Version 10-09/22

User's Manual

Rituximab ELISA Kit

REF **DEIABL238**

Σ **96T**

RUO

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

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

Rituximab ELISA has been especially developed for the quantitative analysis of free rituximab in serum and plasma samples.

General Description

Rituximab is a monoclonal antibody that targets the CD20 antigen, which is expressed on the surface of pre-B and mature B-lymphocytes. After binding to CD20, rituximab mediates B-cell lysis (or breakdown). The possible mechanisms of cell lysis include complement dependent cytotoxicity (CDC) and antibody dependent cell-mediated cytotoxicity (ADCC).

Rituximab belongs to the immunoglobulin G1 (IgG1) sub-class, consisting of a murine variable region (Fab region) and a human constant region (Fc region). The Fab region has variable sections that define a specific target antigen, allowing the antibody to attract and secure its exclusive antigen, specifically the binding of rituximab (IgG1) to CD20 on pre-B and mature B lymphocytes. The Fc region is the tail end of the antibody that communicates with cell surface receptors to activate the immune system, in this case, a sequence of events leading to the depletion of circulating B lymphocytes by complement-dependent cell lysis, antibody-dependent cellular cytotoxicity, as well as apoptosis. Therapeutic drug monitoring (TDM) is the clinical practice of measuring specific drugs at designated intervals to maintain a constant concentration in a patient's bloodstream, thereby optimizing individual dosage regimens. The indications for drug monitoring include efficacy, compliance, drug-drug interactions, toxicity avoidance, and therapy cessation monitoring. Additionally, TDM can help to identify problems with medication compliance among noncompliant patient cases.

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Biologic medicinal products (biologics) have transformed treatment landscapes worldwide for patients with haematological or solid malignancies with the 21st century. Today, as data exclusivity periods of first wave biologics approach expiration/have expired, several biosimilar products (i.e., biologics that are considered to be similar in terms of quality, safety and efficacy to an approved 'reference' biologic) are being developed or have already been approved for human use.

Like all biologics, biosimilars are structurally complex proteins that are typically manufactured using genetically engineered animal, bacterial or plant cell culture systems. As a consequence of this molecular complexity and the proprietary nature of the manufacturing process, which will inevitably result in the use of different host cell lines and expression systems as well as related differences in manufacturing conditions, it is not possible to manufacture exact copies of a reference biologic.

When administered to patients, all therapeutic proteins have the potential to induce an unwanted immune response (i.e., to stimulate the formation of antidrug antibodies [ADAs]). The impact of immune responses can range from no apparent effect to changes in pharmacokinetics, loss of effect and serious adverse events. Furthermore, the immunogenicity profile of a biologic can be significantly altered by even small differences in

its manufacturing process that are accompanied by a change in product attributes, as well as differences in dosing schedules, administration routes or patient populations.

Principles of Testing

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. Standards and samples (serum or plasma) are incubated in the microtiter plate coated with the reactant for rituximab. After incubation, the wells are washed. Then, horse radish peroxidase (HRP) conjugated probe is added and binds to rituximab captured by the reactant on the surface of the wells. Following incubation wells are washed and the bound enzymatic activity is detected by addition of tetramethylbenzidine (TMB) chromogen substrate. Finally, the reaction is terminated with an acidic stop solution. The colour developed is proportional to the amount of rituximab in the sample or standard. Results of samples can be determined directly using the standard curve.

Reagents And Materials Provided

1. Microtiter Plate (1 x 12 x 8): Break apart strips. Microtiter plate with 12 rows each of 8 wells coated with reactant.
2. Standard A-F (1.0 mL (each)):

Standards A-F

Standard A: 300 ng/mL

Standard B: 100 ng/mL

Standard C: 30 ng/mL

Standard D: 10 ng/mL

Standard E: 3 ng/mL

Standard F: 0 ng/mL

Used for the standard curve and control. Contains rituximab, human serum and stabilizer, <0,1% NaN3.

3. Controls (1.0 mL (each)): Control low and high levels. Contains human serum and stabilizer, <0,1% NaN3.
4. Assay Buffer (2x50 mL): Ready to use. Blue coloured. Contains proteins, <0,1% NaN3.
5. Conjugate (1x12 mL): Ready to use. Red coloured. Contains HRP conjugated probe, stabilizer and preservatives.
6. Substrate (1x12 mL): Ready to use. Contains 3,3´,5,5´- Tetramethylbenzidine (TMB).
7. Stop Buffer (1x12 mL): Ready to use. 1N HCl.
8. Wash Buffer (1x50 mL): Prepared concentrated (20x) and should be diluted with the dilution rate given in the "Reagents Preparation" before the test. Contains buffer with tween 20.
9. Foil (2x1): For covering microtiter plate during incubation.

Materials Required But Not Supplied

1. Micropipettes and tips
2. Calibrated measures

3. Tubes for sample dilution
4. Wash bottle, automated or semi-automated microtiter plate washing system
5. Microtiter plate reader capable of measuring optical density with a photometer at OD 450nm with reference wavelength 650 nm (450/650 nm)
6. Distilled or deionised water, paper towels, pipette tips and timer

Storage

The kit is shipped at ambient temperature (10-30°C) and should be stored at 2-8°C for long term storage. Keep away from heat or direct sunlight. The strips of microtiter plate are stable up to the expiry date of the kit in the broken, but tightly closed bag when stored at 2-8°C.

Specimen Collection And Preparation

Serum, Plasma (EDTA, Heparin)

The usual precautions for venipuncture should be observed. Do not use grossly haemolytic, icteric or lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material. Avoid repeated freeze-thaw cycles for serum/plasma samples.

Samples should be diluted with the dilution rate given in the "Reagent Preparation" before the test.

Drug infusions may camouflages/mask the presence of antibody to drugs in serum/plasma samples. Therefore, blood sampling time is critical for detection of antibodies. It is recommended to take the blood sample just before the scheduled dose (trough specimen).

Storage	2-8°C	-20°C
Stability (serum/plasma)	2 days	6 months

Reagent Preparation

Wash Buffer - Dilute the concentrate wash buffer with distilled water at the ratio of 1/20 before starting assay procedure. If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. The diluted wash buffer can stable for 2 weeks at 2-8°C.

Serum/Plasma - Dilute the sample with assay buffer at the ratio of 1/1000 before starting assay procedure.

First step (1/10 dilution): 10 µL sample + 990 µL assay buffer.

Second step (1/100 dilution): 5 µL sample (1/10 diluted) + 495 µL assay buffer.

Assay Procedure

1. Pipette 100 µL of each diluted "Standards", "Low level control", "High level control" and samples into the respective wells of microtiter plate.

Wells

A1: Standard A

B1: Standard B

C1: Standard C

D1: Standard D

E1: Standard E

F1: Standard F

G1: Low level control

H1: High level control

A2 and on: Samples

2. Cover the plate with adhesive foil. Briefly mix contents by gently shaking the plate. Incubate 60 minutes at room temperature (18-25°C).
3. Remove adhesive foil. Discard incubation solution. Wash plate three times each with 300 µL "Wash Buffer". Remove excess solution by tapping the inverted plate on a paper towel.
4. Pipette 100 µL "Conjugate" into each well.
5. Cover the plate with adhesive foil. Incubate 60 minutes at room temperature (18-25°C).
6. Remove adhesive foil. Discard incubation solution. Wash plate three times each with 300 µL "Wash Buffer". Remove excess solution by tapping the inverted plate on a paper towel.
7. Pipette 100 µL "Substrate" into each well.
8. Incubate 15 minutes without adhesive foil at room temperature (18-25°C) in the dark.
9. Stop the substrate reaction by adding 100 µL "Stop Solution" into each well. Briefly mix contents by gently shaking the plate Colour changes from blue to yellow.
10. Measure optical density with a photometer at OD 450nm with reference wavelength 650 nm (450/650 nm) within 30 minutes after pipetting the "Stop Solution".

Quality Control

The test results are only valid if the test has been performed following the instructions. Moreover, the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. For the run to be valid, the OD 450/650 nm of positive control should be>1,500 and the OD 450/650 nm of each negative control should be <0.150. In case of any deviation the following technical issues (but not limited to) should be reviewed: Expiration dates of reagents, storage conditions, pipettes, devices, incubation conditions, washing methods, etc.

Calculation

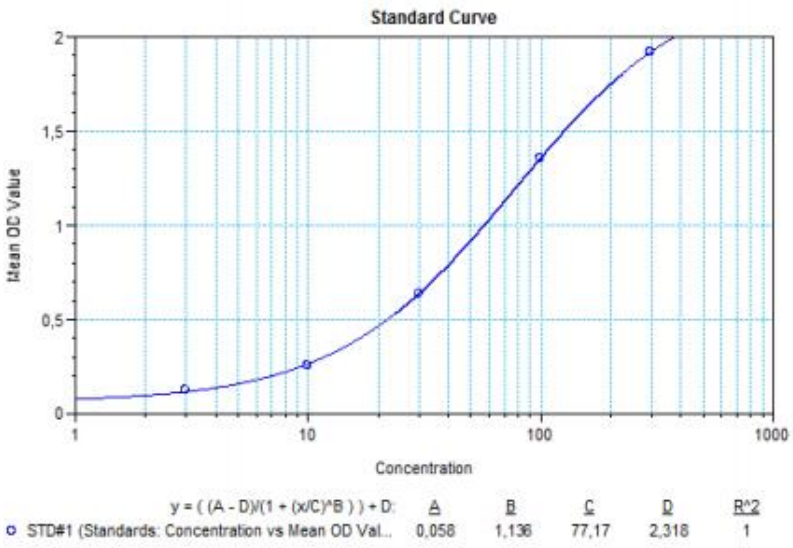
1. Create a standard curve by using the standards. OD 450/650 nm for each standard on the vertical (Y-axis) axis versus the corresponding drug concentration on the horizontal (X-axis) axis.
2. The concentration of the samples can be read directly from this standard curve. Using the absorbance value for each sample, determine the corresponding concentration of drug from the standard curve. Find the absorbance value on the Y-axis and extend a horizontal line to the curve. At the point of intersection, extend

a vertical line to the Xaxis and read the drug concentration of the unknown sample.

3. If computer data is going to be used, we recommend primarily "Four Parameter Logistic (4PL)" or secondly the "point-to-point calculation".
4. To obtain the exact values of the samples, the concentration determined from the standard-curve must be multiplied by the dilution factor (1000x). Any sample reading greater than the highest standard should be further diluted appropriately with assay buffer and retested. Therefore, if the pre-diluted samples have been further diluted, the concentration determined from the standard curve must be multiplied by the further dilution factor. e.g.; If the pre-diluted sample further diluted in a ratio of 1/5 then results should be multiplied by 5000.
5. For low and high level controls values, refer to "Quality Control Certificate" provided by each kit.

Typical Standard Curve

This is only an example. Assay conditions will change in every assay and do not use this curve for your assay calculations.



Precision

Intra-assay and inter-assay CVs <30%.

Sensitivity

The lowest detectable level (Lowest detection limit, LOD) that can be distinguished from the zero standard is 3 ng/mL.

Specificity

There is no cross reaction with native serum immunoglobulin

Recovery

Recovery <100±30%.

Precautions

1. For professional use only.
2. Do not use damaged components in test runs but keep safe for complaint related issues.
3. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
4. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood. For further information (clinical background, test performance, automation protocols, alternative applications, literature, etc.) please refer to the local distributor.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. All reagents of this kit containing human serum or plasma (standards etc.) have been tested and were found negative for HIV I/II, HBsAg and Anti-HCV. However, a presence of these or other infectious agents cannot be excluded absolutely and therefore reagents should be treated as potential biohazards in use and for disposal.
7. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MSDS and labels for details.
8. Chemicals and prepared or used reagents must be treated as hazardous waste according the national biohazard safety guidelines or regulations.