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BioSim™ anti- HER2 (Human) ELISA Kit

rev *06/20*

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

I. Introduction:

Anti-HER2 is a recombinant DNA-derived humanized monoclonal antibody that selectively targets the extracellular domain of the human epidermal growth factor receptor 2 protein (HER2). It 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, anti- HER2 antibody 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™ anti-HER2 ELISA kit has been developed for specific quantification of anti-HER2 antibody concentration in human serum or plasma with high sensitivity and reproducibility.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of anti- HER2 antibody
Detection Range: 10 - 300 ng/ml
Sensitivity: Quantitative limit - 10 ng/ml, Detection limit - 2 ng/ml
Assay Precision: Intra-Assay: CV < 30%; Inter-Assay: CV < 30% (CV (%) = SD/mean X 100)
Recovery rate: <100±30% 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
anti- HER2 Standards (S1 – S7)	0.3 ml X 7	E4376-100-2.x
Assay Buffer	2 X 50 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_2O (25 ml of Assay Buffer stock to 100 ml of ddH_2O)
2. **Wash Buffer**: Dilute the 20X Wash Buffer to 1X solution in ddH_2O (10 ml of Wash Buffer stock to 190 ml of ddH_2O). Mix the 1X solution thoroughly by vortex manually. The working stock can be stable for 2 weeks after preparation at 4°C.
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	300	100	0	High Control	Low Control
Working Conc. (ng/ml)	300	100	30	10	0	-	-

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

4. **Sample Dilution:**
   - **Serum/Plasma**: Initially dilute samples 1:10 (10 µl Serum/Plasma+ 90 µl Assay Buffer). Then Dilute another 1:100 (5 µl Standard + 495 µl Assay Buffer) to a total of 1:1000 dilution.
   - Diluted samples should further be diluted if the concentration of anti- HER2 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 µl 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 µl of diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
4. Add 100 µl 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 µl of 1X **TMB substrate** solution and incubate the plate in dark at RT for 10 minutes
7. Add 100 µl 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 OD450/650 nm for each of 4 standards on the Y-axis versus the corresponding anti- HER2 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.

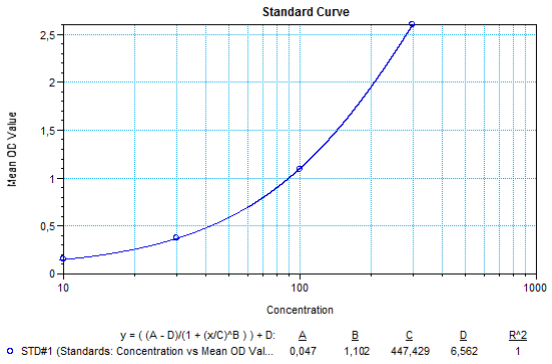

Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

X. RELATED PRODUCTS:

- BioSim™ Rituximab (Human) ELISA Kit (Cat. No. E4371-100)
- BioSim™ Adalimumab (Human) ELISA Kit (Cat. No. E4372-100)
- BioSim™ Bevacizumab (Human) ELISA Kit (Cat. No. E4373-100)
- BioSim™ Etanercept (Human) ELISA Kit (Cat. No. E4374-100)
- BioSim™ Ipilimumab (Human) ELISA Kit (Cat. No. E4375-100)
- BioSim™ Golimumab (Human) ELISA Kit (Cat. No. E4377-100)
- BioSim™ Infliximab (Human) ELISA Kit (Cat. No. E4378-100)
- BioSim™ Cetuximab (Human) ELISA Kit (Cat. No. E4379-100)
- BioSim™ Denosumab (Human) ELISA Kit (Cat. No. E4380-100)
- BioSim™ Omalizumab (Human) ELISA Kit (Cat. No. E4381-100)
- BioSim™ Nivolumab (Human) ELISA Kit (Cat. No. E4382-100)
- BioSim™ Pembrolizumab (Human) ELISA Kit (Cat. No. E4383-100)
- BioSim™ Ipilimumab (Human) ELISA Kit (Cat. No. E4384-100)