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BioSim™ Ustekinumab (Stelara®) (Human) ELISA Kit

12/18

(Catalog # E4695-100 ; 96 Assays ; Storage at 4°C)

I. Introduction:

Ustekinumab is a fully human monoclonal antibody that binds with high specificity and affinity to the cytokines interleukin (IL)-12 and IL-23, thereby suppressing IL-12- and IL-23-mediated inflammation associated with psoriasis. Ustekinumab (STELARA®) is the only FDA-approved medicine that targets IL-12 and IL-23, which are thought to be associated with gastrointestinal inflammation in Crohn's disease. BioSim Ustekinumab (Stelara®) ELISA Kit is a sandwich based ELISA kit. Standards and samples are incubated in the microtitre plate coated with the reactant for Ustekinumab (Stelara®). After incubation, the wells are washed. HRP-conjugated probe is added and binds to ustekinumab 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 ustekinumab in the sample or standard. Results of samples can be determined directly using the standard curve.

II. Applications:

- This ELISA kit is used for in vitro quantitative determination of Ustekinumab
- Detection Range: 3 - 100 ng/ml
- Sensitivity: 3 ng/ml
- Assay Precision: Intra-Assay: CV < 15%; Inter-Assay: CV < 15% (CV (%) = SD/mean X 100)
- Cross Reactivity: No significant cross-reactivity or interference with other proteins present in native human serum or other therapeutic immunoglobulins.
- Recovery rate: 85 – 115% with normal human serum samples with known concentrations

III. Sample Type:

- Plasma
- Serum

IV. Kit Contents:

Components	E4695-100	Part Number
Microtiter Plate	12 strips x 8 wells	E4695-100-1
Standards (S1-S7)	7 x 0.2 mL	E4695-100-2
Assay Buffer	2 x 50 ml	E4695-100-3
HRP-Conjugate	12 ml	E4695-100-4
TMB Substrate	12 ml	E4695-100-5
Stop Solution	12 ml	E4695-100-6
Wash Buffer (20X)	50 ml	E4695-100-7
Plate Sealer	2	E4695-100-8

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450/650 nm

VI. Storage Conditions and Reagent Preparation:

- The kit is shipped at ambient temperature and should be stored at 2-8°C. Keep away from heat or direct sun light.
- **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 after preparation at 4°C.
- **Serum/Plasma Preparation**: First; dilute 10 fold. (10 µl Serum/Plasma + 90 µl Assay Buffer) Second; dilute 20 fold; (20 µl (1:10 diluted) Serum/Plasma + 380 µl Assay Buffer)
- **Standard Preparation**: Dilute standard at 1:10; 25 µl Standard + 225 µl Assay Buffer

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

VII. Assay Protocol:

1. Pipette 75µl of Assay Buffer non-exceptionally into each of the wells to be used.
2. Pipette 25 µL of each Diluted Standards, Diluted High Standard, Low Standard and Diluted Samples into the respective wells of microtiter plate.
3. Cover the plate with adhesive foil. Incubate 30 min at room temperature (18 - 25°C).
4. Remove adhesive foil. Discard incubation solution. Wash plate 3 times each with 300 µL of diluted Wash Buffer. Remove excess solution by tapping the inverted plate on a paper towel.
5. Pipette 100 µL of ready-to use HRP-Conjugated Probe into each well.
6. Cover the plate with adhesive foil. Incubate 30 min at room temperature (18- 25°C).
7. Remove adhesive foil. Discard incubation solution. Wash plate 3 times each with 300 µL of diluted Wash Buffer. Remove excess solution by tapping the inverted plate on a paper towel.
8. Pipette 100 µL of TMB Substrate Solution into each well.
9. Incubate 10 min (without adhesive foil.) at room temperature (18-25°C) in the dark.
10. Stop the substrate reaction by adding 100 µL of Stop Solution into each well. Briefly mix contents by gently shaking the plate.

Color changes from blue to yellow.

11. Measure optical density with a photometer at 450/650 nm within 30 min after pipetting of the Stop Solution.

12. Using the standards (300; 100; 30; 10; 3; 0 ng/mL) disregarding zero standard, construct a standard curve by plotting the OD450/650 nm for each of 5 standards on the Y-axis versus the corresponding rituximab on centration 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.

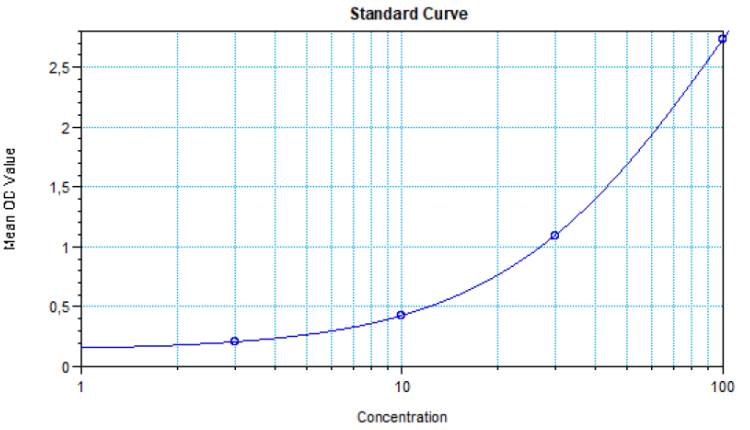

Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

VIII. Related Products:

BioSim™ Rituximab (Mabthera®) (Human) ELISA Kit (E4371)
BioSim™ Trastuzumab (Herceptin®)(Human) ELISA Kit (E4376)
BioSim™ Bevacizumab (Avastin®) (Human) ELISA Kit (E4373)
BioSim™ Adalimumab (Humira®) (Human) ELISA Kit (E4372)
BioSim™ Pembrolizumab (Keytruda®)(Human) ELISA Kit (E4383)

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