

Version 2a Last updated 14 November 2024

ab237654 anti-Rituximab ELISA Kit

For the measurement of the antibody against Rituximab in human serum and plasma.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Anti-Rituximab ELISA Kit (ab237654) is designed to quantify/measure the antibody against Rituximab with high specificity and sensitivity in biological matrices.

Rituximab 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. It is used in treating leukemias and lymphomas, some autoimmune disorders, and organ transplant. However, some patients develop unwanted immunogenicity, which leads to production of anti-drug-antibodies (ADAs) inactivating the therapeutic effects of the treatment and, in rare cases, inducing adverse effects.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed



Add 100 μ L standard, controls, diluted sample and confirmation test mixture to appropriate wells. Cover and incubate for 60 minutes at room temperature



Discard incubation solution and wash plate 3 times with 300 μ L diluted Wash Buffer



Add 100 μ L peroxidase conjugate to each well. Cover and incubate for 60 minutes at room temperature



Discard the solution and wash plate 3 times with 300 μ L diluted Wash Buffer



Add 100 μ L TMB Substrate and incubate the plate in the dark at room temperature for 20 minutes.



Add 100 μ L Stop Solution and read OD at 450 nm within 20 minutes.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors.

6. Materials Supplied

Item	Quantity	Storage
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		condition
Micro ELISA Plate	1 unit	+4°C
Confirmation Reagent	12 mL	+4°C
Rituximab Standard A	1 mL	+4°C
Rituximab Standard B	1 mL	+4°C
Rituximab Standard C	1 mL	+4°C
Rituximab Standard D	1 mL	+4°C
Rituximab Standard E	1 mL	+4°C
Rituximab Standard F	1 mL	+4°C
Control – Low level	1 mL	+4°C
Control – High level	1 mL	+4°C
Assay Buffer	50 mL	+4°C
Peroxidase Conjugate	12 mL	+4°C
TMB Substrate	12 mL	+4°C
Stop Solution	12 mL	+4°C
Wash Buffer (20X)	50 mL	+4°C
Plate sealers	2 units	+4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at OD 450 nm
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for sample dilution.
- Plate shaker for all incubation steps.
- Absorbent paper

8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.
- When generating positive control samples, it is advisable to change pipette tips after each step.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 20X 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.

10. Standard and Control Preparation

Standard (A-F) and controls (low and high levels) are ready to use, please see table below for concentrations:

Name	A	B	C	D	E	F	Control – low level	Control – high level
Conc. ng/mL	160	80	40	20	10	0	Low control	High control

Concentration for high and low controls are indicated on vials.

11. Sample Preparation

General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples for the most reproducible assay.

11.1 Serum/plasma:

1. Dilute samples at 1:10 (20 µL serum/plasma + 180 µL Assay Buffer) and then 1:100 (5 µL serum/plasma + 495 µL Assay Buffer).
2. Diluted samples should further be diluted if the concentration of Rituximab is higher than the measuring range.
3. Samples are stable at 4°C for 7 days and -20°C for 6 months. Avoid freeze-and-thaw cycle.

Δ Note: The usual precautions for venipuncture should be observed.

12. Confirmation test mixture preparation

Mix 20 µL undiluted (positive) serum/plasma sample with 180 µL of confirmation reagent for 60 minutes in a microtube prior to the test.

13. Assay Procedure

- Prepare reagents within 30 minutes before the experiment.
 - Equilibrate all materials and prepared reagents to room temperature 15 minutes prior to use.
 - We recommend that you assay all standards, controls and samples in duplicate.
- 13.1 Add 100 μ L of standards, controls, diluted samples and confirmation test mixture into appropriate wells. Cover wells and incubate for 60 minutes at room temperature.
 - 13.2 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.
 - 13.3 Add 100 μ L of Peroxidase Conjugate into each well. Cover wells with adhesive plate sealer and incubate at room temperature for 60 minutes.
 - 13.4 Discard the solution and wash the wells as step 13.2.
 - 13.5 Add 100 μ L of 1X TMB substrate solution and incubate the plate in the dark at room temperature for 20 minutes.
 - 13.6 Add 100 μ L of Stop solution to stop the reaction.
 - 13.7 Read the absorbance in a microplate reader set to 450 nm within 30 minutes. (Reference wavelength to 650 nm).

14. Calculations

14.1 Quantitative Calculation

- 14.1.1 Calculate the average absorbance value for the blank control (0 ng/mL) standards. Subtract the average blank control standard absorbance value from all other absorbance values.
- 14.1.2 **Create a standard curve** by plotting the average blank control subtracted absorbance value for each standard concentration (y axis) against the target protein concentration (x axis) of the standard.
- 14.1.3 Construct a standard curve of difference data using software capable of generating four-parameter logistic (4PL) or point-to-point calculation curve fit.

- 14.1.4 To obtain the exact values of the samples, the concentration determined from the standard curve should be multiplied by the dilution factor.
- 14.1.5 Determine the concentration of the target protein in the sample by interpolating the blank control subtracted **absorbance values against the standard curve**. Multiply the resulting value by the appropriate dilution factor to obtain the concentration of target protein in the sample.
- 14.1.6 Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at an absorbance values less than that of the lowest standard should be retested in a less dilute form.

14.2 Qualitative Interpretation

- 14.2.1 If "Sample OD450/650 / Zero Standard (S5) OD450/650" is < 3, the sample is NEGATIVE for Antibody to Rituximab (ATR).
- 14.2.2 If "Sample OD450/650 /Zero Standard (S5) OD450/650" is ≥3, the sample is POSITIVE for ATR, and if required samples may be extrapolated for quantitative analysis and confirmation.
- 14.2.3 For the run to be valid, the OD450/650 nm of High Control should be ≥ 1.000 and the OD450/650 nm of each Low Control should be <0.200, if not, improper technique or reagent deterioration may be suspected and the run should be repeated.
- 14.2.4 Interpretation of true and false positive: For true positive sample, inhibition should be equal or greater than 25%.

$$\frac{OD \text{ sample} - OD \text{ sample with confirmation reagent}}{OD \text{ sample}} \times 100 = \text{inhibition \%}$$

15. Typical Data

Typical standard curve - data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

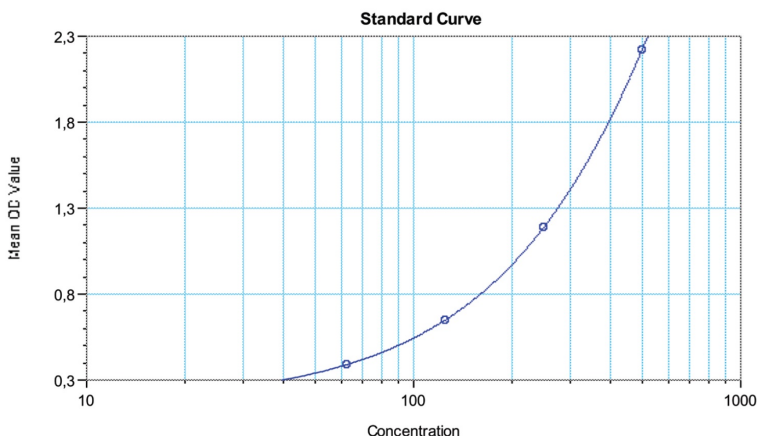


Figure 1. Typical Standard Curve: This standard curve is for demonstration only. A standard curve must be run with each assay.

16. Typical Sample Values

Detection Range: 75 - 500 ng/mL.

Sensitivity: 10 ng/mL.

Assay Precision: Intra-Assay: CV < 30%; Inter-Assay: CV < 30%
(CV (%) = SD/mean X 100)

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

Recovery rate: 70 – 130% with normal human serum samples with known concentrations.

17. Notes

Technical Support

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