Ranibizumab (Lucentis®) (Human) ELISA Kit

(Catalog # E4312-100, 100 assays, Store at -20°C)

I. Introduction:
Ranibizumab (Lucentis®) is a recombinant human IgG1 monoclonal antibody fragment (Fab) that blocks angiogenesis by inhibiting vascular endothelial growth factor-A (VEGF-A) isoforms. The humanized anti-VEGF monoclonal antibody, Ranibizumab, has been approved by the FDA for treatment of patients with wet age-related macular degeneration. Age-related macular degeneration (AMD) is the leading cause of irreversible blindness in people over the age of 50 in the developed world. Although an estimated 80% of patients with AMD have the non-neovascular form, the neovascular (wet or exudative) form is responsible for almost 90% of severe visual loss resulting from AMD. Currently, the most commonly used VEGF antagonists are ranibizumab (Lucentis, Genentech, Inc.) and bevacizumab (Avastin; Genentech, Inc.). Ranibizumab, which is an antibody fragment form the bevacizumab molecule with an increased binding affinity for all forms of VEGF, has been approved for the treatment of patients with neo-vascular AMD by the Food and Drug Administration and by the European Medicines Agency. BioVision’s Ranibizumab ELISA kit is developed for the quantification of Ranibizumab concentration in human serum or plasma with high sensitivity and reproducibility.

II. Application:
This ELISA kit is used for in vitro quantitative determination of Ranibizumab. Detection Range: 1.22 – 625 ng/ml

III. Sample Type:
Human serum and plasma

IV. Kit Contents:

<table>
<thead>
<tr>
<th>Components</th>
<th>E4312-100</th>
<th>Part No.</th>
<th>Storage after Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micro ELISA Plate</td>
<td>1</td>
<td>E4312-100-1</td>
<td>-20°C</td>
</tr>
<tr>
<td>Ranibizumab Standard (10 mg/ml)</td>
<td>10 µl</td>
<td>E4312-100-2</td>
<td>2 - 8°C</td>
</tr>
<tr>
<td>Detection Antibody (500X)</td>
<td>40 µl</td>
<td>E4312-100-3</td>
<td>-20°C</td>
</tr>
<tr>
<td>Assay Diluent</td>
<td>100 ml</td>
<td>E4312-100-4</td>
<td>2 - 8°C</td>
</tr>
<tr>
<td>TMB substrate (20X) (Avoid light)</td>
<td>1 ml</td>
<td>E4312-100-5</td>
<td>2 - 8°C</td>
</tr>
<tr>
<td>Wash buffer-A (20X)</td>
<td>70 ml</td>
<td>E4312-100-6</td>
<td>2 - 8°C</td>
</tr>
<tr>
<td>Plate sealers</td>
<td>2</td>
<td>E4312-100-7</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
- Stop Solution: 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 prepared reagents is listed in section IV.

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-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.
3. TMB Substrate: Dilute the 20X TMB substrate to 1X solution with ddH₂O (600 µl of TMB substrate to 11.4 ml of ddH₂O) Mix the 1X solution thoroughly by vortex manually.
4. Detection Antibody: Dilute the Detection Antibody in assay diluent at 1:500 (Dilute 24 µl of 500X detection antibody to 12 ml of assay diluent). Gently mix the detection antibody before use.
5. Standard Preparation:
- Prepare a main stock of 2000 µg/ml by diluting the Ranibizumab Standard (10 mg/ml) in normal human serum or plasma (5 µl of standard in 20 µl of normal human serum or plasma).

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
• Prepare a sub stock of 10 μg/ml by diluting 5 μl of main stock into 995 μl of Assay Diluent.
• Prepare a Standard #1 of 625 ng/ml by diluting 60 μl of sub stock into 900 μl of Assay Matrix.
• Perform 2-fold serial dilutions of the standards (300 μl standards + 300 μl assay matrix) to make the standard curve within the range of this assay. Use 0.3 ml standard diluent as blank control.
• Suggested standard points are: 625, 312.5, 156.25, 78.13, 39.06, 19.53, 9.77, 4.88, 2.44, 1.22, 0 ng/ml

6. 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.

7. Sample Preparation:
   • Prepare minimum of three QC samples in assay matrix
   • Keep the diluent buffer control in two replicates.

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, QC samples and test samples into appropriate wells. Cover wells and incubate for 1 hour at RT.
   3. Discard the contents of each well and wash 4 times 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.
   4. 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 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 15 minutes
   7. Add 50 μl of Stop solution (2N H₂SO₄) to stop the reaction
   8. 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:
   • BioSim™ Rituximab (Mabthera®) (Human) ELISA Kit (Cat. No. E4385-100)
   • BioSim™ Trastuzumab (Herceptin®) (Human) ELISA Kit (Cat. No. E4386-100)
   • BioSim™ Infliximab (Remicade®) (Human) ELISA Kit (Cat. No. E4387-100)
   • BioSim™ Adalimumab (Humira®) (Human) ELISA Kit (Cat. No. E4388-100)
   • BioSim™ Bevacizumab (Avastin®) (Human) ELISA Kit (Cat. No. E4389-100)
   • BioSim™ Infliximab (Remsima®) (Human) ELISA Kit (Cat. No. E4390-100)
   • BioSim™ Cetuximab (Erbilux®) (Human) ELISA Kit (Cat. No. E4391-100)
   • BioSim™ Etanercept (Enbrel®) (Human) ELISA Kit (Cat. No. E4392-100)
   • BioSim™ Golimumab (Simponi®) (Human) ELISA Kit (Cat. No. E4393-100)
   • BioSim™ Denosumab (Prolia®) (Human) ELISA Kit (Cat. No. E4394-100)
- BioSim™ Omalizumab (Xolair®) (Human) ELISA Kit (Cat. No. E4395-100)
- BioSim™ Nivolumab (Opdivo®) (Human) ELISA Kit (Cat. No. E4396-100)
- BioSim™ Pembrolizumab (Keytruda®) (Human) ELISA Kit (Cat. No. E4397-100)
- BioSim™ Ipilimumab (Yervoy®) (Human) ELISA Kit (Cat. No. E4398-100)
- BioSim™ Filgrastim (Herceptin®) (Human) ELISA Kit (Cat. No. E4399-100)