Human Chromogranin-A ELISA Kit

Enzyme Immunoassay for the quantification of human Chromogranin-A in serum

Catalog number: ARG80446

For research use only. Not for use in diagnostic procedures.

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PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for Chromogranin A has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Chromogranin A present is bound by the immobilized antibody. After washing away any unbound substances, a Horseradish Peroxidase (HRP)-conjugated antibody specific for Chromogranin A is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Chromogranin A bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of Chromogranin A in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
<th>Storage information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody-coated microplate</td>
<td>1 plate</td>
<td>4°C</td>
</tr>
<tr>
<td>Standard A-E</td>
<td>5 x 1 ml (ready for use)</td>
<td>4°C</td>
</tr>
<tr>
<td>Control 1</td>
<td>1 ml (ready for use)</td>
<td>4°C</td>
</tr>
<tr>
<td>Control 2</td>
<td>1 ml (ready for use)</td>
<td>4°C</td>
</tr>
<tr>
<td>HRP conjugated antibody</td>
<td>1 vial (ready for use)</td>
<td>4°C</td>
</tr>
<tr>
<td>Assay Buffer</td>
<td>50 ml</td>
<td>4°C</td>
</tr>
</tbody>
</table>
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<table>
<thead>
<tr>
<th></th>
<th>50X Wash buffer</th>
<th>20 ml</th>
<th>4°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMB substrate</td>
<td>12 ml</td>
<td>4°C (Protect from light)</td>
<td></td>
</tr>
<tr>
<td>STOP solution</td>
<td>12 ml</td>
<td>4°C</td>
<td></td>
</tr>
</tbody>
</table>

**MATERIALS REQUIRED BUT NOT PROVIDED**

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

**TECHNICAL HINTS AND PRECAUTIONS**

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.
SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

**Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **1X Wash buffer**: Dilute 50X Wash buffer into distilled water to yield 1X Wash buffer.
- **Samples**: Dilute patient sample 1:9 with assay buffer before assay, mix well. (e.g. 25 µl of sample + 200 µl of Assay Buffer.) Samples which have been found off-curve should also be diluted accordingly with Assay Buffer and re-assayed.

**Note**: the controls and Standards are ready-to-use and need not further dilution.
ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 50 μl of standards, controls and samples in duplicate into wells.
3. Add 50 μl of HRP-antibody conjugated to each well.
4. Cover wells and incubate for 2 hours at RT.
5. Aspirate each well and wash, repeating the process 3 times for a total 4 washes. Wash by filling each well with 1× Wash Buffer (350 μl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
6. Add 100 μl of TMB mixture to each well. Incubate for 20-30 minutes at room temperature in dark.
7. Add 100 μl of Stop Solution to each well. The color of the solution should change from blue to yellow.
8. Read the OD with a microplate reader at 450 nm immediately.
CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.
QUALITY ASSURANCE

Sensitivity
The minimum detectable dose (MDD) of Chromogranin A ranged from 0-1500 μg/ml. The mean MDD was 12.4 μg/ml

Intra-assay and Inter-assay precision
The CV value of intra-assay precision was 5.55% and inter-assay precision was 8.7%.

Recovery
89-94%