Human Orosomucoid
(Alpha-1-Acid Glycoprotein)
ELISA Quantitation Kit

Manual

Catalog number: 40-288-22927F

For the quantitative determination of human Orosomucoid levels in serum or other biological samples

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Kit Contents* (suitable for one 96-well plate) kit provides enough reagents for ten 96 well plates:

Coating Antibody (Catalog # 15-288-22927F)
Affinity purified Chicken IgY against Human Orosomucoid
Volume: 460 µl
Lot: 130618A-22927F
Concentration: 1.2 mg/ml
Working concentration: 5 µg/ml

Protein Calibrator (Catalog # 10-288-22927F)
Purified Human Orosomucoid Antigen
Volume: 125 µl
Lot: 130618C-22927F
Concentration: 20 µg/ml
Range: 250 – 3.91 ng/mL

Detection Antibody (Catalog # 27-288-22927F)
Affinity purified Chicken IgY against Human Orosomucoid – Horseradish Peroxidase (HRP) Conjugate
Volume: 125 µl
Lot: 130618D-22927F
Concentration: 0.36 mg/ml
Working concentration: 400 ng/ml

Buffers, Horseradish Peroxidase Substrate, and Plates not included.

*Always centrifuge the provided reagent vials before open them!

Notes:

Range of Detection: 3.91 - 250 ng/ml

Shelf life: One year from date of receipt.

Storage: -20°C.

Assay Condition: The kit performance has been optimized for the protocol and materials listed below using standard dilutions of human Orosomucoid in the 3.91- 250 ng/ml range. The operator must determine appropriate dilutions of reagents for alternative assay conditions. ELISA assay reactivity is sensitive to variations in operator, pipetting and washing techniques, incubation time, temperature, composition of reagents, and other experimental variables. Assay optimization may be required to generate the standard curve and fit the samples in the specified detection range.

Country of Origin: United States of America

Assay Use: For Research Use Only (RUO). Not for diagnostic or therapeutic use in humans or animals. Not for animal or human consumption.
Human Orosomucoid Quantitative ELISA Protocol

Buffer Preparation

1. Prepare the following buffers:
   A. Coating Buffer, 0.05 M Carbonate-Bicarbonate, pH 9.6
   B. Wash Solution: 0.05% Tween 20 in PBS, pH 7.4
   C. Blocking Solution, 50 mM Tris, 0.14 M NaCl, 1% BSA, pH 8.0
   D. Sample/Conjugate Diluents, 50 mM Tris, 0.14 M NaCl, 1% BSA, 0.05% Tween 20, pH 8.0
   E. Enzyme Substrate, TMB (KPL, Cat # 50-76-00)
   F. Stopping Solution, 2 N H₂SO₄ or other appropriate solution

Step-by-Step Method (Perform all steps at room temperature)

1. Coating with Capture Antibody
   A. Dilute 46 µl of Capture Antibody in 11ml coating buffer to make a 5 µg/ml solution.
   B. Add 100 µl per well.
   C. Incubate coated plate for 60 minutes.
   D. After incubation, aspirate the Capture Antibody solution from each well.
   E. Wash each well with Wash Solution as follows:
      1. Fill each well with Wash Solution
      2. Remove Wash Solution by aspiration
      3. Repeat for a total of 3 washes.

2. Blocking (Post-coat)
   A. Add 200 µl of Blocking Solution to each well.
   B. Incubate for 60 minutes.
   C. After incubation, remove the Blocking Solution and wash each well three times as in Step 1.E.

3. Standards and Samples
   A. Dilute the standards in Sample Diluent according to the chart below:

<table>
<thead>
<tr>
<th>Step</th>
<th>ng/ml</th>
<th>Calibrator</th>
<th>Sample Diluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>250</td>
<td>12.5 ul from 20 ng/mL Standard</td>
<td>987.5 ul</td>
</tr>
<tr>
<td>2</td>
<td>125</td>
<td>500 ul from step 1</td>
<td>500 ul</td>
</tr>
<tr>
<td>3</td>
<td>62.5</td>
<td>500 ul from step 2</td>
<td>500 ul</td>
</tr>
<tr>
<td>4</td>
<td>31.25</td>
<td>500 ul from step 3</td>
<td>500 ul</td>
</tr>
<tr>
<td>5</td>
<td>15.63</td>
<td>500 ul from step 4</td>
<td>500 ul</td>
</tr>
<tr>
<td>6</td>
<td>7.81</td>
<td>500 ul from step 5</td>
<td>500 ul</td>
</tr>
<tr>
<td>7</td>
<td>3.91</td>
<td>500 ul from step 6</td>
<td>500 ul</td>
</tr>
</tbody>
</table>

   B. Dilute the samples, based on the expected concentration of the analyte, to fit within the concentration range of the standards. (Most Linear Range is between 10-50 ng/mL)
   C. Transfer 100 µl of Protein Calibrator solutions and sample solutions to assigned wells.
   D. Incubate plate for 60 minutes.
   E. After incubation, remove samples and standards and wash each well 5 times as in Step 1.E.
4. Detection Antibody – Horseradish Peroxidase Conjugate
   A. Dilute 12 µl of the HRP conjugate in 11 ml Conjugate diluent to make a 400 ng/ml solution. Adjustments in dilution may be needed depending on substrates used, incubation time, and other experimental conditions.
   B. Transfer 100 µl to each well.
   C. Incubate for 60 minutes.
   D. After incubation, remove HRP Conjugate and wash each well 5 times as in Step 1.E.

5. Enzyme Substrate Reaction
   A. Prepare the Substrate solution according to the manufacturer’s recommendation.
   B. Transfer 100 µl of Substrate solution to each well.
   C. Incubate plate for 5-30 minutes.
   D. To stop the TMB reaction, apply 100 µl of 2 N H₂SO₄ to each well. When using another substrate, follow the manufacturer’s recommendations.

6. Plate Reading
   Using a microtiter plate reader, read the plate at the wavelength that is appropriate for the substrate used (450 nm for TMB).

Calculation of Results
1. Average the duplicate readings from each standard, control, and sample.
2. Subtract the zero reading from each averaged value above.
3. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. Other curve fits may also be used.
4. A standard curve should be generated for each set of samples (see examples below)
5. Determine the concentration of unknowns from the standard curve using absorbance readings for the samples.

Representative Results

![Standard Curve Graph]

\[ y = \frac{(A - D)(1 + (\frac{C}{x})^B)}{1 + (\frac{C}{x})^B} + D \]

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Std</td>
<td>0.045</td>
<td>1.14</td>
<td>19.962</td>
<td>1.768</td>
<td>0.996</td>
</tr>
<tr>
<td>Standard</td>
<td>Concentration (ng/mL)</td>
<td>Calculated Concentration</td>
<td>Absorbance (450 nm)</td>
<td>Average Absorbance</td>
<td>Standard Deviation % CV</td>
</tr>
<tr>
<td>----------</td>
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</tr>
<tr>
<td>St01</td>
<td>250</td>
<td>199.072 91.315 559.100 320.818 461.286 262.554</td>
<td>1.651 1.509 1.730 1.698 1.721 1.681</td>
<td>1.665</td>
<td>0.082</td>
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<tr>
<td>St02</td>
<td>125</td>
<td>1153.202 Range? 105.904 83.263 79.981 95.487</td>
<td>1.751 1.782 1.544 1.485 1.474 1.520</td>
<td>1.593</td>
<td>0.137</td>
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<tr>
<td>St03</td>
<td>62.5</td>
<td>75.334 76.652 55.298 74.819 58.716 60.286</td>
<td>1.457 1.462 1.357 1.455 1.378 1.387</td>
<td>1.416</td>
<td>0.047</td>
</tr>
<tr>
<td>St04</td>
<td>31.25</td>
<td>32.811 28.938 28.150 28.150 26.819 26.209</td>
<td>1.144 1.086 1.073 1.073 1.050 1.039</td>
<td>1.078</td>
<td>0.037</td>
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<tr>
<td>St05</td>
<td>15.625</td>
<td>18.269 18.953 14.954 15.886 14.861 16.556</td>
<td>0.863 0.881 0.766 0.795 0.763 0.815</td>
<td>0.814</td>
<td>0.049</td>
</tr>
<tr>
<td>St06</td>
<td>7.813</td>
<td>8.657 8.856 7.841 8.244 6.962 8.330</td>
<td>0.525 0.534 0.487 0.506 0.444 0.510</td>
<td>0.501</td>
<td>0.032</td>
</tr>
<tr>
<td>St07</td>
<td>3.906</td>
<td>3.688 3.721 3.368 3.251 3.552 3.401</td>
<td>0.265 0.267 0.246 0.239 0.257 0.248</td>
<td>0.254</td>
<td>0.011</td>
</tr>
<tr>
<td>St08</td>
<td>0</td>
<td>0.178 0.158 0.137 0.137 0.095 0.158</td>
<td>0.054 0.053 0.052 0.052 0.050 0.053</td>
<td>0.052</td>
<td>0.001</td>
</tr>
</tbody>
</table>
**Technical Hints**

1. The Capture Antibody should be diluted with Coating Buffer immediately prior to its addition to the wells. Coated plates can be incubated overnight at 4°C.

2. Change pipette tips between each addition of standard, sample and reagents to avoid cross-contamination.

3. Standards and samples should be pipetted to the bottom of the wells; all other reagents should be added to the side of the wells to avoid contamination. When pipetting, avoid bubbles and foam.

4. Make sure that all buffers are not contaminated or expired. When troubleshooting ELISA results, it is recommended to prepare new buffers.

5. Do not add sodium azide to the buffers.

6. Sample and Conjugate dilutions should be prepared shortly before use.

7. When preparing serial dilutions, wipe excess antibody/analyte from pipette tips to ensure accurate dilutions.

8. Incubation time of the HRP Substrate will depend on the formulation used and the intensity of the color change.

9. The Stopping solution should be added to the wells in the same order as the HRP Substrate to ensure uniform chromogen reaction.
Troubleshooting

1. **Problem: Low absorbance**
   - Incorrect dilutions or pipetting errors.
   - Improper incubation times.
   - Improper preparation of the TMB substrate.
   - Wrong filter on microtiter reader. The wavelength should be 450 nm for TMB, 490 nm for OPD, or 405 nm for ABTS.
   - Kit materials or reagents are contaminated or expired.
   - Incorrect reagents used.

2. **Problem: High Absorbance**
   - Cross contamination from other samples or positive control.
   - Incorrect dilutions or pipetting errors.
   - Improper washing.
   - Wrong filter on microtiter reader.
   - Contaminated buffers or enzyme substrate.
   - Improper incubation times.
   - Kit materials or reagents are contaminated or expired.

3. **Problem: Poor Duplicates**
   - Inadequate preparation (e.g., mixing) of specimens.
   - Incorrect dilutions or pipetting errors.
   - Technical errors.
   - Inconsistency in following ELISA protocol.
   - Inefficient washing.

4. **Problem: All wells are positive**
   - Contaminated buffers or enzyme substrate.
   - Incorrect dilutions or pipetting errors.
   - Kit materials or reagents are contaminated or expired.
   - Inefficient washing.

5. **Problem: All wells are negative**
   - Procedure not followed correctly.
   - Contaminated buffers or enzyme substrate.
   - Contaminated conjugate.
   - Kit materials or reagents are contaminated or expired.

TECHNICAL SUPPORT:

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