



# BioSim™ Secukinumab (Human) ELISA Kit

(Catalog # E5026-100, 96 assays, Store at 4°C)

## I. Introduction:

Secukinumab is a human IgG1 monoclonal antibody that targets IL-17 $\alpha$  and prevents its association with the receptor IL-17R. This results in decreased release of proinflammatory cytokines and chemokines and downregulation of inflammation. IL-17 represents a family of cytokines (IL-17 A-F) released by T-helper-17 (Th17) cells, mast cells, neutrophils, and dendritic cells and promotes inflammation. Studies show that IL-17 is associated with multiple autoimmune diseases such as rheumatoid arthritis, spondyloarthritis, psoriasis, Crohn's disease, and multiple sclerosis. The original monoclonal antibody received approval from the FDA to treat plaque psoriasis, psoriatic arthritis, and ankylosing spondylitis. BioSim™ Secukinumab ELISA kit measures free Secukinumab 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 Secukinumab. After incubation, the wells are washed. The HRP conjugated probe is added and binds to Secukinumab 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 Secukinumab 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 Secukinumab in human serum and plasma samples

**Detection Range:** 6.25 - 100 ng/ml

**Sensitivity:** 6.25 ng/ml

**Cross Reactivity:** Except for Secukinumab, there is no cross reaction with other therapeutic antibodies and native serum immunoglobulins

## III. Sample Type:

Human Plasma and Serum

## IV. Kit Contents:

Components	E5025-100	Part Number
Microtiter Plate	8 x 12 strips	E5025-100-1
Secukinumab 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 ddH<sub>2</sub>O (10 ml of **Wash Buffer (20X)** + 190 ml ddH<sub>2</sub>O). 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  $\mu$ l standard/control + 180  $\mu$ l Assay Buffer)

Name	S1	S2	S3	S4	S5	S6	S7	S8
<b>Conc. (ng/ml)</b>	1000	500	250	125	62.5	0	High Control	Low Control
<b>Working conc. (ng/ml)</b>	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 µl sample + 90 µl Assay Buffer). Next, prepare 1:100 dilution (5 µl previously diluted sample + 495 µl Assay Buffer).

#### VIII. Assay Protocol:

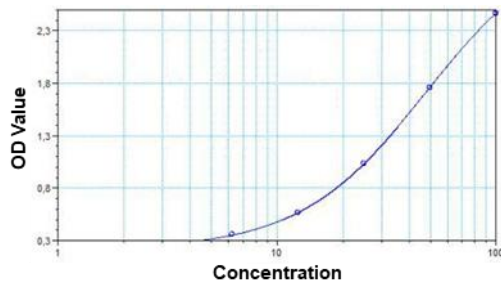
**Note:** Bring all reagents, samples and microtiter plate to room temperature (RT)

It is recommended that all standards and samples be run at least in duplicates  
A standard curve must be run with each assay

- Prepare samples (serum/plasma) and reagents as instructed in **Section VII**.
- Add 100 µl of **Assay Buffer** in each of the wells. Then add 20 µl 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 30 mins.
- Remove the sealer and discard the incubation solution. Wash the plate 3 times with 300 µl of **Wash Buffer (1X)**. 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 Secukinumab 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™ Golimumab (Human) ELISA Kit (E4377)
- BioSim™ Ustekinumab (Human) ELISA Kit (E4695)
- BioSim™ Canakinumab (Human) ELISA Kit (E4867)
- BioSim™ anti-Tocilizumab (Human) ELISA Kit (E4859)
- BioSim™ anti-Ustekinumab (Human) ELISA Kit (E4872)
- BioSim™ Tocilizumab (Human) ELISA Kit (E4858)

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