaction
8. 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_{450/650} nm for each of 5 standards on the Y-axis versus the corresponding Ramucirumab 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 (x1000).

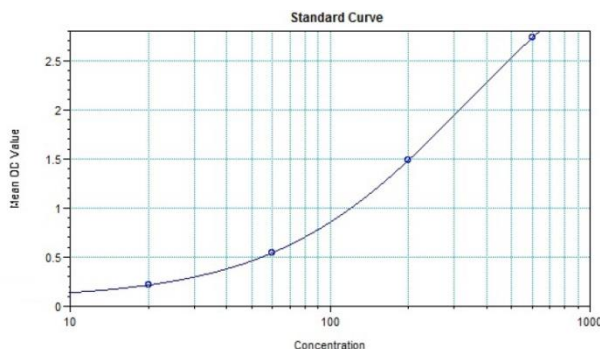


Figure: Typical Standard Curve:
These standard curves are for demonstration only. A standard curve must be run with each assay.

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™ Ramucirumab (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™ Ramucirumab (Human) ELISA Kit (Cat. No. E4698-100)
- BioSim™ Pembrolizumab (Keytruda®)(Human) ELISA Kit (Cat. No. E4383-100)
- BioSim™ Ustekinumab (Stelara®) (Human) ELISA Kit (Cat. No. E4695-100)
- BioSim™ Vedolizumab (Entyvio®)(Human) ELISA Kit (Cat. No. E4697-100)
- BioSim™ Certolizumab pegol (Cimzia®)(Human) ELISA Kit (Cat. No. E4698-100)