ab282904 – Anti-Rituximab ELISA Kit

For *in vitro* qualitative determination of antibodies to Rituximab in human serum and plasma samples.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab282904

Storage and Stability

The entire ELISA kit may be stored at 4°C for up to 12 months from the date of shipment.

Materials Supplied

Item	Quantity	Storage Condition
Anti-Rituximab Negative Control	1 x 1 ml	4°C
Anti-Rituximab Positive Control	1 x 300 µl	4°C
Assay Buffer	1 x 12 ml	4°C
Rituximab coated microtitre plate	1 unit	4°C
HRP-conjugate Probe	1 x 12 ml	4°C
Plate sealers	2 units	4°C
Stop Solution	1 x 12 ml	4°C
TMB substrate	1 x 12 ml	4°C
Wash Buffer (20X)	1 x 50 ml	4°C

Materials Required, Not Supplied

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

- Micropipettes and tips
- Eppendorf tubes
- Absorbent paper
- Microtiter plate reader capable of measuring absorbance at 450 nm

Reagent Preparation

- Before using the kit, spin the tubes and bring down all the components to the bottom of the tubes.

Wash Buffer:
Dilute 20X Wash Buffer to 1X solution in ddH_2O (10 ml of 20X Wash Buffer + 190 ml ddH_2O). To dissolve the crystals, warm the Wash Buffer at 37°C. Mix vigorously. The working stock is stable for 2 weeks after preparation at 4°C.

- All other reagents are supplied ready to use.

Sample Preparation

- The usual precautions should be observed for venipuncture. Samples that are hemolytic, icteric or lipemic should be avoided.
- If the sample is turbid, then it must be centrifuged to separate particulates from solution.
- Freeze/thawing of serum/plasma samples should be avoided.
- Drug infusions may interfere with the detection of antibodies to drugs in serum/plasma samples. Hence, it is advisable to take blood samples prior to the scheduled dose.
- Collected samples are stable for 2 days at 4°C or for 6 months at -20°C.

Assay Procedure

- Bring all the reagents, samples, and microtiter plate to room temperature 15 minutes prior to the assay.
- It is recommended that all samples be run at least in duplicates.

1. Prepare all reagents and samples as instructed.
2. Pipette 100 µl of Assay Buffer into each of the wells to be used, no exceptions.
3. Add 10 µl of Negative control (2 wells), Positive control (1 well), and samples into appropriate wells. Cover wells and incubate at room temperature for 60 minutes.
4. Discard the incubation solution. Wash plate 3 times with 300 µl of 1X Wash Buffer. Remove excess solution by tapping the inverted plate on an absorbent paper.
5. Add 100 µl of HRP-conjugated probe into each well. Cover the plate with adhesive plate sealer and incubate at room temperature for 60 minutes.
6. Discard the solution and wash the wells as in step 4.
7. Add 100 µl of TMB substrate solution and incubate the plate in the dark at room temperature for 20 minutes.
8. Add 100 µl of Stop solution to stop the reaction. Color changes from blue to yellow.
9. Read the absorbance in micro plate reader set to 450 nm within 30 minutes after pipetting the Stop solution. (Use reference wavelength as 650 nm).

Interpretation of Results

1. For the run to be valid, the optical density (OD) 450/650 nm of the Positive control should be > 1.500 and the OD 450/650 nm of each Negative control should be < 0.150. If the results do not comply with the aforementioned information, then improper technique or reagent deterioration may be suspected and therefore the assay must be repeated.
2. 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 OD450/650 / the mean Negative Control OD450/650' is < 3, the sample is NEGATIVE for Antibody to Rituximab.
    - If 'Sample OD450/650 / the mean Negative Control OD450/650' is ≥ 3, the sample is POSITIVE for Antibody to Rituximab.

Δ 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.*

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus