MATRIKS BIOTEK
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"trace & catch"

Instructions for Use

Trastuzumab

(Herclon®, Herceptin®) ELISA

SHIKARI®

Q-TRAS

Enzyme immunoassay for the quantitative determination of Trastuzumab (Herceptin®, Herclon®)in serum and plasma

REF **TR-TRASv2** 12 x8 2-8°C

Revision # 2.1 August 2017

CE
IVD

Matriks Biotek® Laboratories
www.matriksbiotek.com

Intended Use

Enzyme immunoassay for the quantitative determination of **free Trastuzumab** (Herclon®, Herceptin®) in serum and plasma. *Matriks Biotek® trastuzumab ELISA* has been developed for the quantitative analysis of free trastuzumab in serum and plasma samples at high specificity.

Summary and Explanation

The HER (or ErbB) family of transmembrane tyrosine kinase receptors is composed of four members, HER1 to HER4 . HER2, a ligand-less Mr 185,000 receptor encoded by the neu proto-oncogene, is overexpressed in 25–30% of human breast cancer and has been associated with enhanced tumor aggressiveness and a high risk of relapse and death. Recent evidence indicates that HER2 amplifies the signal provided by other receptors of the ErbB family by heterodimerizing with them. The important biological role of HER2 in the signaling network that drives epithelial cell proliferation and transformation, together with its extracellular accessibility and its overexpression in some human tumors led to considering HER2 as an appropriate target for tumor-specific therapies.

The neu gene encodes a 185-kDa transmembrane glycoprotein, referred to as p185neu, HER2, or erbB-2, possessing intrinsic protein tyrosine kinase activity. The receptor consists of an extracellular domain, with four subdomains including two cysteine rich domains, a transmembrane domain, and an intracellular domain, consisting of a juxtamembrane region, a tyrosine kinase domain, and a carboxyl tail harboring autophosphorylation sites HER2 is homologous to, but distinct from, other members of the erbB family, which includes the epidermal growth factor receptor (EGFR or erbB-1), erbB-3, and erbB-4. The binding of cognate growth factors to these receptors regulates cell growth, proliferation, and diferentiation through the activation of receptor tyrosine kinases, triggering an incompletely defined signal transduction cascade. Signal transduction by these receptors is believed to involve dimerization and oligomerization, both in the form of homo-oligomers and hetero-oligomers in various erbB receptor combinations.

Trastuzumab (Herclon®, Herceptin®) is a recombinant DNA-derived humanized monoclonal antibody that selectively targets the extracellular domain of the human epidermal growth factor receptor 2 protein (HER2). The antibody is an IgG1 kappa that contains human framework regions with the complementarity-determining regions of a murine anti-p185 HER2 antibody that binds to HER2. Trastuzumab is composed of 1,328 amino acids and has a molecular weight of 148 kDa.

Trastuzumab has antitumor activity against HER2-positive human breast tumor cells in laboratory models and is active for the treatment of women with HER2-overexpressing breast cancers. On the basis of trastuzumab clinical efficacy, this antibody was approved in 1998 for clinical use for HER2 overexpressing metastatic breast cancer. Trastuzumab seems to exert its antitumor effects by several mechanisms that are not yet completely understood. In HER2 overexpressing cells, trastuzumab markedly down-regulates HER2 expression by accelerating receptor endocytosis and degradation and inhibits cell cycle progression by inducing the formation of p27Kip1/Cdk2 complexes. Trastuzumab also induces antibody-dependent cell-mediated cytotoxicity against the HER2 expressing tumor cells in animal models. This process is regulated by antibody receptors FcγRIII and FcγRIIB on myeloid cells. Other additional mechanisms that have been proposed include suppression by trastuzumab of angiogenesis and metastasis.

The pharmacokinetics of trastuzumab according to the prescribing data; The pharmacokinetics of trastuzumab were studied in women with metastatic breast cancer. Short duration intravenous infusions of 10 to 500 mg trastuzumab once weekly demonstrated dose-pendent pharmacokinetics. Mean half-life increased and clearance decreased with increasing dose level. The half-life averaged 2 and 12 days at the 10 and 500 mg dose levels, respectively. The volume of distribution of trastuzumab was approximately that of serum volume (44 mL/kg). At the highest weekly dose studied (500 mg), mean peak serum concentrations were 377 mcg/mL. In studies using an initial dose of 4 mg/kg followed by a weekly dose of 2 mg/kg, a mean half-life of 6 days (range 1-32 days) was observed. Between weeks 16 and 32, trastuzumab serum concentrations reached a steady state with mean trough and peak concentrations of approximately 9 mcg/mL and 123 mcg/mL, respectively.

In a study of women receiving adjuvant therapy for breast cancer, a mean half-life of trastuzumab of 16 days (range: 11-23 days) was observed after an initial dose of 8 mg/kg followed by a dose of 6 mg/kg every three weeks. Between weeks 6 and 37, trastuzumab serum concentrations reached a steady-state with mean trough and peak concentrations of 63 mcg/mL and 216 mcg/mL, respectively.

In this context, identification of biomarkers for (non-)response and risk factors for adverse drug reactions relating to serum concentrations and therapeutic drug monitoring of trastzumab might be very helpful.

Test Principle

Solid phase enzyme-linked immunosorbent assay (ELISA). Standards and samples (serum or plasma) are incubated in the microtitre plate coated with ligand antigen

(recombinant human HER2/ErbB2/CD340) for trastuzumab (Herclon®, Herceptin®). After incubation, the wells are washed. In the next step, a horse radish peroxidase (HRP) conjugated is added. Following incubation, wells are washed and the bound enzymatic activity is detected by addition of TMB chromogen-substrate. The colour developed is proportional to the amount of trastuzumab in the sample or standard. Results of samples are determined using the standard curve constructed.

Warnings and Precautions

1. For professional use only.

2. 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.

3. In case of severe damage of the kit package please contact Matriks Biotek® 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.

4. Obey lot number and expiry date. Do not mix reagents of different lots.

5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.

6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details.

7. Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guidelines or regulations.

8. Avoid contact with Stop solution. It may cause skin irritations and burns.

9. Some reagents contain sodium azide (NaN_3) as preservatives. In case of contact with eyes or skin, flush immediately with water. NaN_3 may react with lead and copper plumbing to form explosive metal azides. When disposing reagents, flush with large volume of water to avoid azide build-up.

10. All reagents of this test kit containing human serum or plasma have been tested and were found negative for HIV I/II, HBsAg and HCV by FDA approved procedures. 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.

Storage and Stability

The kit is shipped at ambient temperature and should be stored at 2-8°C. Keep away from heat or direct sun light. The storage and stability of specimen and prepared reagents is stated in the corresponding chapters.

Specimen Collection and Storage

Serum, Plasma (EDTA, Heparin)*

The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material.

Storage:	2-8°C	-20°C	Keep away from heat or direct sun light
Stability:	2 d	6 mon	Avoid repeated freeze-thaw cycles

*Trastuzumab (Herclon®,Herceptin®) infusion camouflages/masks the presence of antibody to trastuzumab in serum/plasma samples. Therefore, blood sampling time is critical for detection of TRAS. The Matriks Biotek® Laboratories suggests to obtain blood sample just before the infusion of Trastuzumab (Herclon®,Herceptin®) or at least 2 weeks after the infusion of Trastuzumab (Herclon®,Herceptin®).

Materials Supplied

1 x 12 x 8	MTP	**Microtiter Plate** Break apart strips. Microtiter plate with 12 rows each of 8 wells precoated with recombinant human HER2/ErbB2/CD340.
7 x 0.3 mL	STND A-E HIGH CNTRL LOW CNTRL	**Trastuzumab Standards A-E, Concentrate (10X),High Level Control, Low Level Control** 3000; 1000; 333; 111; 0 nanogram/mL. Ready to use. Contains trastuzumab, human serum, stabilizers and <0.1% NaN_3.
1 x 30 mL	ASSAY BUF	**Assay Buffer, Concentrate (5X)** Blue colored. Ready to use. Contains proteins and <0.1% NaN_3.
1 x 12 mL	HRP CONJ	**Horse radish peroxidase-Conjugated Probe** Red colored. Ready to use. Contains HRP-conjugated preservatives and stabilizers.
1 x 12 mL	TMB SUBS	**TMB Substrate Solution** Ready to use. Contains TMB
1 x 12 mL	TMB STOP	**TMB Stop Solution** Ready to use. 1N HCl.
1 x 50 mL	WASHBUF CONC	**Wash Buffer, Concentrate (20x)** Contains Buffer with Tween 20.
2 x 1	ADH FILM	**Adhesive Film** For sealing of Microtiter Plate during incubation
2 x 1	SLGP	**Semi-Log Graph Paper** For constructing standard curve and calculation of results.

Materials Required But Not Supplied

1. Micropipettes (< 3% CV) and tips to deliver 5-1000 µL.
2. Calibrated measures.
3. Tubes (1 mL) for sample dilution.
4. Wash bottle, automated or semi-automated microtiter plate washing system
5. Microtiter plate reader capable of reading absorbance at 450/650 nm.
6. Bidistilled or deionised water, paper towels, pipette tips and timer.

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 pretreatment steps have to 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 8-channel Micropipettor 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 multchannel pipete or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiraton. Rinse and fll all reagents with care. While rinsing, check that all wells are flled 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.

Pre-test Setup Instructons

Preparaton of Components

Dilute/ disolve	Component	With	Diluent	Relation	Remarks	Storage	Stability
10 mL	Wash Buffer*	Up to 200 mL	bidist. Water	1:20	Warm up at 37°C to dissolve crystals. Mix vigorously.	2-8 °C	2 w
10 mL	Assay Buffer*	Up to 50 mL	bidist. Water	1:5		2-8 °C	2 w

*****Prepare Wash Buffer and Assay Buffer before starting assay procedure.

Dilution of Standards and Samples (serum/plasma)*

Sample	To be diluted	With	Remarks
Standards	1:10	Assay Buffer	For dilution at 1:10; 20 µl Standard + 180 µl Assay Buffer
Serum/Plasma	1:1000	Assay Buffer	First; for dilution at 1:10; 10 µl Standard + 90 µl Assay Buffer Second; for dilution at 1:100; 5 µl Standard + 495 µl Assay Buffer

Patient samples with a concentration of trastuzumab 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.

Calculation & Interpretation of Results

1. Using the diluted standards (300; 100; 33; 11; 0 ng/mL) disregarding zero standard, construct a standard curve by plotting the OD450/650 nm for each of 4 standards on the vertical (Y-axis) axis versus the corresponding trastuzumab concentration on the horizontal (X-axis) axis, thus creating a standard curve by 4 points obtained.

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 trastuzumab 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 trastuzumab concentration for the unknown sample.

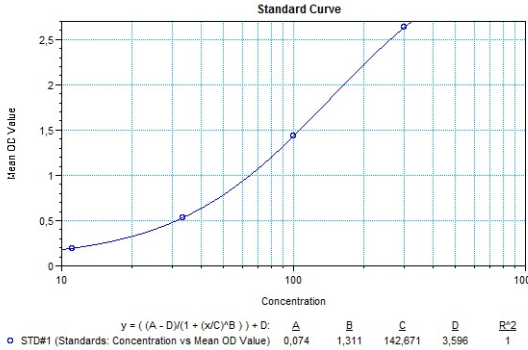
3. If computer data regation is going to be used, we recommend primarily "4 Parameter Logistic (4PL)" or secondly the "point-to-point calculation".

4. To obtain the exact values of the samples, the concentration determined from the standard-curve must be multiplied by the dilution factor (10x). 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 determind 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. Automated method: Computer programs can also generally give a good fit.

Typical Calibration Curve
(Example. Do not use for calculation!)



Standard	Concentration (ng/mL)	Mean OD 450/650
A	300	2,631
B	100	1,432
C	33	0,530
D	11	0,194
E	0	0,032

Assay Characteristics

1. **Specificity:** Except for trastuzumab, there is no cross reaction with other therapeutic antibodies and native serum immunoglobins.
2. **Sensitivity:** The lowest detectable level that can be specifically distinguished from the zero standard is 111 pg/mL.
3. **Precision Of Kit:**
   Intra-assay CV: <15% for trastuzumab range 300-11 ng/mL.
   Inter-assay CV: <15% for trastuzumab range 300-11 ng/mL.
4. **Recovery:** Recovery rate was found to be between 85-115% with native human serum spiked with Cmax (123µgr/mL) and Cmin (27mgr/mL) values of Trastuzumab.

Automation

Experiments have shown that the *Matriks Biotek®* SHIKARI® Trastuzumab ELISA is also suitable to run on an automated ELISA processor.

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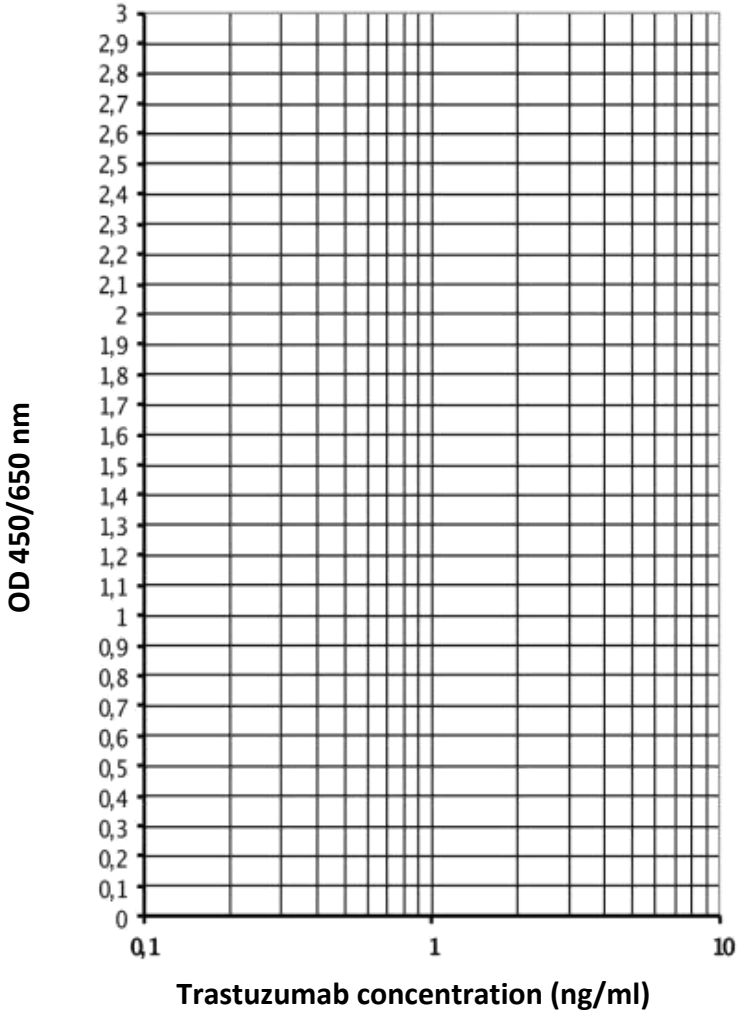
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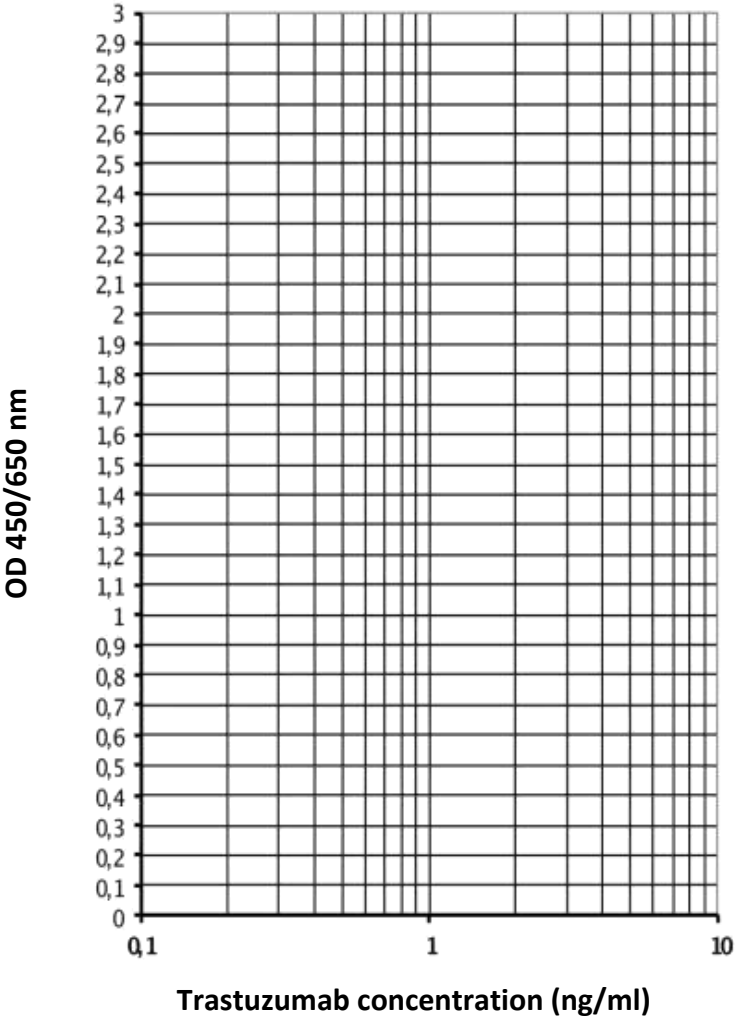
0,1
1
10
Trastuzumab concentration (ng/ml)


OD 450/650 nm
3
2,9
2,8
2,7
2,6
2,5
2,4
2,3
2,2
2,1
2
1,9
1,8
1,7
1,6
1,5
1,4
1,3
1,2
1,1
1
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
0,1
1
10
Trastuzumab concentration (ng/ml)