Taiclone®
Quality Guarantee

USER MANUAL



ELISA KIT

Trastuzumab(Herceptin®)
Pharmacokinetic ELISA Kit

Catalog No.tcae2182 96 Tests

FOR RESEACH USE ONLY!

Please read completely user manual and storage condition.

Web: www.taiclone.com
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Trastuzumab(Herceptin®) Pharmacokinetic ELISA Kit
Catalog No.tcae2182

Available Sizes

Size: 96 Tests

Specifications

Research Area:	Cancer Reseach
Species Reactivity:	Human
Sample Type:	Serum, plasma and other biological fluids
Sensitivity:	hIgG1, Rituximab, and Infliximab prepared at 250 ng/mL were assayed and exhibited no cross-reactivity or interference.
Recovery:	1000ng/mL of Trastuzumab was spiked in 10 lots of human serum. Recovery ranges are from 91-117% with an average recovery of 106%.
Detection Limit:	1.25ng/ml
Assay Time:	3h
Detection Method:	Colorimetric; absorbance at 450 nm with reference absorbance at 620 nm
Precision:	The precision was determined by analyzing samples prepared at 1000 ng/mL in 6 replicates on 6 different occasions. Intra-assay coefficient of variation (CV) <10%. Inter-assay CV <10%.

Introduction

Trastuzumab (Herceptin®) is a humanized monoclonal antibody used in the treatment of HER2-positive cancers. Trastuzumab binds to domain IV of the extracellular domain of the HER2/neu receptor. Cells treated with Trastuzumab undergo cell cyle arrest during the G1 phase. It has been suggested that trastuzumab does not alter HER-2 expression, but decreases activation of AKT.
The Trastuzumab ELISA kit is designed to measure Trastuzumab with high specificity and enhanced sensitivity. The assay design utilizes a pair of antibodies that allows the detection of the whole Trastuzumab molecule in biological matrices.

Test Principle

This assay employs the sandwich enzyme immunoassay technique. Anti- Trastuzumab is coated onto a 96 well microplate. Calibrator, quality control samples (if desired) and test samples are pipetted into the appropriate wells. Trastuzumab present in biological matrices is bound by the immobilized anti- Trastuzumab antibody. After washing away any unbound substances, enzyme linked antiTrastuzumab antibody is added to the wells. The plate is washed to remove any unbound antibody-enzyme reagent and a substrate solution is added to the wells for color development. The color development is proportional to the amount of Trastuzumab present in test samples. The color development is stopped and the intensity of the color is measured.

All products are for RESEARCH USE ONLY. Not for diagnostic & therapeutic purposes!

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Materials and Storage.

Store kit components at -20 °C unless specified otherwise.
DO NOT USE past kit expiration date. Some vials contain a small amount of reagents.
Spin tubeson pulse setting prior to opening

Components

Each kit includes:	Units
Coated microtiter plate, 96 wells (1x8 strips).	1
Calibrator diluent.	1.8ml
Calibrator (1000 µg/mL)	12µl
10X wash buffer	25ml
Assay buffer	50ml
1000X detection reagent	17µl
TMB	12ml
TMB stop solution	12ml
Do not mix or substitute reagents with those from other lots.	

Materials and instruments required but not supplied

- Precision pipettes calibrated to deliver 5-1000µL
- Multi-channel pipette calibrated to deliver 50-250µL
- Plate shaker
- Disposable tips
- Vortex-Mixer
- Distilled or de-ionized water
- Microplate reader capable of reading 450nm with background subtraction at 620nm.

Safety Precautions

- The test protocol must be followed strictly.
- All reagents containing human material should be handled as if potentially infectious. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.
- The kit reagents contain antimicrobial agents, acid and 3,3',5,5'-tetramethylbenzidine. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
- Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Disposal must be performed in accordance with local regulations.
- Disposal must be performed in accordance with local regulations.
- Only trained laboratory personnel should execute this test.

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Preparation of Reagents.

Prepare only the appropriate amount of required reagent on the day of use. Store all reagents as per instructions stated on the label.

1. Wash Buffer (1X) Preparation: Dilute wash buffer concentrate with ultra-pure water 1/10 before use (for example add 10mL concentrate to 90mL ultra-pure water). Mix well.
2. Detection Reagent (1X) Preparation: Dilute detection reagent with assay buffer 1/1000 beforeuse (for example add 10µl concentrate to 10ml of assay buffer). Mix welll.
3. Preparation of Calibrators: Prepare calibrators with concentrations ranging from 2000 ng/mL to 62.5 ng/mL.

The following is an example calibrator curve.

Sol'n ID	Source	Source Vol (µL)	Cal* Diluent (µL)	Final Vol (µL)	Final Concen-tration (ng/mL)
1**	Stock Cal* (1000µg/mL)	5	995	1000	5,000
1*	Inter-mediate 1**	40	60	100	2,000
2*	1*	50	50	100	1,000
3*	2*	50	50	100	500
4*	3*	50	50	100	250
5*	4*	50	50	100	125
6*	5*	50	50	100	62.5
7*	-	-	100	100	0
*Calibrator **Intermediate					

Specimen Storage

This kit is compatible with EDTA-plasma, heparin plasma and serum samples. Samples can be stored at or below -20°C for up to 1 year.

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Assay Procedures.

1. Remove coated microtiter plate from -20ºC and allow it to acclimate to room temperature for 15-20 minutes.
2. Dilute calibrators and test samples 1/50 with assay buffer (for example add 5µL of prepared calibrator or sample to 245µL of assay buffer). Mix well. Do not store diluted samples.
3. Add 100µL diluted calibrators and samples to appropriate wells on the plate. Incubate for 1 hour at room temperature on a plate shaker at approx 300rpm.
4. Discard the content of the plate and wash the wells 3x with 200µL wash buffer per well.
5. Add 100µL detection reagent to appropriate wells on the plate. Incubate for 1 hour at room temperature on a plate shaker at approx 300rpm.
6. Discard the content of the plate and wash the wells 3x with 200µL wash buffer per well.
7. Add 100µL of TMB to each well on plate. Incubate for 6-10 minutes at room temperature protected from light. Alternatively stop reaction when a strong signal has developed in the highest concentration positive control if not using the supplied calibrator.
8. Add 100µL of TMB stop solution to each well on plate.
   Mix by gently tapping the side of the plate.
9. Determine absorbance with a microplate reader at 450nm against 620nm.

Calculations and result.

1. Construct a standard curve by plotting the absorbance obtained from each standard against concentration. Use a 4 or 5 parameter curve fit. Alternatively a log-log curve fit may be used.
2. The concentration of the unknowns can be read directly from this standard curve using the absorbance value for each sample.
3. Any sample undiluted or diluted still reading greater than the highest standard should be diluted appropriately with calibrator diluent and retested.
   If the samples have been diluted, the concentration determined from the standard curve must be multiplied by the dilution factor.

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Performance Characteristics.

Precision: The precision was determined by analyzing samples prepared at 1000 ng/mL in 6 replicates on 6 different occasions. Intra-assay coefficient of variation (CV) <10%. Inter-assay CV <10%.

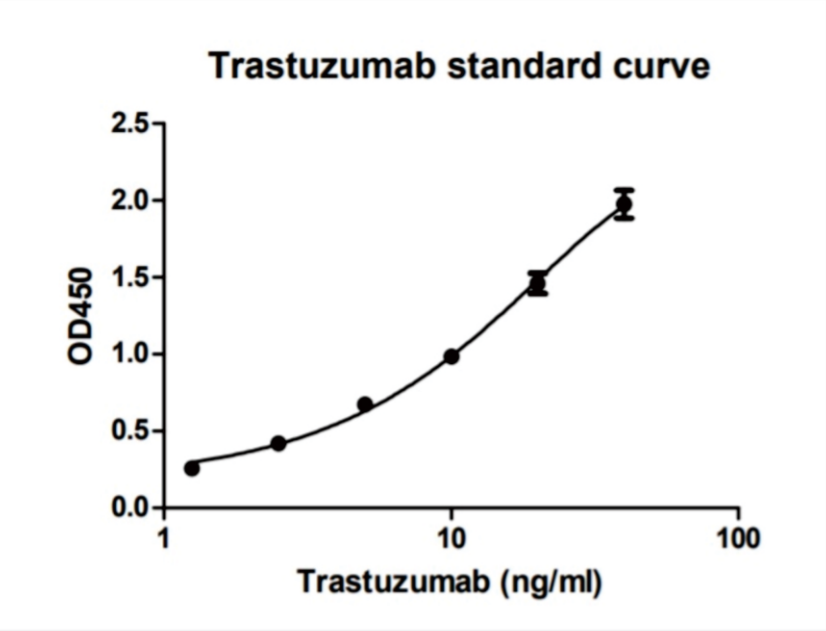
Detection Limit: The detection limit is 1.25 ng/mL.

Recovery: 1000ng/mL of Trastuzumab was spiked in 10 lots of human serum. Recovery ranges are from 91-117% with an average recovery of 106%.

Specificity: hIgG1, Rituximab, and Infliximab prepared at 250 ng/mL were assayed and exhibited no cross-reactivity or interference.

Sample Standard Curve:

The standard curve below was generated with the supplied calibrator using the recommended conditions and diluted 1 in 50 to give a final concentration range of 40ng/ml to 1.25ng/ml. Each sample was run with 6 replicates. Intra-assay coefficient of variance is <10%



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