Instructions for use

Chromogranin A ELISA
Chromogranin A ELISA

1. Introduction

1.1 Intended use and principle of the test

Enzyme Immunoassay for the quantitative determination of human Chromogranin A in serum and plasma.

The quantitative determination of Chromogranin A (CgA) follows the basic principles of the enzyme immunoassay. First, the Chromogranin A in the samples, controls and standards binds to CgA-specific antibodies fixed to a 96 wells microtiter plate. After incubation and following washing steps, a sandwich is formed by adding CgA antibodies conjugated to horseradish peroxidase. After incubation the wells are washed thoroughly and the complex bound to the solid phase is detected by using TMB as a substrate. The reaction is monitored at 450 nm. By means of a standard curve the CgA concentrations in the samples are determined.

1.2 Clinical application

Chromogranin A or parathyroid secretory protein 1 (gene name CHGA) is a member of the chromogranin/secretogranin (granins) family of neuroendocrine secretory proteins, i.e. it is located in secretory vesicles of neurons and endocrine cells. Examples of cells producing Chromogranin A are chromaffin cells of the adrenal medulla, enterochromaffin-like cells and beta cells of the pancreas. Chromogranin A (CgA) is the precursor to several functional peptides including vasostatin, pancreastatin, catestatin and parastatin. These peptides negatively modulate the neuroendocrine function of the releasing cell (autocrine) or nearby cells (paracrine). Other peptides derived from chromogranin A with uncertain function include chromostatin, WE-14 and GE-25. CgA has become the most important circulating tumour marker for different kinds of neuroendocrine tumours. CgA levels are increased in carcinoid tumours, neuroblastoma, pheochromocytoma, and gastro-entero-pancreatic tumours such as gastrinoma, glucagonoma, insulinoma. An increase of CgA levels in patients with prostate carcinoma is a hint for an unfavourable outcome of the disease. In addition Chromogranin A-levels show a high correlation to the tumour mass and are therefore widely used to monitor the outcome of therapies.

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as under point “Procedural cautions, guidelines and warnings”. Any laboratory result is only a part of the total clinical picture of the patient. Only in cases where the laboratory results are in an acceptable agreement with the overall clinical picture of the patient it can be used for therapeutic consequences. The test result itself should never be the sole determinant for deriving any therapeutic consequences.

2. Procedural Cautions, Guidelines, Warnings and Limitations

2.1 Procedural Cautions, Guidelines and Warnings

(1) This kit is intended for professional use only. Users should have a thorough understanding of this protocol for the successful use of this kit. Only the test instruction provided with the kit is valid and has to be used to run the assay. Reliable performance will only be attained by strict and careful adherence to the instructions provided.

(2) This assay was validated for a certain type of sample as indicated in Intended Use (please refer to Chapter 1). Any off-label use of this kit is in the responsibility of the user and the manufacturer cannot be held liable.

(3) Reagents of this kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.

(4) The principles of Good Laboratory Practice (GLP) have to be followed.

(5) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.

(6) All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.

(7) For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water.

(8) The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch with desiccant and used in the frame provided.

(9) Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.

(10) Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials and devices are prepared ready at the appropriate time.

(11) Incubation times do influence the results. All wells should be handled in the same order and time intervals.
(12) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
(13) A standard curve must be established for each run.
(14) The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report.
(15) Do not mix kit components with different lot numbers within a test and do not use reagents beyond expiry date as shown on the kit labels.
(16) Avoid contact with Stop Solution containing 0.25 M H₂SO₄. It may cause skin irritation and burns. In case of contact with eyes or skin, rinse off immediately with water.
(17) Some reagents contain sodium azide (NaN₃) as preservatives. In case of contact with eyes or skin, rinse off immediately with water. NaN₃ may react with lead and copper plumbing to form explosive metal azides. When disposing reagents, flush with a large volume of water to avoid azide build-up.
(18) TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them.
(19) For information on hazardous substances included in the kit please refer to Material Safety Data Sheets (MSDS). The Material Safety Data Sheet for this product is made available directly on the website of the manufacturer or upon request.
(20) The expected reference values reported in this test instruction are only indicative. It is recommended that each laboratory establishes its own reference intervals.
(21) The results obtained with this test kit should not be taken as the sole reason for any therapeutic consequence but have to be correlated to other diagnostic tests and clinical observations.
(22) Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

2.2 Limitations

Any inappropriate handling of samples or modification of this test might influence the results.

2.2.1 Interfering substances

Serum/Plasma
Samples containing precipitates or fibrin strands or which are haemolytic or lipemic might cause inaccurate results.

2.2.2 Drug interferences

There are no known substances (drugs) which ingestion interferes with the measurement of Chromogranin A level in the sample.

2.2.3 Measuring range

Do not extrapolate measured values found higher than the highest standard. Samples with higher concentrations have to be pre-diluted.

2.2.4 High-Dose-Hook effect

This assay will not show any kind of high dose hook effect due to separated incubation steps of the antigen and antibody.

3. Storage and stability

Store the unopened reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Once opened the reagents are stable for 1 month when stored at 2 - 8 °C. Once the resealable pouch has been opened, care should be taken to close it tightly with desiccant again.

4. Materials

4.1 Content of the kit

**BA E-0030**  **WASH-CONC 50X**  **Wash Buffer Concentrate** - Concentrated 50x  
Content: Buffer with a non-ionic detergent and physiological pH  
Volume: 1 x 20 ml/vial, light purple cap

**TM E-9010**  **CONJUGATE**  **Antibody Conjugate** - Ready to use  
Content: Rabbit anti-chromogranin A antibody, conjugated with peroxidase  
Volume: 1 x 6 ml/vial, red cap
**TM E-9055**  **Substrate** - Ready to use
Content: Chromogenic substrate containing tetramethylbenzidine, substrate buffer and hydrogen peroxide
Volume: 1 x 12 ml/vial, black cap

**BA E-0080**  **Stop Solution** - Ready to use
Content: 0.25 M sulfuric acid
Volume: 1 x 12 ml/vial, light grey cap

**TM E-9031**  **Chromogranin A Microtiter Strips** - Ready to use
Content: 1 x 96 well (12x8) antibody precoated microwell plate in a resealable pouch with desiccant

**Standards** and **Controls** - Ready to use

<table>
<thead>
<tr>
<th>Cat. no.</th>
<th>Component</th>
<th>Colour/Cap</th>
<th>Concentration µg/l</th>
<th>Volume/ Vial</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM E-9001</td>
<td>STANDARD A</td>
<td>white</td>
<td>0</td>
<td>1 ml</td>
</tr>
<tr>
<td>TM E-9002</td>
<td>STANDARD B</td>
<td>light yellow</td>
<td>50</td>
<td>1 ml</td>
</tr>
<tr>
<td>TM E-9003</td>
<td>STANDARD C</td>
<td>orange</td>
<td>150</td>
<td>1 ml</td>
</tr>
<tr>
<td>TM E-9004</td>
<td>STANDARD D</td>
<td>dark blue</td>
<td>500</td>
<td>1 ml</td>
</tr>
<tr>
<td>TM E-9005</td>
<td>STANDARD E</td>
<td>light grey</td>
<td>1 000</td>
<td>1 ml</td>
</tr>
<tr>
<td>TM E-9051</td>
<td>CONTROL 1</td>
<td>light green</td>
<td>Refer to QC-Report for expected value and acceptable range!</td>
<td>1 ml</td>
</tr>
<tr>
<td>TM E-9052</td>
<td>CONTROL 2</td>
<td>dark red</td>
<td></td>
<td>1 ml</td>
</tr>
</tbody>
</table>

Content: Assay buffer spiked with defined quantity of human Chromogranin A

**TM E-9013**  **Assay Buffer** - Ready to use
Content: Buffer with proteins and non-mercury preservatives
Volume: 1 x 50 ml/vial, blue cap

### 4.2 Additional materials and equipment required but not provided in the kit

- Calibrated precision pipettes to dispense volumes of 25, 50, 100, and 200 μl
- Microtiter plate washing device (manual, semi-automated or automated)
- ELISA reader capable of reading absorbance at 450 nm and if possible 620 - 650 nm
- Microtiter plate shaker (shaking amplitude 3 mm; approx. 600 rpm)
- Absorbent material (paper towel)
- Water (deionized, distilled, or ultra-pure)
- Vortex mixer

### 5. Sample collection and storage

**Serum**
Collect blood by venipuncture (Monovette™ or Vacuette™), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time. Haemolytic and lipemic samples should not be used for the assay.
Storage: for longer period (up to 6 month) at -20 °C. Repeated freezing and thawing should be avoided.

**Plasma**
Whole blood should be collected into centrifuge tubes containing EDTA as anti-coagulant (Monovette™ or Vacuette™) and centrifuged according to manufacturer’s instructions immediately after collection. Haemolytic and lipemic samples should not be used for the assay.
Storage: for longer period (up to 6 month) at -20 °C. Repeated freezing and thawing should be avoided.
6. **Test procedure**
Allow all reagents to reach room temperature and mix thoroughly by gentle inversion before use. Duplicate determinations are recommended.

The binding of the antisera and the enzyme conjugates and the activity of the enzyme used are temperature dependent, and the absorption values may vary if a thermostat is not used. The higher the temperature, the higher the absorption values will be. The extinction values also depend on the incubation times. The optimal temperature during the Enzyme Immunoassay is between 20 – 25 °C.

⚠️ *In case of overflow, read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 405 nm*

6.1 **Preparation of reagents and samples**

**Wash Buffer**
Dilute the 20 ml Wash Buffer Concentrate with water (deionized, distilled, or ultra-pure) to a final volume of 1000 ml.

Storage: 1 month at 2 – 8 °C

**Predilution of samples**
Prior to use, the samples have to be diluted 1+8 with Assay Buffer (TM E-9013), 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.

6.2 **Chromogranin A ELISA**

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Pipette 50 µl of the standards, controls and diluted samples into the wells of the Chromogranin A Microtiter Strips and incubate 1 h at RT (20 – 25 °C) on a shaker (approx. 600 rpm).</td>
</tr>
<tr>
<td>2.</td>
<td>Discard or aspirate the content of the wells. Wash the plate 4 x by adding 300 µl of Wash Buffer, discarding the content and blotting dry each time by tapping the inverted plate on absorbent material.</td>
</tr>
<tr>
<td>3.</td>
<td>Pipette 50 µl of the Antibody-Conjugate into all wells and incubate 1 h at RT (20 – 25 °C) on a shaker (approx. 600 rpm).</td>
</tr>
<tr>
<td>4.</td>
<td>Discard or aspirate the content of the wells. Wash the plate 4 x by adding 300 µl of Wash Buffer, discarding the content and blotting dry each time by tapping the inverted plate on absorbent material.</td>
</tr>
<tr>
<td>5.</td>
<td>Pipette 100 µl of the Substrate into all wells.</td>
</tr>
<tr>
<td>6.</td>
<td>Incubate for 25 ± 5 min at RT (20 – 25 °C) on a shaker (approx. 600 rpm).</td>
</tr>
<tr>
<td>7.</td>
<td>Add 100 µl of the Stop Solution to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.</td>
</tr>
<tr>
<td>8.</td>
<td>Read the absorbance of the solution in the wells within 10 minutes, using a microtiter plate reader set to 450 nm (if available a reference wavelength between 620 nm and 650 nm is recommended).</td>
</tr>
</tbody>
</table>

7. **Calculation of results**

<table>
<thead>
<tr>
<th>Measuring range</th>
<th>Serum</th>
<th>EDTA-Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>19 – 1000 µg/l</td>
<td>13 – 1000 µg/l</td>
</tr>
</tbody>
</table>

The standard curve is obtained by plotting the absorbance readings (calculate the mean absorbance) of the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis). Use a non-linear regression for curve fitting (e.g. spline, 4-parameter, akima).

**Samples and controls**
The concentrations of the samples and the controls can be read directly from the standard curve.

Samples found off-curve should be diluted with Assay Buffer and re-assayed.

**Expected reference values**
It is strongly recommended that each laboratory should determine its own reference values.
In a study conducted with apparently normal healthy adults, using the Chromogranin A ELISA the following values are observed:

<table>
<thead>
<tr>
<th>Expected reference value</th>
<th>Serum/EDTA-Plasma</th>
<th>&lt; 100 µg/l</th>
</tr>
</thead>
</table>

7.1 Quality control

It is recommended to use control samples according to national regulations. Use controls at both normal and pathological levels. The kit, or other commercially available controls should fall within established confidence limits. The confidence limits of the kit controls are listed in the QC-Report.

7.2 Typical standard curve

⚠️ Example, do not use for calculation!

![Typical standard curve](image)

<table>
<thead>
<tr>
<th>µg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
</tr>
<tr>
<td>100</td>
</tr>
<tr>
<td>1000</td>
</tr>
<tr>
<td>10000</td>
</tr>
</tbody>
</table>

8. Assay characteristics

<table>
<thead>
<tr>
<th>Analytical Sensitivity</th>
<th>Serum</th>
<th>EDTA-Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limit of Detection (LOD)</td>
<td>13 µg/l</td>
<td>13 µg/l</td>
</tr>
<tr>
<td>Limit of Quantification (LOQ)</td>
<td>19 µg/l</td>
<td>13 µg/l</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Precision – Intra Assay Variation</th>
<th>Serum, n = 15</th>
<th>EDTA-Plasma, n = 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>Mean ± SD (µg/l)</td>
<td>CV (%)</td>
</tr>
<tr>
<td>1</td>
<td>51 ± 3</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>102 ± 3</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>222 ± 9</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>547 ± 46</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>983 ± 89</td>
<td>6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Precision – Inter Assay Variation</th>
<th>Serum, n = 6</th>
<th>EDTA-Plasma, n = 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>Mean ± SD (µg/l)</td>
<td>CV (%)</td>
</tr>
<tr>
<td>1</td>
<td>50 ± 1</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>100 ± 6</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>221 ± 6</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>569 ± 25</td>
<td>5</td>
</tr>
</tbody>
</table>
**Recovery**

<table>
<thead>
<tr>
<th></th>
<th>Range (µg/l)</th>
<th>Range (%)</th>
<th>Mean (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>50 - 569</td>
<td>101 - 111</td>
<td>105</td>
</tr>
<tr>
<td>EDTA-Plasma</td>
<td>43 - 507</td>
<td>94 - 96</td>
<td>95</td>
</tr>
</tbody>
</table>

**Linearity**

<table>
<thead>
<tr>
<th></th>
<th>Serial dilution up to</th>
<th>Range (%)</th>
<th>Mean (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>1:1024</td>
<td>93 - 111</td>
<td>106</td>
</tr>
<tr>
<td>EDTA-Plasma</td>
<td>1:1024</td>
<td>87 - 120</td>
<td>107</td>
</tr>
</tbody>
</table>

**High-dose hook effect**

Despite the fact that a high dose hook effect is theoretically eliminated, we tested samples with concentrations higher than 200,000 µg/l Chromogranin A. A high dose hook effect was not detected.

**Method comparison versus RIA***

ELISA = 1.07xLDN – 8.73; r² = 0.95; n = 46

*commercially available RIA

9. **References/Literature**


⚠️ **For updated literature or any other information please contact your local supplier.**

**Symbols:**

- **Storage temperature**

- **Manufacturer**

- **Contains sufficient for <n> tests**

- **Expiry date**

- **Batch code**

- **For in-vitro diagnostic use only!**

- **Consult instructions for use**

- **Content**

- **CE labelled**

- **Caution**

- **Catalogue number**

- **For research use only!**

**Version:** 13.0 **Effective:** 2015-03-09