

# BioSim™ Trastuzumab(Herceptin®)(Human) ELISA Kit rev 12/18

(Catalog # E4376-100, 100 assays, Store at 4°C)

## I. Introduction:

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). 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. This antibody was approved in 1998 for clinical use for HER2 overexpressing metastatic breast cancer. 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. BioSim™ Trastuzumab ELISA kit has been developed for specific quantification of Trastuzumab concentration in human serum or plasma with high sensitivity and reproducibility.

## II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Trastuzumab.

Detection Range: 11 - 300 ng/ml

Sensitivity: Quantitative limit - 11 ng/ml, Detection limit - 2 ng/ml

Assay Precision: Intra-Assay: CV < 15%; Inter-Assay: CV < 15% (CV (%) = SD/mean X 100)

Recovery rate: 85 – 115% with normal human serum samples with known concentrations

Cross Reactivity: No significant cross-reactivity or interference with other proteins present in native human serum or other therapeutic immunoglobulins.

## III. Sample Type:

Human serum and plasma

## IV. Kit Contents:

Components	E4376-100	Part No.
Micro ELISA Plate	1 plate	E4376-100-1
Trastuzumab Standards (S1 – S7)	0.3 ml X 7	E4376-100-2.x
Assay Buffer (5X)	25 ml	E4376-100-3
HRP-conjugate Probe	12 ml	E4376-100-4
TMB substrate (Avoid light)	12 ml	E4376-100-5
Stop Solution	12 ml	E4376-100-6
Wash buffer (20X)	50 ml	E4376-100-7
Plate sealers	2	E4376-100-8

## V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- Calibrated measures
- Precision pipettes with disposable tips
- Clean eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

## VI. Storage and Handling:

The entire kit may be stored at 4°C for up to 12 months from the date of shipment.

## VII. Reagent and Sample Preparation:

Note: Prepare reagents within 30 minutes before the experiment.

Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Assay Buffer:** Dilute 5X assay buffer to 1X in ddH<sub>2</sub>O (25 ml of Assay Buffer stock to 100 ml of ddH<sub>2</sub>O)
2. **Wash Buffer:** Dilute the 20X Wash Buffer to 1X solution in ddH<sub>2</sub>O (10 ml of Wash Buffer stock to 190 ml of ddH<sub>2</sub>O). Mix the 1X solution thoroughly by vortex manually. The working stock can be stable for 2 weeks after preparation at 4°C.A
3. **Standard Preparation:**

Dilute 10X stock with Assay Buffer. (20 µl Standards + 180 µl Assay Buffer)

Name	S1	S2	S3	S4	S5	S6	S7
Conc. (ng/ml)	3000	1000	333	111	0	High Control	Low Control
Working Conc. (ng/ml)	300	100	33	11	0	-	-

**FOR RESEARCH USE ONLY! Not to be used on humans.**

#### 4. Sample Dilution:

- **Serum/Plasma:** Initially dilute samples 1:10 (20  $\mu$ l Serum/Plasma+ 180  $\mu$ l Assay Buffer). Then Dilute another 100X (5  $\mu$ l Standard + 495  $\mu$ l Assay Buffer) to a total of 1:1000 dilution.
- Diluted samples should further be diluted if the concentration of Trastuzumab is higher than the measuring range.
- The usual precautions for venipuncture should be observed. Samples are stable at 4°C for 2 days and -20°C for 6 months. Avoid freeze-and-thaw cycle.

#### VIII. Assay Protocol:

**Note:** Bring all reagents, microplate and samples to room temperature 15 minutes prior to the assay.

It is recommended that all standards and samples be run at least in duplicate.

A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in section VII.
2. Add 100  $\mu$ l of **standards** and **diluted-samples** into appropriate wells. Cover wells and incubate for 30 minutes at room temperature (RT).
3. Discard incubation solution. Wash plate 3 times each with 300  $\mu$ l of diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
4. Add 100  $\mu$ l of **HRP-conjugate** into each well. Cover wells with adhesive plate sealer and incubate at RT for 30 minutes.
5. Discard the solution and wash the wells as step 3.
6. Add 100  $\mu$ l of 1X **TMB substrate** solution and incubate the plate in dark at RT for 10 minutes
7. Add 100  $\mu$ l of **Stop solution** to stop the reaction
8. Read the absorbance in micro plate reader set to 450 nm within 20 minutes. (reference wavelength to 650 nm)

#### IX. CALCULATION:

Using the standards disregarding zero standard, construct a standard curve by plotting the OD<sub>450/650 nm</sub> for each of 4 standards on the Y-axis versus the corresponding Trastuzumab concentration on the X-axis. Construct a standard curve of difference data using software capable of generating four parameter logistic (4PL) or point-to-point calculation curve fit. To obtain the exact values of the samples, the concentration determined from the standard-curve should be multiplied by the dilution factor.



**Figure:** Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

#### X. RELATED PRODUCTS:

- BioSim™ Rituximab (Mabthera®) (Human) ELISA Kit (Cat. No. E4371-100)
- BioSim™ Adalimumab (Humira®) (Human) ELISA Kit (Cat. No. E4372-100)
- BioSim™ Bevacizumab (Avastin®) (Human) ELISA Kit (Cat. No. E4373-100)
- BioSim™ Etanercept (Enbrel®) (Human) ELISA Kit (Cat. No. E4374-100)
- BioSim™ Ipilimumab (Remicade®) (Human) ELISA Kit (Cat. No. E4375-100)
- BioSim™ Trastuzumab(Herceptin®)(Human) ELISA Kit (Cat. No. E4376-100)
- BioSim™ Golimumab (Simponi®)(Human) ELISA Kit (Cat. No. E4377-100)
- BioSim™ Infliximab (Remsima®)(Human) ELISA Kit (Cat. No. E4378-100)
- BioSim™ Cetuximab (Erbitux®)(Human) ELISA Kit (Cat. No. E4379-100)
- BioSim™ Denosumab (Prolia®)(Human) ELISA Kit (Cat. No. E4380-100)
- BioSim™ Omalizumab (Xolair®)(Human) ELISA Kit (Cat. No. E4381-100)
- BioSim™ Nivolumab (Opdivo®)(Human) ELISA Kit (Cat. No. E4382-100)
- BioSim™ Pembrolizumab (Keytruda®)(Human) ELISA Kit (Cat. No. E4383-100)
- BioSim™ Ipilimumab (Yervoy®)(Human) ELISA Kit (Cat. No. E4384-100)

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