Rituximab(Rituxan®) Immunogenicity ELISA Catalog EL-141-181

For the qualitative determination of Rituximab binding antibodies in serum and plasma.

Introduction

Rituximab (Rituxan®) is a chimeric mouse/human monoclonal antibody used for treating B cell malignancy, autoimmune conditions, and graft rejection by depleting B cells from the body. This immunogenicity assay employs the bridging ELISA technique. Capture antibody is precoated onto a 96 well microplate. Quality control and test samples are pipetted into the appropriate wells. Anti-Rituximab present in biological matrices is bound by the immobilized capture antibody. After washing away any unbound substances, secondary antibody is added to the wells and after a final wash a detection reagent is added. The plate is washed to remove any unbound antibody-enzyme reagent and a substrate solution is added to the wells for color development. The color development is proportional to the amount of anti-Rituximab present in test samples. Four levels of QC samples give a qualitative reference signal which can be used to determine the level of anti-Rituximab antibody in the unknown samples. The color development is stopped and the intensity of the color is measured.

Materials and storage

Store kit components at -20°C unless specified otherwise. DO NOT USE past kit expiration date. Some vials contain a small amount of reagents. Spin tubes on pulse setting prior to opening.

Each kit includes:	Units
Coated microtiter plate, 96 wells (1x8 strips)	1
QC Samples	7
QC1 (50µl) anti-Rituximab	1250ng/ml
QC2 (50µl) anti-Rituximab	625/ml
QC3 (50µl) anti-Rituximab	312.5ng/ml
QC4 (50µl) anti-Rituximab	156.25ng/ml
QC5 (50µl) anti-Rituximab	78.125ng/ml
QC6 (50µl) anti-Rituximab	39.0625ng/ml
QC7 (50µl) Negative control	0ng/ml
Do not mix or substitute reagents with those from other lots.	

Each kit includes:	Units
10X wash buffer	50ml
Assay buffer	50ml
1000X secondary antibody	17µl
1000X detection reagent	17µl
TMB	12ml
TMB stop solution	12ml
Plate sealers	3
Do not mix or substitute reagents with those from other lots.	

Materials and instruments required but not supplied

- Precision pipettes calibrated to deliver 5-1000µL
- Multi-channel pipette calibrated to deliver 50-200µL
- Plate shaker
- Disposable tips
- Vortex-Mixer
- Distilled or de-ionized water
- Microplate reader capable of reading 450nm with background subtraction at 620nm

Safety precautions

- The test protocol must be followed strictly.
- All reagents containing human material should be handled as if potentially infectious. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.
- The kit reagents contain antimicrobial agents, acid and 3,3',5,5'-tetramethylbenzidine. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
- Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Disposal must be performed in accordance with local regulations.
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- Only trained laboratory personnel should execute this test.

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Preparation of reagents

Prepare only the appropriate amount of required reagent on the day of use. Store all reagents as per instructions stated on the label.

1. **Wash Buffer (1X) Preparation:** Dilute wash buffer concentrate with ultra-pure water 1/10 before use (for example add 50mL concentrate to 450mL ultra-pure water). Mix well.
2. **Secondary antibody (1X) Preparation:** Dilute secondary antibody with assay buffer 1/1000 before use (for examples add 12µl concentrate to 12ml of assay buffer). Mix well.
3. **Detection Reagent (1X) Preparation:** Dilute detection reagent with assay buffer 1/1000 before use (for example add 12µl concentrate to 12ml of assay buffer). Mix well.

Specimen storage

This kit is compatible with EDTA-plasma, heparin-plasma and serum samples. Samples can be stored at or below -20°C for up to 1 year.

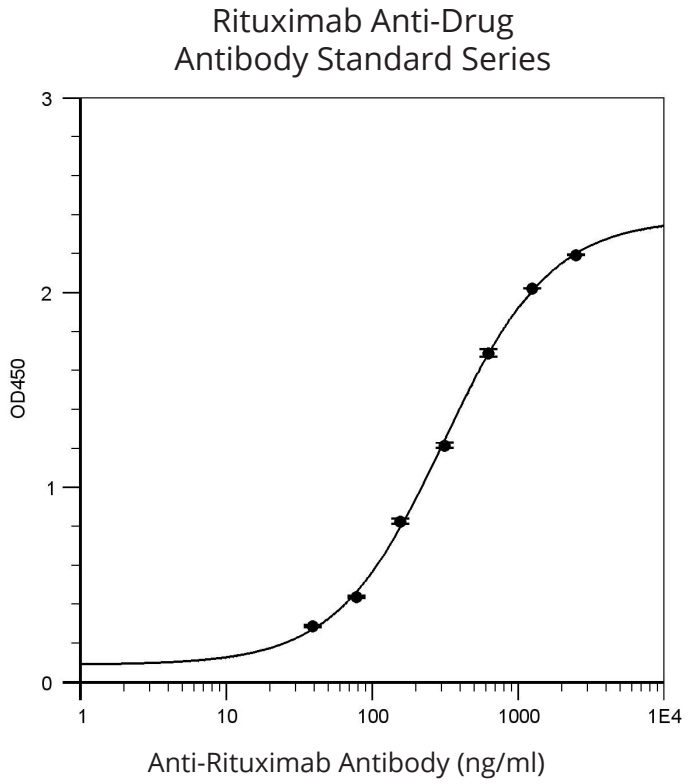
Assay procedure

1. Remove coated microtiter plate from -20ºC and allow it to acclimate to room temperature for 15-20 minutes.
2. Dilute QC samples and test samples 1/10 with assay buffer (for example add 30µL of prepared calibrator or sample to 270µL of assay buffer). Mix well. Do not store diluted samples. If test samples are out of range, then they may be further diluted.
3. Add 100µL diluted calibrators and samples to appropriate wells on the plate. Incubate for 1 hour at room temperature on a plate shaker at approximately 300rpm.
4. Discard the content of the plate and wash the wells 3x with 200µL wash buffer per well.
5. Add 100µL secondary antibody to appropriate wells on the plate. Incubate for 1 hour at room temperature on a plate shaker at approx 300rpm.
6. Discard the content of the plate and wash the wells 3x with 200µL wash buffer per well.
7. Add 100µL detection reagent to appropriate wells on the plate. Incubate for 1 hour at room temperature on a plate shaker at approx 300rpm.
8. Discard the content of the plate and wash the wells 3x with 200µL wash buffer per well.
9. Add 100µL of TMB to each well on plate. Incubate for 3-6 minutes at room temperature protected from light.
10. Add 100µL of TMB stop solution to each well on plate. Mix by gently tapping the side of the plate.
11. Determine absorbance with a microplate reader at 450nm against 620nm.

Calculations and results

1. Because anti-drug antibodies will vary in terms of affinity and concentration, the user should use comparable positive controls when assessing interassay results. The provided controls provided are tested for comparability between lots and can be traced.
2. We recommend each lab develop their own statistical cutpoint using methodologies as described by G. Shankar, et al. (2008). (Recommendations for the validation of immunoassays used for detection of host antibodies against biotechnology products. J. Pharmaceutical and Biomedical Analysis 48:1267–1281).
3. Any sample undiluted or diluted still reading greater than the highest standard should be diluted appropriately with assay buffer and retested. If the samples have been diluted, the concentration determined from the standard curve must be multiplied by the dilution factor.

Typical Results



6 replicates of each QC sample diluted 1 in 10 and then used to generate a standard series. Assay developed for 6 minutes in this example.

Performance characteristics

Precision: The precision was determined by analyzing samples spiked at 250ng/mL anti-drug antibody in 6 replicates on 6 different occasions. Intra-assay coefficient of variation (CV) < 10%. Inter-assay CV < 10%.

Ordering Information

Please vist www.affinityimmuno.com to order this product. Visa, Mastercard, AMEX and PayPal are accepted in our online store.

Your order will be processed immediately and you will be notified with a delivery timeframe.