



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

07/20

(Catalog # E4857-100, 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 $\alpha 4$ -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). BioVision's BioSim™ anti- Natalizumab ELISA kit is designed to detect antibody against Natalizumab with high specificity and sensitivity in serum and plasma samples. anti-Natalizumab ELISA is based on the sandwich ELISA principle. Controls and samples are incubated in the microtiter plate coated with the drug Natalizumab. After incubation, the wells are washed. Then, HRP conjugated probe is added and binds to Natalizumab antibodies captured by the drug Natalizumab on the surface of the wells. Following incubation wells are washed and the bound enzymatic activity is detected by addition of chromogen-substrate. Finally, the reaction is terminated with an acidic stop solution. The color developed is proportional to the amount of Natalizumab antibodies in the sample or controls. The results can be evaluated with using cut-off value.

II. Application:

This ELISA kit is used for *in vitro* qualitative determination of antibody against Natalizumab in serum and plasma

Cross Reactivity: Natalizumab (Tysabri®) infusion camouflages/masks the presence of antibody to Natalizumab in serum/plasma samples. Therefore, blood sampling time is critical for detection of anti-drug-antibodies. It is convenient to obtain blood sample just before the infusion or at least 2 weeks after the infusion of Natalizumab.

III. Sample Type:

Human serum and plasma

IV. Kit Contents:

Components	E4857-100	Part No.
Micro ELISA Plate	8 X 12 Strips	E4857-100-1
Positive Control	0.3 ml	E4857-100-2
Negative Control	1 ml	E4857-100-3
Assay Buffer	12 ml	E4857-100-4
Peroxidase Conjugate	12 ml	E4857-100-5
TMB substrate (Avoid light)	12 ml	E4857-100-6
Stop Solution	12 ml	E4857-100-7
Wash buffer (20X)	50 ml	E4857-100-8
Plate sealers	2	E4857-100-9

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes with disposable tips
- 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: 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 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.
2. **Samples preparation:** The usual precautions for venipuncture should be observed. Samples are stable at 4°C for 7 days and -20°C for 6 months. Avoid freeze-and-thaw cycle.

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.

1. Prepare all reagents, samples and standards as instructed in section VII.
2. Pipette 100 μ l of **Assay Buffer** into each of the wells to be used.
3. Add 10 μ l of **negative control** (2 wells), **positive control** (1 well), and **samples** into appropriate wells. Cover wells and incubate for 60 minutes at room temperature (RT).
4. Discard incubation solution. Wash plate 3 times each with 300 μ l of diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
5. Add 100 μ l of **Peroxidase Conjugate** into each well. Cover wells with adhesive plate sealer and incubate at RT for 60 minutes.
6. Discard the solution and wash the wells as step 3.
7. Add 100 μ l of **TMB substrate** solution and incubate the plate in dark at RT for 20 minutes

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



8. Add 100 µl of **Stop solution** to stop the reaction
9. Read the absorbance in micro plate reader set to 450 nm within 20 minutes. (reference wavelength to 650 nm)

IX. QUALITATIVE INTERPRETATION:

- For the run to be valid, the OD_{450/650} nm of Positive Control (Standard A) should be ≥ 1.500 and the OD_{450/650} nm of each Negative Control should be <0.150, if not, improper technique or reagent deterioration may be suspected and the run should be repeated.

The results are evaluated by a cut-off value which is estimated by multiplying the mean OD 450/650 nm of the negative controls by 3

- If "Sample OD_{450/650} / the mean Negative Control OD_{450/650}" is < 3, the sample is **NEGATIVE** for Antibody to Natalizumab.
- If "Sample OD_{450/650} / the mean Negative Control OD_{450/650}" is ≥ 3, the sample is **POSITIVE** for Antibody to Natalizumab.

Note: The cut-off information provided with this kit can only be considered as a recommendation. Cut-off values must be calculated/set or verified according to scientific standards by the users.

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™ Infliximab (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 (Erbix®) (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™ Nivolumab (Opdivo®) (Human) ELISA Kit (Cat. No. E4382-100)
- BioSim™ Pembrolizumab (Keytruda®) (Human) ELISA Kit (Cat. No. E4383-100)
- BioSim™ Avelumab (Bavencio®) (Human) ELISA Kit (Cat. No. E4384-100)
- BioSim™ anti-Rituximab (Mabthera®) (Human) ELISA Kit (Cat. No. E4385-100)
- BioSim™ anti-Trastuzumab (Herceptin®) (Human) ELISA Kit (Cat. No. E4386-100)
- BioSim™ anti-Infliximab (Remicade®) (Human) ELISA Kit (Cat. No. E4387-100)
- BioSim™ anti-Adalimumab (Humira®) (Human) ELISA Kit (Cat. No. E4388-100)
- BioSim™ anti-Bevacizumab (Avastin®) (Human) ELISA Kit (Cat. No. E4389-100)
- BioSim™ anti-Infliximab (Remsima®) (Human) ELISA Kit (Cat. No. E4390-100)
- BioSim™ anti-Cetuximab (Erbix®) (Human) ELISA Kit (Cat. No. E4391-100)
- BioSim™ anti-Etanercept (Enbrel®) (Human) ELISA Kit (Cat. No. E4392-100)
- BioSim™ anti-Golimumab (Simponi®) (Human) ELISA Kit (Cat. No. E4393-100)
- BioSim™ anti-Denosumab (Prolia®) (Human) ELISA Kit (Cat. No. E4394-100)
- BioSim™ anti-Omalizumab (Xolair®) (Human) ELISA Kit (Cat. No. E4395-100)
- BioSim™ anti-Nivolumab (Opdivo®) (Human) ELISA Kit (Cat. No. E4396-100)
- BioSim™ anti-Pembrolizumab (Keytruda®) (Human) ELISA Kit (Cat. No. E4397-100)
- BioSim™ Avelumab (Bavencio®) (Human) ELISA Kit (Cat. No. E4556-100)
- BioSim™ anti-Filgrastim (Herceptin®) (Human) ELISA Kit (Cat. No. E4399-100)
- BioSim™ anti-Ipilimumab (Yervoy®) (Human) ELISA Kit (Cat. No. E4398-100)
- BioSim™ Avelumab (Bavencio®) (Human) ELISA Kit (Cat. No. E4556-100)