Version 13-12/24

User's Manual

Anti-Ramucirumab ELISA Kit

REF **DEIAZ0009**

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

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PRODUCT INFORMATION

Intended Use

Qualitative Antibodies to Ramucirumab ELSA has been especially developed for the qualitative analysis of antibodies to ramucirumab in serum and plasma samples.

General Description

Ramucirumab is a human monoclonal antibody (IgG1) against vascular endothelial growth factor receptor 2 (VEGFR2), a type II trans-membrane tyrosine kinase receptor expressed on endothelial cells. By binding to VEGFR2, ramucirumab prevents binding of its ligands (VEGF-A, VEGF-C, and VEGF-D), thereby preventing VEGF-stimulated receptor phosphorylation and downstream ligand-induced proliferation, permeability, and migration of human endothelial cells. VEGFR stimulation also mediates downstream signalling required for angiogenesis and is postulated to be heavily involved in cancer progression, making it a highly likely drug target. In contrast to other agents directed against VEGFR-2, ramucirumab binds a specific epitope on the extracellular domain of VEGFR-2, thereby blocking all VEGF ligands from binding to it.

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.

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. Controls and samples (serum or plasma) are incubated in the microtiter plate coated with the drug ramucirumab. After incubation, the wells are washed. Then, horse radish peroxidase (HRP) conjugated probe is added and binds

to ramucirumab antibodies captured by the drug ramucirumab on the surface of the wells. Following incubation wells are washed and the bound enzymatic activity is detected by addition of chromogen-substrate. Finally, the reaction is terminated with an acidic stop solution. The colour developed is proportional to the amount of ramucirumab antibodies in the sample or controls. The results can be evaluated with using cut-off value.

Reagents And Materials Provided

1. Microtiter Plate: 1 × 12 × 8

Break apart strips. Microtiter plate with 12 rows each of 8 wells coated with ramucirumab.

2. Controls 1.0 mL (negative); 1.0 mL (positive)

Ready to use. Contains human serum and stabilizer, <0.1% NaN_3.

3. Assay Buffer 1 × 50 mL

Ready to use. Blue coloured. Contains proteins, <0.1% NaN_3.

4. Conjugate 1 × 12 mL

Horse radish peroxidase conjugated probe. Ready to use. Red coloured. Contains HRP conjugated probe, stabilizer and preservatives.

5. Substrate 1 × 12 mL

TMB substrate solution Ready to use. Contains 3,3´,5,5´-Tetramethylbenzidine (TMB).

6. Stop Buffer 1 × 12 mL

TMB stop solution. Ready to use. 1N HCl.

7. Wash Buffer 1 × 50 mL

Prepared concentrated (20×) and should be diluted with the dilution rate given in the "Reagent Preparation" before the test. Contains buffer with tween 20.

8. Foil 2 × 1

Adhesive Foil. 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. Distiled 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).

Samples can be stored at 2-8°C for 2 days and at -20°C for 6 months

Dilution ofsamples

Sample: Serum/Plasma

Diluent: Assay buffer

Dilution Ratio: 1/10

Remarks: 1/10 dilution; 20 µl sample + 180 µl assay buffer

Patient samples with a concentration of drug above the measuring range are to be rated as > "Highest Standard (Standard A)", The result must not be extrapolated. The patient sample in question should be further diluted with assay buffer and retested

Reagent Preparation

Component	Wash buffer (must be prepared before starting assay procedure)
Dilute	10 mL (e.g.)
With	Up to 200 mL
Diluent	Distilled water
Dilution Ratio	1/20
Remarks	Warm up 37°C to dissolve crystals. Mix vigorously.
Storage	2-8°C
Stability	2 weeks

Assay Procedure

Total assay time: 140 minutes

1. Pipette 100 µL of each "Negative contro!", "Positive control" and diluted samples into the respective wells of microtiter plate.

   Wells

   A1: Negative control*

   B1: Negative control*

   C1: Positive control

   D1 and on: Samples

*It is advised to run more than one "Negative control" samples. Negative control studies can be duplicated or triplicated in order to take the mean value.

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 Butter". 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 20 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 450 nm 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 >1.000 and the OD 450/650 nm of each negative control should be <0.200.In case of any deviation the following technical issues (but not limited to) should be reviewed: Expiratior dates of reagents, storage conditions, pipettes, devices, incubation conditions washing methods, etc.

Calculation

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.

e.g.

If "Sample OD 450/650 / the mean negative control OD 450/650≥3" then the sample is POSITIVE

If"Sample OD 450/650 /the mean negative control OD 450/650<3" then the sample is NEGATIVE

Note: The cutoff information provided with this kit can only be considered as a recommendation. Cutoff values must be calculated/set or verified according to scientific standards by the users/laboratories.

Cut-off: Cut-off values must be calculated/set or verified according to scientific standards by the users/laboratories.

The "Quality control certificate" contains lot specifc analytical performance data and is supplied separately with each kit.

Precision

Intra-assay and inter-assay CVs <30%

Specificity

There is no cross reaction with native serum immunoglobulin

Precautions

1. For professional use only.
2. In case of severe damage of the kit package please contact Creative Diagnostics or your supplier in written form, latest one week after receiving the kit. 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 "Reagents And Materials Provided", 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.