Infliximab (Remicade®) (Human) ELISA Kit

(Catalog # K4256-100, 100 assays, Store at -20°C)

I. Introduction:
Infliximab (Remicade®) is a therapeutic chimeric monoclonal antibody against tumor necrosis factor (TNF) and is used to treat rheumatic arthritis, intestinal disorders, dermatological diseases and cancer. Infliximab drug inhibits the action of TNF and reduces the inflammation and subsequently improves the patient's health. Drug level quantification can be important to adapt patient prescription or to switch to an alternative TNF inhibitor drug. EMA Bio-analytical Method Validation Guidelines and industry-recommended practices for ligand-binding assays were used for validation of this kit. BioVision's Infliximab ELISA kit has been developed for specific quantification of Infliximab concentration in human serum or plasma with high sensitivity and reproducibility.

II. Application:
This ELISA kit is used for in vitro quantitative determination of Infliximab.
Detection Range: 4.7 - 300 ng/ml

III. Sample Type:
Human serum and plasma

IV. Kit Contents:

<table>
<thead>
<tr>
<th>Components</th>
<th>K4256-100</th>
<th>Part No.</th>
<th>Storage After Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micro ELISA Plate</td>
<td>1 plate</td>
<td>K4256-100-1</td>
<td>-20°C</td>
</tr>
<tr>
<td>Infliximab Standard (10 mg/ml)</td>
<td>10 μl</td>
<td>K4256-100-2</td>
<td>-20°C</td>
</tr>
<tr>
<td>Detection Antibody (2000X)</td>
<td>20 μl</td>
<td>K4256-100-3</td>
<td>-20°C</td>
</tr>
<tr>
<td>Assay Diluent</td>
<td>1 g</td>
<td>K4256-100-4</td>
<td>2 - 8°C</td>
</tr>
<tr>
<td>TMB substrate (20X) (Avoid light)</td>
<td>1 ml</td>
<td>K4256-100-5</td>
<td>2 - 8°C</td>
</tr>
<tr>
<td>Wash buffer-A (20X)</td>
<td>70 ml</td>
<td>K4256-100-6</td>
<td>2 - 8°C</td>
</tr>
<tr>
<td>Plate sealers</td>
<td>2</td>
<td>K4256-100-7</td>
<td>RT</td>
</tr>
</tbody>
</table>

V. User Supplied Reagents and Equipment:
- Microplate reader capable of measuring absorbance at 450 nm
- Normal human serum or plasma
- Tween-20
- 2N H₂SO₄
- Precision pipettes with disposable tips
- Distilled or deionized water
- Clean eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

VI. Storage and Handling:
The entire kit may be stored at -20°C for up to 12 months from the date of shipment. Storage condition for opened or prepared reagents is listed in section IV.

VII. Reagent and Sample Preparation:

1. **Wash Buffer-A:** Thaw the wash buffer at room temperature (RT) until it is a clear solution. Dilute 50 ml of 20X Wash buffer-A with ddH₂O to a total volume of 1000 ml.
2. **Wash Buffer-B:** Aliquot 500 ml of wash buffer-A. Add 250 μl of Tween-20 solution (to a final concentration of 0.05%) and mix it for 10 minutes at RT.
3. **Assay Diluent:** Add 100 ml of 1X wash buffer-A into the Assay Diluent Bottle. Allow it to mix on rocker for 10 minutes or by gentle manual mixing at room temperature. Assay diluent should appear as a clear solution after mixing. Use this assay diluent buffer for assay matrix and test sample dilution.
4. **Assay Matrix:** Dilute normal human serum or plasma at minimum dilution of 1:250 in assay diluent. Use this assay matrix for standards and quality control preparation.
5. **TMB Substrate:** Dilute the 20X TMB substrate to 1X solution in ddH₂O (600 μl of TMB substrate to 11.4 ml of ddH₂O) Mix the 1X solution thoroughly by vortex manually.
6. **Detection Antibody:** Dilute the Detection Antibody in assay diluent at 1:2000 (Dilute 10 μl of 2000X detection antibody to 20 ml of assay diluent). Gently mix the detection antibody before use.
7. **Standard Preparation:**
   - Prepare a main stock of 1000 μg/ml by diluting the Infliximab Standard (10 mg/ml) in normal human serum or plasma (5 μl of Infliximab standard in 245 μl of human serum or plasma).
   - Prepare a sub stock of 5 μg/ml (5000 ng/ml) by diluting 5 μl of main stock into 995 μl of Assay Diluent.

FOR RESEARCH USE ONLY! Not to be used on humans.
- Prepare the **first standard** (300 ng/ml) by diluting 60 μl of sub stock into 940 μl of Assay Matrix. Perform 2-fold serial dilutions of the standards to make the standard curve within the range of this assay. Use 0.3 ml standard diluent as blank control.

- Suggested standard points are: 300, 150, 75, 37.5, 18.75, 9.38, 4.69, 0 ng/ml

### 8. Sample Preparation:

- **Serum**: Use serum clot tube and allow the blood sample to coagulate at room temperature (RT) for 30 minutes. Centrifuge at 5000 rpm for 10 minutes at RT. Aliquot the clear serum and store at -20°C. Avoid repeated freeze/thaw cycles.

- **Plasma**: Use K₂ EDTA as anticoagulant for blood collection and allow at RT for 30 minutes. Centrifuge the sample at 5000 rpm for 10 minutes at RT. Aliquot the clear plasma and store at -20°C. Avoid repeated freeze/thaw cycles.

### 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. Wash the plate with 1X wash buffer-A, incubate wells 2 minutes during each wash.
3. Decant off the contents, Add 100 μl of standards, QC and samples into appropriate wells. Cover wells and incubate for 1 hour at RT.
4. Discard the contents of each well and wash 3 times with 1X wash buffer-B and followed by 3 washes with 1X wash buffer-A, allowing 2 minutes for incubation between each wash step. Blot the microtiter plate on absorbent paper to remove any residual reagent.
5. Add 100 μl of **Detection Antibody** solution to each well of the microtiter plate. Cover wells with adhesive plate sealer and incubate at RT for 20 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 15 minutes.
8. Add 50 μl of **Stop solution** (2N H₂SO₄) to stop the reaction.
9. Read the absorbance in micro plate reader set to 450 nm, set the reference wavelength to 600 nm

### IX. CALCULATION:

After the absorbance is read at 450 nm and 600 nm as reference wave length, construct a standard curve of difference data using software capable of generating four or five parameter logistic (4PL or 5PL) curve fit. Absorbance of the test/specimen and the QC samples are interpolated from the standard curve. Report the values of test/specimen samples within the assay range.

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

### X. RELATED PRODUCTS:

- Adalimumab (Humira) (Human) ELISA Kit (Cat. No. K4256-100)
- Bevacizumab (Avastin) (Human) ELISA Kit (Cat. No. K4254-100)
- Etanercept (Enbrel) (Human) ELISA Kit (Cat. No. K4255-100)
- Rituximab (Mabthera) (Human) ELISA Kit (Cat. No. K4257-100)