BioSim™ Adalimumab (Human) ELISA Kit
(Catalog # E4372-100, 96 assays, Store at 4°C)

I. Introduction:
Adalimumab is a recombinant human IgG1 monoclonal antibody specific for Tumor Necrosis Factor-Alpha (TNF-α). Adalimumab specifically binds to TNF alpha and blocks its interaction with p55 and p75 cell surface TNF receptors. TNF is a naturally occurring cytokine that plays a role in normal inflammatory and immune responses. Adalimumab also lyses surface tumor necrosis factor expressing cells in vitro when in the presence of complement and it does not bind or inactivate lymphotoxin. BioSim™ Adalimumab (Human) ELISA Kit has been developed for specific quantification of Adalimumab concentration in human serum or plasma with high sensitivity and reproducibility. It is based on the principle of sandwich ELISA. Standards and samples (serum or plasma) are incubated in the microtiter plate coated with TNF-alpha. The wells are washed after incubation. HRP-Streptavidin is added and unbound conjugates are washed away with wash buffer. The HRP enzymatic reaction is detected using TMB-substrate. Finally, an acidic stop solution terminates the enzymatic reaction. The color developed is directly proportional to the amount of adalimumab antibodies in the sample. Results of the sample can be determined using the standard curve.

II. Application:
This ELISA kit is used for in vitro quantitative determination of Adalimumab.
Detection Range: 30 - 1000 ng/ml
Sensitivity: 10 ng/ml
Assay Precision: Intra-Assay: CV < 30%; Inter-Assay: CV < 30% (CV (%) = SD/mean X 100)
Cross Reactivity: No significant cross-reactivity or interference with other proteins present in native human serum or other immunoglobulins.
Recovery rate: < 100 ± 30% with normal human serum samples with known concentrations

III. Sample Type:
Human serum and plasma

IV. Kit Contents:

<table>
<thead>
<tr>
<th>Components</th>
<th>E4372-100</th>
<th>Part No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micro ELISA Plate</td>
<td>1 plate</td>
<td>E4372-100-1</td>
</tr>
<tr>
<td>Adalimumab Standards (S1 – S7)</td>
<td>1 ml X 7</td>
<td>E4372-100-2.x</td>
</tr>
<tr>
<td>Assay Buffer</td>
<td>50 ml</td>
<td>E4372-100-3</td>
</tr>
<tr>
<td>HRP-conjugate Probe</td>
<td>12 ml</td>
<td>E4372-100-4</td>
</tr>
<tr>
<td>TMB substrate (Avoid light)</td>
<td>12 ml</td>
<td>E4372-100-5</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>12 ml</td>
<td>E4372-100-6</td>
</tr>
<tr>
<td>Wash buffer (20X)</td>
<td>50 ml</td>
<td>E4372-100-7</td>
</tr>
<tr>
<td>Plate sealers</td>
<td>2</td>
<td>E4372-100-8</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. Standards:

<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. (ng/ml)</td>
<td>1000</td>
<td>300</td>
<td>100</td>
<td>30</td>
<td>0</td>
<td>High Standard</td>
<td>Low Standard</td>
</tr>
</tbody>
</table>

3. Sample Dilution:
- Serum/Plasma: Dilute samples at 1:10 (20 μl Serum/Plasma + 180 Assay Buffer)
- Diluted samples should further be diluted if the concentration of Adalimumab 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.

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

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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 100μl of **Assay Buffer** non-exceptionally into each of the wells to be used.
3. Add 20 μl of **standards** 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 3.
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 Adalimumab 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.](image)

Conc. (μg/ml)

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)