



BioSim™ Evolocumab (Human) ELISA Kit

(Catalog # E5025-100, 96 assays, Store at 4°C)

I. Introduction:

Evolocumab is a human IgG2 monoclonal antibody that targets proprotein convertase subtilisin/kexin type 9 (PCSK9). PCSK9 is a serine protease that binds to LDL receptors on the hepatocyte cell surface and promotes the lysosome-mediated degradation of these receptors. This results in decreased uptake and clearance of LDL-C (bad cholesterol) by the receptors. The monoclonal antibody binds to PCSK9 and prevents interaction with the LDL receptor. This promotes the recycling of the internalized LDL receptor to the hepatocyte cell surface and increased removal of LDL-C from the blood. The original drug received approval from the FDA to treat homozygous and heterozygous familial cholesterolemia. The drug has also been approved to treat patients with high cholesterol, whose LDL-C was not controlled by the statin treatment. BioSim™ Evolocumab ELISA kit measures free Evolocumab in human serum or plasma with high sensitivity and reproducibility. The kit is based on the Sandwich ELISA principle. Standards and samples (serum or plasma) are added to the microtiter plate coated with the reactant for Evolocumab. After incubation, the wells are washed. The HRP conjugated probe is added and binds to Evolocumab captured by the reactant on the surface of the wells. Following incubation, wells are washed and the 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 Evolocumab in the sample or standard. The sample results can be determined directly using the standard curve.

II. Features and Benefits:

For *in vitro* quantitative determination of free Evolocumab in human serum and plasma samples

Detection Range: 6.25 - 100 ng/ml

Sensitivity: 6.25 ng/ml

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

III. Sample Type:

Human Plasma and Serum

IV. Kit Contents:

Components	E5025-100	Part Number
Microtiter Plate	8 x 12 strips	E5025-100-1
Evolocumab Standards (S1 – S7)	8 x 0.3 ml	E5025-100-2.x
Assay Buffer	2 x 50 ml	E5025-100-3
HRP-conjugate Probe	12 ml	E5025-100-4
TMB substrate (Avoid light)	12 ml	E5025-100-5
Stop Solution	12 ml	E5025-100-6
Wash buffer (20X)	50 ml	E5025-100-7
Plate sealers	2	E5025-100-8

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 (1X):** Dilute **Wash Buffer (20X)** to 1X solution in ddH₂O (10 ml of **Wash Buffer (20X)** + 190 ml ddH₂O). To dissolve the crystals, warm the **Wash Buffer (20X)** at 37°C. Mix vigorously. The working stock is stable for 2 weeks after preparation at 4°C.
- 2. Standard and Controls Dilution:** Standards and controls must be diluted 1:10 prior to the experiment (20 µl standard/control + 180 µl Assay Buffer)

Name	S1	S2	S3	S4	S5	S6	S7	S8
Conc. (ng/ml)	1000	500	250	125	62.5	0	High Control	Low Control
Working conc. (ng/ml)	100	50	25	12.5	6.25	0	-	-



- Sample Dilution:** Dilute Serum/Plasma samples 1:1000 in Assay Buffer. First, make 1:10 dilution (10 μ l sample + 90 μ l Assay Buffer). Next, prepare 1:100 dilution (5 μ l previously diluted sample + 495 μ l 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 samples (serum/plasma) and reagents as instructed in **Section VII**.
- Add 100 μ l of **diluted 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 60 mins.
- Remove the sealer and discard the incubation solution. Wash the plate 3 times with 300 μ l of **Wash Buffer (1X)**. Remove excess solution by tapping the inverted plate on an absorbent paper.
- Add 100 μ l of **HRP-conjugate Probe** into each well. Cover the plate and incubate at RT for 60 mins.
- Discard the incubation solution and wash wells as mentioned in **Step 3**.
- Add 100 μ l of **TMB Substrate** into each well. Incubate the plate without plate sealer in the dark at RT for 20 mins.
- Add 100 μ l 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 Evolocumab 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).

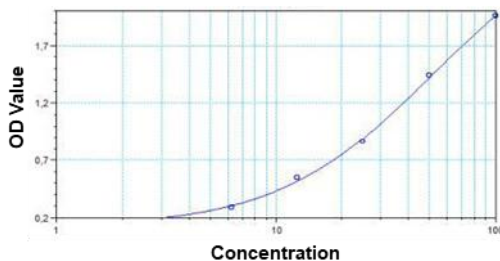


Figure: Typical Standard Curve. These standard curves are for demonstration only. A standard curve must be run with each assay.

X. Related Products:

- BioSim™ anti-Ustekinumab (Human) ELISA Kit (E4872)
- BioSim™ Ustekinumab (Human) ELISA Kit (E4695)
- BioSim™ Canakinumab (Human) ELISA Kit (E4867)
- BioSim™ Denosumab (Human) ELISA Kit (E4380)
- BioSim™ Golimumab (Human) ELISA Kit (E4377)
- BioSim™ anti-Canakinumab (Human) ELISA Kit (E4868)

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