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BioSim™ Natalizumab (Tysabri®) (Human) ELISA Kit

07/20

(Catalog # E4856-100, 96 assays, Store at 4°C)

I. Introduction:

Natalizumab is a humanized IgG4k monoclonal antibody produced in murine myeloma cells. Natalizumab contains human framework regions and the complementarity-determining regions of a murine antibody that binds to a4-integrin. Natalizumab is used to treat multiple sclerosis-MS. It is thought to help by preventing patient's immune system from attacking the nerves in the brain and spinal cord. Natalizumab is also used to treat a bowel condition called Crohn's disease (CD). BioSim™ Natalizumab ELISA kit has been developed for specific quantification of Natalizumab concentration in human serum or plasma with high sensitivity and reproducibility. Natalizumab ELISA 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 Natalizumab. After incubation, the wells are washed. The HRP conjugated probe is added and binds to Natalizumab 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 TMB chromogen substrate. Finally, the reaction is terminated with an acidic stop solution. The color developed is proportional to the amount of Natalizumab in the sample or standard. Results of samples can be determined directly using the standard curve.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Natalizumab
Detection Range: 30 - 1000 ng/ml
Sensitivity: 3 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 Natalizumab, there is no cross reaction with other therapeutic antibodies and native serum immunoglobins.

III. Sample Type:

Human serum and plasma

IV. Kit Contents:

Components	E4856-100	Part No.
Micro ELISA Plate	8 x 12 strips	E4856-100-1
Natalizumab Standards (S1 – S7)	0.3 ml X 7	E4856-100-2.x
Assay Buffer (5X)	50 ml X 2	E4856-100-3
HRP-conjugate Probe	12 ml	E4856-100-4
TMB substrate (Avoid light)	12 ml	E4856-100-5
Stop Solution	12 ml	E4856-100-6
Wash buffer (20X)	50 ml	E4856-100-7
Plate sealers	2	E4856-100-8

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 ddH_2O (10 ml of Wash Buffer stock to 190 ml of ddH_2O). Mix the 1X solution thoroughly by vortex manually. The working stock can be stable for 4 weeks after preparation at 4°C.
2. **Standard Preparation:**
Dilute standards 1:10 with Assay Buffer (100 µl Standard + 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 Con. (ng/ml)	100	30	10	3	0	-	-

3. **Sample Dilution:**
   - **Serum/Plasma**: Dilute samples 1:2000. First dilution-1:20 (10 µL sample + 190 µL assay buffer). Second dilution-1:100 (5 µL diluted sample + 495 µL assay buffer)

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

- Diluted samples should further be diluted if the concentration of Natalizumab 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 100 µl of **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 µl of diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
4. Add 100 µl 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 µl of **TMB substrate** solution and incubate the plate in dark at RT for 10 minutes
7. Add 100 µl 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 OD450/650 nm for each of 5 standards on the Y-axis versus the corresponding Natalizumab 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.

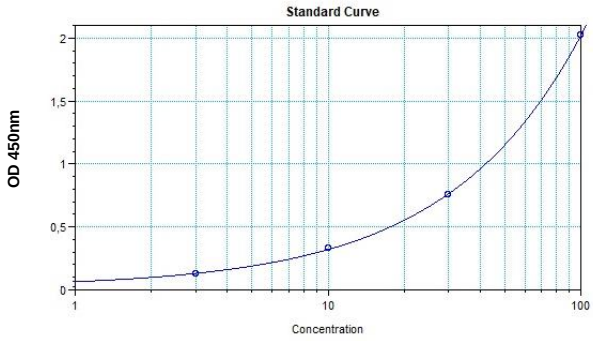

Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

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™ Avelumab (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 (Erbitux®) (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™ Avelumab (Bavencio®) (Human) ELISA Kit (Cat. No. E4856-100)
- BioSim™ Pembrolizumab (Keytruda®) (Human) ELISA Kit (Cat. No. E4383-100)
- BioSim™ Ipilimumab (Yervoy®) (Human) ELISA Kit (Cat. No. E4384-100)
- BioSim™ Avelumab (Bavencio®) (Human) ELISA Kit (Cat. No. E4856-100)