FOR RESEARCH USE ONLY!

09/20

BioSim™ Canakinumab (Human) ELISA Kit

(Catalog # E4867-100, 96 assays, Store at 4°C)

I. Introduction:

Canakinumab is a recombinant human IgG1 kappa monoclonal antibody that binds to and inhibits human IL-1β by preventing the interaction with its receptor and subsequent inflammatory activity. The antibody neither has cross-reactivity with IL-1α nor with the Interleukin 1 receptor, type I (IL1R1). The antibody has been approved to treat cryopyrin-associated periodic syndromes (CAPS). BioSim™ Canakinumab ELISA kit has been developed for specific quantification of Canakinumab concentration 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 in the microtiter plate coated with the reactant for Canakinumab. After incubation, the wells are washed. The HRP conjugated probe is added and binds to Canakinumab 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 Canakinumab 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 Canakinumab in human serum and plasma samples
Detection Range: 3-100 ng/ml
Sensitivity: 3 ng/ml
Assay Precision: Intra-Assay and Inter-Assay CV < 30%
Recovery rate: < 100 ± 30% with known concentrations of normal human serum samples
Cross Reactivity: Except for Canakinumab, there is no cross reaction with other therapeutic antibodies and native serum immunoglobins

III. Sample Type:

Human Plasma and Serum

IV. Kit Contents:

Components	E4867-100	Part Number
Microtiter Plate	8 x 12 strips	E4867-100-1
Canakinumab Standards (S1 – S7)	0.3 ml	E4867-100-2
Assay Buffer	50 ml x 2	E4867-100-3
HRP-conjugate Probe	12 ml	E4867-100-4
TMB substrate (Avoid light)	12 ml	E4867-100-5
Stop Solution	12 ml	E4867-100-6
Wash buffer (20X)	50 ml	E4867-100-7
Plate sealers	2	E4867-100-8

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_2O (10 ml of 20X Wash Buffer + 190 ml ddH_2O). 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 (100 µl Standard & Control + 900 µl Assay Buffer).

Name	S1	S2	S3	S4	S5	S6	S7
Conc. (ng/ml)	1000	300	100	30	0	High Control	Low Control
Working Conc. (ng/ml)	100	30	10	3	0	-	-

3. **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

1. Prepare standards, controls and samples (serum/plasma) as instructed in **Section VII**.
2. 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.
3. 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.
4. Add 100 µl of **HRP-conjugate Probe** into each well. Cover the plate and incubate at RT for 30 mins.
5. Discard the incubation solution and wash wells as mentioned in Step 3.
6. Add 100 µl of **TMB Substrate** into each well. Incubate the plate without plate sealer in the dark at RT for 10 mins.
7. Add 100 µl of **Stop Solution** to stop the reaction. Gently mix the plate. The color changes from blue to yellow.
8. 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 Canakinumab 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).

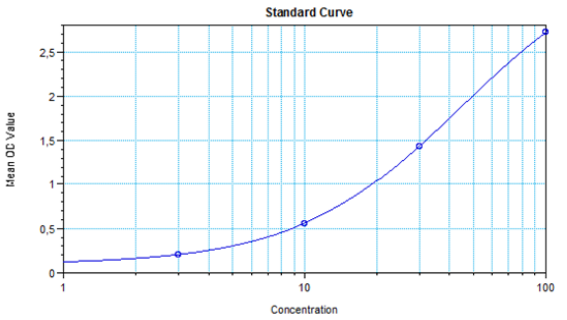

Figure: Typical Standard Curve. These standard curves are for demonstration only. A standard curve must be run with each assay.

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™ Aflibercept (Human) ELISA Kit (E4854)
- BioSim™ Certolizumab pegol (Human) ELISA Kit (E4699)

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