Version 12-12/24

User's Manual

Anti-Certolizumab ELISA Kit

REF **DEIA-JY2148**

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.

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PRODUCT INFORMATION

Intended Use

Enzyme immunoassay for determination of quantitative antibodies to Certolizumab in serum and plasma samples.

General Description

Certolizumab pegol is a pegylated monoclonal antibody against the tumour necrosis factoralpha (TNF-alpha). It is formed with a humanized Fab fragment of 50 kDa, from an IgG 1 isotype, fused to a 40 kDa polyethylene glycol moiety replacing the Fc antibody region. The absence of the Fc region was ideated to prevent complement fixation and antibodymediated cytotoxicity as well as to markedly increase its half-life. Certolizumab does not require glycosylation for active function and hence, its production is significantly more affordable when compared to other existing TNF-alpha therapies as it can be done directly in bacterial hosts such as E. coli.

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 drug Certolizumab. After incubation, the wells are washed. Then, horse radish peroxidase (HRP) conjugated probe is added and binds to Certolizumab antibodies captured by the drug Certolizumab 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 color developed is proportional to the amount of Certolizumab antibodies in the sample or standard. Results of samples can be determined directly using the standard curve.

Reagents And Materials Provided

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

   Standard A: 160 ng/mL

   Standard B: 80 ng/mL

   Standard C: 40 ng/mL

   Standard D: 20 ng/mL

   Standard E: 10 ng/mL

   Standard F: 0 ng/mL

   Ready to use. Used for the standard curve and control. Contains antibodies to Certolizumab, human serum and stabilizer, < 0,1 % NaN_3.
3. Control low and high levels: 1 mL (each) Ready to use. Contains human serum and stabilizer, < 0,1 % NaN_3

Control concentrations are given in "Quality control certificate".

4. Assay Buffer: 1 × 50 mL, Ready to use. Blue colored. Contains proteins, < 0,1 % NaN_3.
5. Conjugate: 1 × 12 mL, Horse radish peroxidase. Conjugated probe ready to use. Red colored. Contains HRP conjugated probe, stabilizer and preservatives.
6. Substrate: 1 × 12 mL, TMB substrate solution. Ready to use. Contains 3,3´,5,5´- Tetramethylbenzidine (TMB).
7. Stop Buffer: 1 × 12 mL, Ready to use. 1N HCl.
8. Wash Buffer (20×): 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.
9. Foil: 2 × 1, 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 450 nm 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

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 freezethaw 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).

Stability (serum/plasma):

2-8°C, 2 days

-20°C, 6 months

Reagent Preparation

Procedure Notes:

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pre-treatment steps must be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25°C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each reagent, standard or specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. Use a pipetting scheme to verify an appropriate plate layout.
5. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an eight-channel micropipette for pipetting of solutions in all wells.
6. Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with wash buffer, and that there are no residues in the wells.
7. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.

Component:

Wash buffer (Must be prepared before starting assay procedure)

Dilute: 10 mL (e.g.)

With Distilled water: Up to 200 mL

Dilution Ratio: 1/20

Remarks: Warm up 37°C to dissolve crystals. Mix vigorously

Storage: 2-8°C

Stability: 2 weeks

Dilution of samples

Sample: Serum/Plasma

Diluent: Assay buffer

Dilution Ratio: 1/100

Remarks: Dilution 1/100,

5 µL sample + 495 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.

Preparation of confirmation test mixture

Sample: Serum/Plasma

Diluent: Confirmation Reagent

Dilution Ratio: 1/10

Remarks: 1/10 dilution

20 µL sample + 180 µL confirmation reagent.

Assay Procedure

Total assay time: 140 minutes

1. Pipette 100 µL of each "Standards", "Low level control","High level control" and diluted 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

*It is advised to run more than one "Standard F (negative control)" samplesfor qualitative assay. 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 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 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 color 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"

The ELISA is also suitable to run on automated ELISA processors.

Confirmation test for positive samples*

Prepare confirmation test mixture as described in "Reagent Preparation"

Incubate this mixture for 60 minutes in a microtube

After the incubation proceed the test procedure from step one given above by pipetting 100 µL of this solution to respective well

Instructions are followed givenin the test procedure in table

*It is recommended to run only positive samples for confirmation testing. Negative samples may give improper results in confirmation step.

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 a valid study, the OD 450/650 of the highest standard should be > 1.000 and the OD 450/650 of the lowest standard should be < 0.200.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 X-axis 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 (10×). 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 1/10 then results should be multiplied by 100.

5. For low and high level controls values, refer to "Quality Control Certificate" provided by each kit.

Interpretation Of Results

Interpretation of true and false positives

(OD 450/650 sample – OD 450/650 sample with confirmation reagent) / OD 450/650 sample × 100% = inhibition%

If the inhibition is ≥ 25% then the sample is "true positive".

e.g.: If the OD 450/650 of the tested sample is 0.800 and after incubation of the sample with confirmation reagent and retested and the OD 450/650 of the sample is 0.200, then:

$(0.800 - 0.200 / 0.800) \times 100\% = 75\%$

(the sample is "true positive" for anti − drug antibody)

Qualitative interpretation

The results are evaluated by a cut-off value which is estimated by multiplying the mean OD 450/650 nm of the "standard F" by 3.

e.g. If "Sample OD 450/650 / the mean "standard F" OD 450/650 ≥ 3"

then the sample is POSITIVE

If "Sample OD 450/650 / the mean "standard F" OD 450/650 < 3"

then the sample is NEGATIVE

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 scientificstandards by the users/laboratories.

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 5 ng/mL

Functional sensitivity (Limit of quantification-LOQ): 10 ng/mL

Specificity

There is no cross reaction with native serum immunoglobulin

Recovery

< 100 ± 30%

Precautions

1. For professional use only.
2. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
3. 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.
4. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
5. 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.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations.
7. Chemicals and prepared or used reagents must be treated as hazardous waste according the national biohazard safety guidelines or regulations.