Version 21-04/24

User's Manual

Anti-Adalimumab ELISA Kit

REF **DEIABL201**

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

Qualitative Antibodies to Adalimumab ELISA has been especially developed for the qualitative analysis of antibodies to adalimumab in serum and plasma samples.

General Description

Adalimumab binds with specificity to tumour necrosis factor-alpha (TNF-alpha) and inhibits its interaction with the p55 and p75 cell surface TNF receptors. Adalimumab also lyses surface tumour necrosis factor expressing cells in vitro when in the presence of complement. Adalimumab does not bind or inactivate lymphotoxin (Tumour necrosis factorbeta). TNF is a naturally occurring cytokine that plays a role in normal inflammatory and immune responses. Increased levels of TNF are found in the joint synovial fluid of rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis patients, and play an imperative role in pathologic inflammation and the joint destruction that are major complications of these diseases. Increased levels of TNF are also measured in psoriasis plaques. In plaque psoriasis, treatment with adalimumab may decrease the epidermal thickness and inflammatory cell infiltration. The relationship between this pharmacodynamics and the mechanism(s) by which adalimumab achieves its clinical effects is not known. Additionally, adalimumab alters biological responses that are induced/regulated by TNF, including changes in the levels of adhesion molecules responsible for leukocyte migration during inflammation (ELAM-1, VCAM-1, and ICAM-1 with an IC50 of 1-2 X 10-10M).

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 adalimumab. After incubation, the wells are washed. Then, horse radish peroxidase (HRP) conjugated probe is added and binds to adalimumab antibodies captured by the drug adalimumab 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 adalimumab antibodies in the sample or controls. The results can be evaluated with using cut-off value.

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. Controls (Negative 1.0 mL, Positive 0.3 mL): Ready to use. Contains human serum and stabilizer, <0,1% NaN3.
3. Assay Buffer (1x12 mL): Ready to use. Blue coloured. Contains proteins, <0,1% NaN3.
4. Conjugate (1x12 mL): Ready to use. Red coloured. Contains HRP conjugated probe, stabilizer and preservatives.
5. Substrate (1x12 mL): Ready to use. Contains 3,3´,5,5´- Tetramethylbenzidine (TMB).

6. Stop Buffer (1x12 mL): Ready to use. 1N HCl.
7. 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.
8. 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.

Assay Procedure

1. Pipette 100 µL "Assay Buffer" into each of the wells to be used.
2. Pipette 10 µL of each "Negative control", "Positve control" and samples into the respective wells of microtiter plate.

Wells

A1: Negative control

B1: Negative control

C1: Positive control

D1 and on: Sampels

3. Cover the plate with adhesive foil. Briefly mix contents by gently shaking the plate. Incubate 60 minutes at room temperature (18-25°C).
4. 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.
5. Pipette 100 µL "Conjugate" into each well.
6. Cover the plate with adhesive foil. Incubate 60 minutes at room temperature (18-25°C).
7. 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.
8. Pipette 100 µL "Substrate" into each well.
9. Incubate 20 minutes without adhesive foil at room temperature (18-25°C) in the dark.
10. 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.
11. 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.

Interpretation Of Results

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 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/laboratories.

Precision

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

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.