



User's Manual

Anti-Trastuzumab ELISA Kit

REF DEIABL220

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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 Trastuzumab ELISA has been especially developed for the qualitative analysis of antibodies to trastuzumab in serum and plasma samples.

General Description

Trastuzumab is a recombinant humanized IgG1 monoclonal antibody against the HER-2 receptor, a member of the epidermal growth factor receptors which is a proto-oncogene. Over-expressed in breast tumour cells, HER-2 overamplifies the signal provided by other receptors of the HER family by forming heterodimers. The HER-2 receptor is a transmembrane tyrosine kinase receptor that consists of an extracellular ligand-binding domain, a transmembrane region, and an intracellular or cytoplasmic tyrosine kinase domain. It is activated by the formation of homodimers or heterodimers with other EGFR proteins, leading to dimerization and autophosphorylation and/or transphosphorylation of specific tyrosine residues in EGFR intracellular domains. Further downstream molecular signalling cascades are activated, such as the Ras/Raf/mitogen-activated protein kinase (MAPK), the phosphoinositide 3-kinase/Akt, and the phospholipase C γ (PLC γ)/protein kinase C (PKC) pathways that promote cell growth and survival and cell cycle progression. Due to upregulation of HER-2 in tumour cells, hyperactivation of these signalling pathways and abnormal cell proliferation is observed. Trastuzumab binds to the extracellular ligand-binding domain and blocks the cleavage of the extracellular domain of HER-2 to induce its antibody-induced receptor downmodulation, and subsequently inhibits HER-2-mediated intracellular signalling cascades. Inhibition of MAPK and PI3K/Akt pathways lead to an increase in cell cycle arrest, and the suppression of cell growth and proliferation. Trastuzumab also mediates the activation of antibody-dependent cell-mediated cytotoxicity (ADCC) by attracting the immune cells, such as natural killer (NK) cells, to tumour sites that overexpress HER-2. Intrinsic trastuzumab resistance has been noted for some patients with HER-2 positive breast cancer. Mechanisms involving trastuzumab resistance include deficiency of phosphatase and tensin homologue and activation of phosphoinositide 3-kinase, and the overexpression of other surface receptors, such as insulin-like growth factor.

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 trastuzumab. After incubation, the wells are washed. Then, horse radish peroxidase (HRP) conjugated probe is added and binds to trastuzumab antibodies captured by the drug trastuzumab 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 trastuzumab 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 1.0mL, Positive 0.3mL): 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", "Positive 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 $<100\pm 30\%$.

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.