BioSim™ Trastuzumab (Herceptin®) (Human) ELISA Kit

(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:

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

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 ddH₂O (25 ml of Assay Buffer stock to 100 ml of ddH₂O)
2. **Wash Buffer**: Dilute the 20X Wash Buffer to 1X solution in ddH₂O (10 ml of Wash Buffer stock to 190 ml of ddH₂O). 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)

<table>
<thead>
<tr>
<th>Name</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
<th>S6 High Control</th>
<th>S7 Low Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc. (ng/ml)</td>
<td>3000</td>
<td>1000</td>
<td>333</td>
<td>111</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Working Conc. (ng/ml)</td>
<td>300</td>
<td>100</td>
<td>33</td>
<td>11</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

155 S. Milpitas Blvd., Milpitas, CA 95035 USA | T: (408)493-1800 F: (408)493-1801 | www.biovision.com | tech@biovision.com
4. Sample Dilution:
   - **Serum/Plasma:** Initially dilute samples 1:10 (20 μl Serum/Plasma + 180 μl Assay Buffer). Then dilute another 100X (5 μl Standard + 495 μl 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 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 (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 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™ Ipaliumumab (Remicade®) (Human) ELISA Kit (Cat. No. E4375-100)
   - BioSim™ Trastuzumab (Herceptin®) (Human) ELISA Kit (Cat. No. E4376-100)
   - BioSim™ Golimumumab (Simponi®) (Human) ELISA Kit (Cat. No. E4377-100)
   - BioSim™ Infliximab (Remsima®) (Human) ELISA Kit (Cat. No. E4378-100)
   - BioSim™ Cetuximab (Erbixus®) (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)