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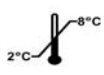
Instructions for use

Chromogranin A ELISA

REF TM E-9000R



RUO

For research use only – Not for use in diagnostic procedures

English

1. Introduction

1.1 Intended use and principle of the test

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

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 Background

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.

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) 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.
(3) The principles of Good Laboratory Practice (GLP) have to be followed.
(4) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.
(5) 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.
(6) For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water.
(7) 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.
(8) Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.
(9) 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.
(10) Incubation times do influence the results. All wells should be handled in the same order and time intervals.
(11) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
(12) A standard curve must be established for each run.
(13) The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report.
(14) 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.
(15) Avoid contact with Stop Solution containing 0.25 M H_2SO_4. It may cause skin irritation and burns. In case of contact with eyes or skin, rinse off immediately with water.
(16) 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.
(17) For information on hazardous substances included in the kit please refer to Safety Data Sheets (SDS). The Safety Data Sheet for this product is made available directly on the website of the manufacturer or upon request.
(18) 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

Samples containing precipitates or fibrin strands might cause inaccurate results. Biotin (up to 600 ng/ml), hemolytic samples (up to 0.5 mg/ml hemoglobin), icteric samples (up to 12.5 mg/dl bilirubin) and lipemic samples (up to 1657 mg/dl triglycerides) have no influence on the assay results.

2.2.2 Drug interferences

Medications like proton pump inhibitors and histamine type-2 receptor antagonists can influence CgA level in serum. People who are taking such medication should consult with their doctor before specimen collection. Sport and ingestion of a meal can also influence CgA level.

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

BA E-0055 SUBSTRATE **Substrate** - Ready to use

Contents: Chromogenic substrate containing tetramethylbenzidine, substrate buffer and hydrogen peroxide

Volume: 1 x 12 ml/black vial, black cap

BA E-0080 STOP-SOLN **Stop Solution** - Ready to use

Content: 0.25 M sulfuric acid

Volume: 1 x 12 ml/vial, light grey cap

Hazards identification:

H290 May be corrosive to metals.

TM E-9031 96 **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

Cat. no.	Component	Colour / Cap	Concentration µg / l	Volume / Vial
TM E-9001	STANDARD A	white	0	1 ml
TM E-9002	STANDARD B	light yellow	30	1 ml
TM E-9003	STANDARD C	orange	110	1 ml
TM E-9004	STANDARD D	dark blue	450	1 ml
TM E-9005	STANDARD E	light grey	900	1 ml
TM E-9051	CONTROL 1	light green	Refer to QC-Report for expected value and acceptable range!	1 ml
TM E-9052	CONTROL 2	dark red		1 ml

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

TM E-9013 ASSAY-BUFF **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 according to manufacturer's instructions at room temperature. Do not centrifuge before complete clotting has occurred. Donors receiving anticoagulant therapy may require increased clotting time. Haemolytic, icteric and lipemic samples should not be used for the assay.
If the samples are not used immediately for the assay, they have to be stored frozen.
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. It is recommended to number the strips of the microwell plate before usage to avoid any mix-up.

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.

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.

Chromogranin A Microtiter Strips
In rare cases residues of the blocking and stabilizing reagent can be seen in the wells as small, white dots or lines. These residues do not influence the quality of the product.

6.2 Chromogranin A ELISA

1.	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).
2.	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.
3.	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).
4.	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.
5.	Pipette **100 µl** of the **Substrate** into all wells.
6.	Incubate for **25 ± 5 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm).
⚠	***Avoid exposure to direct sunlight!***
7.	Add **100 µl** of the **Stop Solution** to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
8.	**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).

7. Calculation of results

Measuring range	Serum	7.4 – 900 µg/l

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.

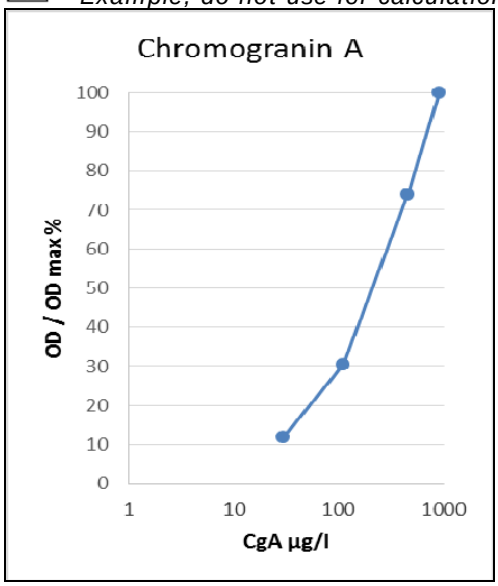
Expected reference value	Serum	< 100 µg/l

7.1 Quality control

The confidence limits of the kit controls are listed in the QC-Report.

7.2 Typical standard curve

⚠ *Example, do not use for calculation!*



8. Assay characteristics

Analytical Sensitivity	**Serum**
Limit of Detection (LOD)	6.5 µg/l
Limit of Quantification (LOQ)	7.4 µg/l

Precision – Intra Assay Variation		
Serum, n = 13		
Sample	Mean ± SD (µg/l)	CV (%)
1	26.6 ± 1.2	4.7
2	50.2 ± 2.6	5.1
3	112.8 ± 4.8	4.3
4	329.8 ± 15.6	4.8
5	545.3 ± 30.3	5.6

Precision – Inter Assay Variation		
Serum, n = 10		
Sample	Mean ± SD (µg/l)	CV (%)
1	55.1 ± 5.7	10.3
2	119.1 ± 13.1	11.0
3	331.7 ± 31.1	9.4

Recovery		Range (µg/l)	Range (%)	Mean (%)
	Serum	26.6 – 545.3	93 - 96	95

Linearity		Serial dilution up to	Range (%)	Mean (%)
	Serum	1:1024	94 - 109	101

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 Kryptor CgA II	Kryptor = 0.96 x(ELISA) + 25.2; r^2 = 0.91 ; n = 97

9. References/Literature

(1) Deftos, L. J.. Chromogranin A: its role in endocrine function and as an endocrine and neuroendrocrine tumor marker. Endocr Rev 12(2): 181-187 (1991)

(2) Ramage, J. K., A. Ahmed, et al. (2012). "Guidelines for the management of gastroenteropancreatic neuroendocrine (including carcinoid) tumors (NETs)". Gut 61(1): 6-32 (2012)

(3) Bilek, R., L. Safarik, et al. "Chromogranin A, a member of neuroendocrine secretory as a selective marker for laboratory diagnosis of pheochromocytoma." Physiol Res 57(1): 171-179: (2008)

(4) Cătălina Poiană et al. The neuroendocrine markers assay and the glycemia profile in patients with neuroendocrine tumors under octreotide therapy: a 2 years study. Revista Română de Medicină de Laborator, Vol. 22(3):369-375 (2014)

(5) Giovinazzo et al. Chromogranin A and its fragments as regulators of small intestinal neuroendocrine neoplasmn proliferation. PloS One, 8(11):e81111 (2013)

(6) Bieglmayer. Chromogranin A: Ein universieller Marker für neuroendokrine Tumoren. Journal of Clinical Endocrinology and Metabolism 3 (4):8-14 (2010)

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

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code		
	Consult instructions for use	CONT	Content		
	Caution	REF	Catalogue number	RUO	For research use only!