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BioSim™ Rituximab (Human) ELISA Kit

rev 12/20

(Catalog # E4371-100, 96 assays, Store at 4°C)

I. Introduction:

Rituximab (Mabthera®) is a genetically engineered chimeric murine/human monoclonal antibody specific to CD20. CD20 is an approximately 35 KDa transmembrane phosphoprotein involved in the activation, proliferation, and differentiation of B-lymphocytes. It is absent in terminally differentiated plasma cells. The Fab domain of rituximab binds to the CD20 antigen on B-lymphocytes and the Fc domain recruits immune effector functions to induce apoptosis in B cells and is used in the treatment of leukemia s and lymphomas, some autoimmune disorders, and organ transplant. BioVision's BioSim™ Rituximab ELISA kit is designed to quantify/measure the Rituximab with high specificity and sensitivity in biological matrices.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Rituximab.
Detection Range: 3 - 300 ng/ml
Sensitivity: 3 ng/ml
Assay Precision: Intra-Assay: CV < 30%; Inter-Assay: CV < 30% (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: < 100 ± 30% with normal human serum samples with known concentrations

III. Sample Type:

Human serum and plasma

IV. Kit Contents:

Components	E4371-100	Part No.
Micro ELISA Plate	1 plate	E4371-100-1
Rituximab Standards (S1 – S8)	1 ml X 8	E4371-100-2.x
Assay Buffer	2 x 50 ml	E4371-100-3
HRP-conjugate Probe	12 ml	E4371-100-4
TMB substrate (Avoid light)	12 ml	E4371-100-5
Stop Solution	12 ml	E4371-100-6
Wash buffer (20X)	50 ml	E4371-100-7
Plate sealers	2	E4371-100-8

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- Calibrated measures
- 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: 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 2 weeks after preparation at 4°C.
2. **Standard Preparation:**
Ready to use

Name	S1	S2	S3	S4	S5	S6	S7	S8
Conc. (ng/ml)	300	100	30	10	3	0	High Standard	Low Standard

3. **Sample Dilution:**
   - **Serum/Plasma**: First dilute samples at 1:10 (10 µl Serum/Plasma + 90 µl Assay Buffer). Secondly, dilute samples 1:100 (5 µl diluted sample + 495 µl Assay Buffer) (Dilution factor : 1000)
   - Diluted samples should further be diluted if the concentration of rituximab is higher than the measuring range.
   - 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.

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

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 60 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 60 minutes.
5. Discard the solution and wash the wells as step 3.
6. Add 100 µl of 1X **TMB substrate** solution and incubate the plate in dark at RT for 15 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 20 minutes. (reference wavelength to 650 nm)

IX. CALCULATION:

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 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.

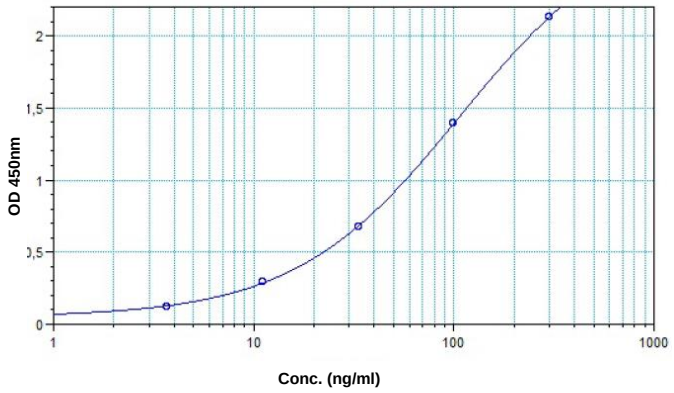

Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

X. RELATED PRODUCTS:

- BioSim™ Rituximab (Human) ELISA Kit (Cat. No. E4371-100)
- BioSim™ Adalimumab (Human) ELISA Kit (Cat. No. E4372-100)
- BioSim™ Bevacizumab (Human) ELISA Kit (Cat. No. E4373-100)
- BioSim™ Etanercept (Human) ELISA Kit (Cat. No. E4374-100)
- BioSim™ anti-HER2 (Human) ELISA Kit (Cat. No. E4376-100)
- BioSim™ Golimumab (Human) ELISA Kit (Cat. No. E4377-100)
- BioSim™ Cetuximab (Human) ELISA Kit (Cat. No. E4379-100)
- BioSim™ Denosumab (Human) ELISA Kit (Cat. No. E4380-100)
- BioSim™ Omalizumab (Human) ELISA Kit (Cat. No. E4381-100)
- BioSim™ Nivolumab (Human) ELISA Kit (Cat. No. E4382-100)
- BioSim™ Pembrolizumab (Human) ELISA Kit (Cat. No. E4383-100)
- BioSim™ Ipilimumab (Human) ELISA Kit (Cat. No. E4384-100)