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BioSim™ anti-HER2 Mab (Human) ELISA Kit

rev 03/21

(Catalog # E4386-100, 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. In HER2 overexpressing cells, this 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. However, some patients develop unwanted immunogenicity, which leads to production of anti-drug-antibodies (ADAs) inactivating the therapeutic effects of the treatment and, in rare cases, inducing adverse effects. **BioVision's BioSim™ anti-HER2 Mab ELISA kit** is designed to quantify/measure the antibody against anti-HER2 monoclonal antibody with high specificity and sensitivity in biological matrices. The kit is based on the Sandwich principle. Standards and samples (serum or plasma) are added to the microtiter plate coated with anti-HER2 monoclonal antibody. After incubation, the wells are washed. The HRP conjugated probe is added and binds to the antibodies against anti-HER2 that is captured by monoclonal antibody coated on the wells. Following incubation, wells are washed and the enzymatic activity is detected by the addition of TMB chromogen substrate. Finally, the reaction is terminated with an acidic stop solution. The color developed is proportional to the amount of antibodies against anti-HER2 in the sample or standard. The quantitative test results can be evaluated using the standard curve.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of antibody against anti-HER2 monoclonal antibody in serum and plasma
Detection Range: 62.5 - 500 ng/ml
Sensitivity: 62.5 ng/ml
Assay Precision: Intra-Assay: CV < 30%; Inter-Assay: CV < 30% (CV (%) = SD/mean X 100)
Cross Reactivity: anti-HER2 humanized antibody infusion camouflages/masks the presence of antibody to anti-HER2 humanized antibody in serum/plasma samples. Therefore, blood sampling time is critical for detection of antibodies. It is convenient to obtain blood sample just before the infusion of anti-HER2 humanized antibody or at least 2 weeks after the infusion.
Recovery rate: 100 ± 30% with normal human serum samples with known concentrations

III. Sample Type:

Human serum and plasma

IV. Kit Contents:

Components	E4386-100	Part No.
Micro ELISA Plate	1 plate	E4386-100-1
anti-HER2 Mab Standards (S1 – S7)	1 ml X 7	E4386-100-2.x
Assay Buffer	50 ml	E4386-100-3
Confirmation Reagent	12 ml	E4386-100-4
Peroxidase Conjugate	12 ml	E4386-100-5
TMB substrate (Avoid light)	12 ml	E4386-100-6
Stop Solution	12 ml	E4386-100-7
Wash buffer (20X)	50 ml	E4386-100-8
Plate sealers	2	E4386-100-9

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. **Wash Buffer**: Prepare 1:20 dilution of 20X Wash Buffer with ddH_2O (20 ml of Wash Buffer stock + 180 ml of ddH_2O). Mix this Wash Buffer solution thoroughly by vortex manually. The working stock can be stable for 2 weeks after preparation at 4°C.
2. **Standard Preparation:**
Ready to use

Name	S1	S2	S3	S4	S5	S6	S7
Conc. (ng/ml)	500	250	125	62.5	0	High Control	Low Control

3. **Sample Dilution:**
   - **Serum/Plasma**: Dilute samples at a ratio of 1:10 (20 µl Serum/Plasma + 180 µl Assay Buffer)
     - Diluted samples should further be diluted if the concentration of anti-HER2 Mab is higher than the measuring range.
   - The usual precautions for venipuncture should be observed. Samples are stable at 4°C for 7 days and -20°C for 6 months. Avoid freeze-and-thaw cycle.
4. **Confirmation Test Mixture:** Mix 10 µl undiluted (positive) serum/plasma sample with 90 µl confirmation reagent (dilution ratio 1:10) and incubate for 60 minutes in a microtube prior to the test. After incubation, add 100 µl of this test mixture to the respective well and perform the assay as instructed below. The purpose of performing a confirmation test is to confirm that the anti-drug antibodies in positive samples are true positives. In the case of true positives, the inhibition % of the reaction would be atleast 25% after incubation with the confirmation reagent.

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, controls, diluted-samples**, and **confirmation test mixture** (if applicable) into appropriate wells. Cover wells and incubate for 60 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 **Peroxidase Conjugate** into each well. Cover wells with adhesive plate sealer and incubate at RT for 60 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 20 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 30 minutes. (reference wavelength to 650 nm)

IX. QUANTITATIVE CALCULATION:

Using the standards disregarding zero standard, construct a standard curve by plotting the OD450/650 nm for each standard on the Y-axis versus the corresponding anti-HER2 Mab 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.

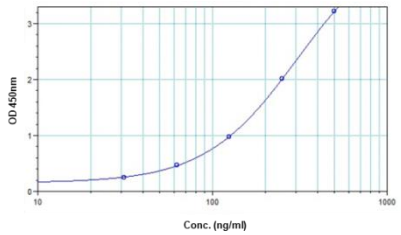

Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

X. QUALITATIVE INTERPRETATION:

- If "Sample $OD_{450/650}$ / Zero Standard (S5) $OD_{450/650}$" is < 3, the sample is NEGATIVE for Antibody to anti-HER2 Mab (ATR)
- If "Sample $OD_{450/650}$ /Zero Standard (S5) $OD_{450/650}$" is ≥3, the sample is POSITIVE for ATR and if required samples may be extrapolated for quantitative analysis and confirmation.
- For the run to be valid, the OD450/650 nm of Positive Control (Standard A) should be ≥ 1.000 and the OD450/650 nm of each Negative Control should be <0.200, if not, improper technique or reagent deterioration may be suspected and the run should be repeated.
- Interpretation of true and false positive: For true positive sample, inhibition should be equal or greater than 25%

$$\frac{OD_{(450/650)}\ \text{sample} - OD_{(450/650)}\ \text{sample w/confirmation reagent}}{OD_{(450/650)}\ \text{sample}} \times 100 = \text{inhibition \%}$$

XI. 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™ Infliximab (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)

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