BioSim™ Cetuximab (Human) ELISA Kit

(Catalog # E4379-100, 100 assays, Store at 4°C)

I. Introduction:
Cetuximab is a chimeric monoclonal antibody of the immunoglobulin G1 (IgG1) and FDA-approved epidermal growth factor receptor (EGFR) inhibitor. Preclinical studies have shown that cetuximab enhances the antitumour effects of chemotherapy as well as radiotherapy by inhibiting cell proliferation, angiogenesis and metastasis and by promoting apoptosis is used for the treatment of metastatic colorectal cancer, metastatic non-small cell lung cancer and head and neck cancer. Cetuximab blocks growth factor-induced activation of the downstream mitogen-activated protein kinase, inhibiting cell proliferation. It also decreases angiogenic factors, inhibits tumor-cell invasion and metastasis via downregulation of matrix metalloproteinases (MMPs) and VEGF, and promotes apoptosis by upregulating apoptotic protein, Bax, with the help of other chemotherapeutic agents. BioSim™ Cetuximab ELISA kit has been developed for specific quantification of Cetuximab concentration in human serum or plasma with high sensitivity and reproducibility.

II. Application:
This ELISA kit is used for in vitro quantitative determination of Cetuximab
Detection Range: 100 - 3000 ng/ml
Sensitivity: 100 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: There is no cross reaction with native serum immunoglobulins

III. Sample Type:
Human serum and plasma

IV. Kit Contents:

<table>
<thead>
<tr>
<th>Components</th>
<th>E4379-100</th>
<th>Part No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micro ELISA Plate</td>
<td>1 plate</td>
<td>E4379-100-1</td>
</tr>
<tr>
<td>Cetuximab Standards (S1 – S7)</td>
<td>0.3 ml x 7</td>
<td>E4379-100-2.x</td>
</tr>
<tr>
<td>Assay Buffer</td>
<td>50 ml x 2</td>
<td>E4379-100-3</td>
</tr>
<tr>
<td>HRP-conjugate Probe</td>
<td>12 ml</td>
<td>E4379-100-4</td>
</tr>
<tr>
<td>TMB substrate (Avoid light)</td>
<td>12 ml</td>
<td>E4379-100-5</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>12 ml</td>
<td>E4379-100-6</td>
</tr>
<tr>
<td>Wash buffer (20X)</td>
<td>50 ml</td>
<td>E4379-100-7</td>
</tr>
<tr>
<td>Plate sealers</td>
<td>2</td>
<td>E4379-100-8</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. 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.

2. Standard Preparation:
Dilute all standards 100X with assay buffer. (10 μl Standard + 990 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</th>
<th>S7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc. (μg/ml)</td>
<td>300</td>
<td>100</td>
<td>30</td>
<td>10</td>
<td>0</td>
<td>High Control</td>
<td>Low Control</td>
</tr>
<tr>
<td>Working conc. (ng/ml)</td>
<td>3000</td>
<td>1000</td>
<td>300</td>
<td>100</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

3. Sample Dilution:
- Serum/Plasma: Dilute samples 1:100 (10 μl Sample + 990 μl Assay Buffer).
- Diluted samples should further be diluted if the concentration of Cetuximab is higher than the measuring range.

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

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. Pipette 100 μl of Assay Buffer non-exceptionally into each of the wells to be used.
3. Add 10 μl of standards, controls and diluted-samples into appropriate wells. Cover wells and incubate for 30 minutes at room temperature (RT).
4. 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.
5. Add 100 μl of HRP-conjugate into each well. Cover wells with adhesive plate sealer and incubate at RT for 30 minutes.
6. Discard the solution and wash the wells as step 4.
7. Add 100 μl of 1X TMB substrate solution and incubate the plate in dark at RT for 10 minutes.
8. Add 100 μl of Stop solution to stop the reaction.
9. 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 Cetuximab 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™ anti-HER2 (Human) ELISA Kit (Cat. No. E4376-100)
• BioSim™ Golimumab (Human) ELISA Kit (Cat. No. E4377-100)
• 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)