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Instructions for use / Gebrauchsanweisung

Chromogranin A ELISA

REF **TM E-9000**

2°C 8°C | Σ 96 | IVD | CE

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1. Introduction

1.1 Intended use and principle of the test

Enzyme immunoassay for the quantitative determination of Chromogranin A in serum. The determination of Chromogranin A helps in the detection of neuroendocrine tumors and is used to assess the course of the cancer treatment.
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. Manual processing is recommended. The use of automatic laboratory equipment is the responsibility of the user. This IVD is for professional use only.

1.2 Clinical application

Chromogranin A (CgA) is an acid glycoprotein with 439 amino acids that is present in the secretory dense core granules of most neuroendocrine cells [1]. The chromogranin family consists of at least three different water-soluble acidic glycoproteins (CgA, CgB, and secretogranin II, sometimes called Chromogranin C) [1].
Upon stimulation, CgA and other peptide hormones and neuropeptides are released. CgA is also secreted from neuroendocrine-derived tumors [1].
Neuroendocrine tumors (NETs), which originate from neuroendocrine cells, are found widely distributed throughout the body [2]. The most common sites of NET are the lung, stomach, appendix, cecum, duodenum, pancreas, jejunum/ileum, colon and rectum [3]. NET arising from the gastrointestinal tract are collectively known as gastroenteropancreatic neuroendocrine tumors (GEP-NET) and account for approximately 2/3 of incident NET [3]. The annual incidence of NET is estimated as 2 – 5 cases per 100,000 population [2].
CgA is widely expressed throughout the neuroendocrine system and serves as a general biomarker for a wide variety of neuroendocrine tumors [3]. The determination of Chromogranin A helps in the detection of neuroendocrine tumors and is used to assess the course of cancer treatment [3 – 6].

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 must 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) The principles of Good Laboratory Practice (GLP) must be followed.
(4) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.
(5) If serious incidents should occur in connection with this product, they should be reported to the manufacturer and the competent national authorities.
(6) All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water. Avoid repeated freezing and thawing of reagents and specimens.
(7) The microplate contains snap-off strips. Unused wells must be stored at 2 – 8 °C in the sealed foil pouch with desiccant and used in the frame provided. Microtiter strips which are removed from the frame for usage should be marked accordingly to avoid any mix-up.
(8) Duplicate determination of sample is highly recommended.
(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 for use 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 provided with the kit.
(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.
(17) For information about hazardous substances included in the kit please refer to Safety Data Sheet (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.
(19) The expected reference values reported in this test instruction are only indicative. It is recommended that each laboratory establishes its own reference intervals.
(20) In case of any severe damage to the test kit or components, the manufacturer has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components must not be used for a test run. They must be stored properly until the manufacturer decides what to do with them. If it is decided that they are no longer suitable for measurements, they must be disposed of in accordance with national regulations.
(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) 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.

2.2 Limitations

Any inappropriate handling of samples or modification of this test might influence the results.
In slightly more than 10% of the samples used in the method comparison, a discrepancy was detected between the Kryptor II and the ELISA measurements. These were samples whose CgA concentrations were in the range of 350 – 900 µg/l.
The sequence of the specific antibodies used was checked for possible cross-reactions. Even if no significant cross-reactivities could be detected, it cannot be excluded that in rare individual cases and depending on medication or disease status, influences on the values may occur.
If the Chromogranin A determination is used as part of a patient's follow-up, we therefore recommend the following procedure:

- A patient's sample should always be examined using the same method during the course of his treatment.
- In case of abnormalities during the follow-up, it should be investigated whether changes in medication or lifestyle have taken place.

If you have any further questions, please contact the manufacturer.

2.2.1 Interfering substances

Serum samples containing precipitates or fibrin strands might cause inaccurate results. Biotin (up to 1,200 ng/ml), hemolytic samples (up to 1 mg/ml hemoglobin), icteric samples (up to 50 mg/dl bilirubin) and lipemic samples (up to 1,700 mg/dl triglycerides) have no influence on the assay results. When in doubt, it is recommended that hemolytic, icteric, and lipemic samples not be used in the assay.

2.2.2 Drug interferences

Medications like proton pump inhibitors, selective serotonin reuptake inhibitors, histamine type-2 receptor antagonists and somatostatin analogues can influence CgA level in serum.

2.2.3 High-Dose-Hook effect

No hook effect was observed in this test.

3. Storage and stability

Store kit and 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 2 months when stored at 2 – 8 °C. Once the resealable pouch has been opened, care should be taken to close it tightly again including the desiccant.

4. Materials

4.1 Contents 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, purple cap
BA E-0055	**SUBSTRATE** **Substrate** – ready to use
Content:	Chromogenic substrate containing 3,3',5,5'-tetramethylbenzidine, substrate buffer and hydrogen peroxide
Volume:	1 x 12 ml/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, grey cap
Hazards identification:	
	H290 May be corrosive to metals.
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
Description:	Species is rabbit
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
Description:	Species of protein in the buffer is bovine
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
Description:	Species is rabbit

4.2 Calibration and Controls

Standards and Controls – ready to use

Cat. no.	Component	Colour/Cap	Concentration [µg/l] CgA	Volume/ Vial
TM E-9001	**STANDARD A**	white	0	1 ml
TM E-9002	**STANDARD B**	yellow	30	1 ml
TM E-9003	**STANDARD C**	orange	110	1 ml
TM E-9004	**STANDARD D**	blue	450	1 ml
TM E-9005	**STANDARD E**	grey	900	1 ml
TM E-9051	**CONTROL 1**	green	Refer to QC-Report for expected value and acceptable range.	1 ml
TM E-9052	**CONTROL 2**	red		1 ml
Content:	Assay Buffer with a defined quantity of human Chromogranin A and stabilizing protein			
Description:	Chromogranin A is derived from human, the stabilizing protein is from bovine origin			

4.3 Additional materials required but not provided in the kit

- Absorbent material (paper towel)
- Water (deionized, distilled, or ultra-pure)

4.4 Additional equipment required but not provided in the kit

- Calibrated precision pipettes to dispense volumes between 20 – 400 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)
- Vortex mixer

5. Sample collection and storage

Serum

Collect blood by venipuncture, allow to clot, and separate serum by centrifugation according to manufacturer's instructions. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.
When in doubt, it is recommended that hemolytic, icteric, and lipemic samples not be used in the assay (see 2.2.1). If the samples are not used immediately for the assay, they must be stored frozen.
Storage: for longer period (up to 6 months) at -20 °C.
Repeated freezing and thawing should be avoided.

6. Test procedure

Allow all reagents and samples to reach room temperature and mix thoroughly by gentle inversion before use. Determinations in duplicate are recommended. Number the microwell plates (microtiter strips which are removed from the frame for usage should be marked accordingly to avoid any mix-up).
The binding of the antisera and of the enzyme conjugate and the activity of the enzyme are temperature dependent. The higher the temperature, the higher the absorption values will be. Varying incubation times will have similar influences on the absorbance. The optimal temperature during the enzyme immunoassay is between 20 – 25 °C.

⚠ *The use of a microtiter plate shaker with the following specifications is mandatory: shaking amplitude 3 mm; approx. 600 rpm. Shaking with differing settings might influence the results.*

6.1 Preparation of reagents and further notes

Wash Buffer

Dilute the 20 ml Wash Buffer Concentrate **WASH-CONC** **50X** with water to a final volume of 1000 ml.
Storage: 2 months at 2 – 8 °C

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 Preparation of samples – Dilution

1.	Prior to use, the serum samples have to be diluted **1+20** with **ASSAY-BUFF** e.g. 20 µl of serum sample + 400 µl of **ASSAY-BUFF**.
	Serum samples which have been found off-curve should also be diluted accordingly with **ASSAY-BUFF** and re-assayed.

6.3 Chromogranin A ELISA

1.	Pipette **50 µl** of the **standards, controls and diluted samples** into the wells of the **Chromogranin A Microtiter Strips** **96** 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 times** 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 **CONJUGATE** into each well 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 times** 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 each well.
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-SOLN** 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 min, 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	**Chromogranin A in serum**
	2.3 – 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) using a concentration of 0.001 µg/l for Standard A (this alignment is mandatory because of the logarithmic presentation of the data).
Use non-linear regression for curve fitting (e. g. 4-parameter, marquardt).
The concentrations of the **samples and controls** can be read directly from the standard curve.
Samples found with concentrations higher than the highest standard (Standard E) should be diluted accordingly with **ASSAY-BUFF** and have to be re-assayed.

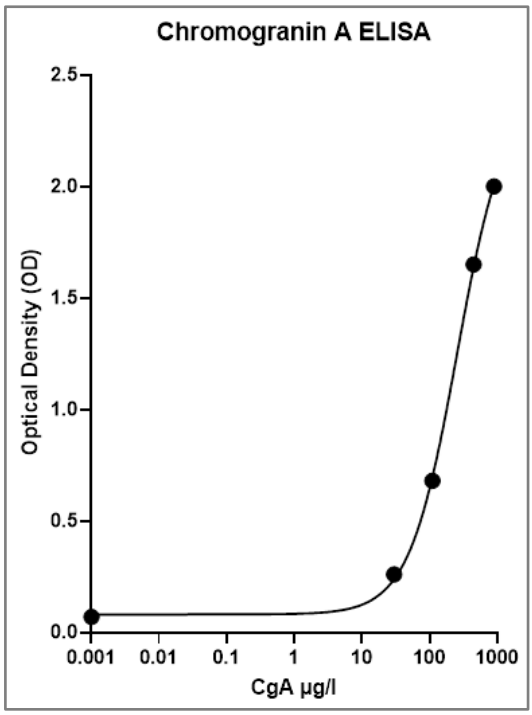
7.1 Expected reference value

It is strongly recommended that each laboratory should determine its own reference values.

	Chromogranin A in serum
Reference value (ULN) [7]	< 100 µg/l
Typical pathological range [7]	Up to 143,500 µg/l

7.2 Typical standard curve

⚠ *Example, do not use for calculation!*



8. Quality control

It is recommended to use control samples according to national regulations. Use controls at both normal and pathological levels. Commercially obtained control samples should be treated like unknown samples. Control samples should fall within established confidence limits. The confidence limits of the kit controls are printed on the QC-Report.

9. Assay characteristics

9.1 Performance data

Precision					
Intra-Assay			**Inter-Assay**		
n = 12			n = 10		
Sample	Mean ± SD [µg/l]	CV [%]	Sample	Mean ± SD [µg/l]	CV [%]
1	43.6 ± 1.2	2.8	1	73.0 ± 3.8	5.2
2	73.5 ± 3.0	4.2	2	102 ± 3.5	3.5
3	103 ± 3.4	3.3	3	161 ± 5.7	3.6
4	161 ± 10.1	6.3	4	300 ± 16.0	5.3
5	283 ± 14.6	5.1			
6	502 ± 15.9	3.2			

Analytical Sensitivity	
Limit of Blank (LOB)	0.9 µg/l
Limit of Detection (LOD)	1.4 µg/l
Limit of Quantification (LOQ)	2.3 µg/l

Recovery			
	Range [µg/l]	Mean [%]	Range [%]
Chromogranin A	43.6 – 502	101	95 – 104

Linearity			
	Serial Dilution up to	Mean [%]	Range [%]
Chromogranin A	1:64	92	91 – 96

Method Comparison: B.R.A.H.M.S CgA II Kryptor	CgA ELISA = 1.05 x (Kryptor) – 15; R^2 = 0.97; n = 57

Lot-to-Lot			
	Sample	Range [ng/ml] mean ± SD	CV [%]
Chromogranin A in serum (n = 4)	1	42.5 ± 2.5	6
	2	111 ± 3.2	3
	3	500 ± 20.9	4

Diagnostic Performance [7] GEP-NET			
Diagnostic Specificity [%]	Diagnostic Sensitivity [%]	Positive Predictive Value (PPV) [%]	Negative Predictive Value (NPV) [%]
83	56	87	49
Positive Likelihood Ratio (LR+)		Negative Likelihood Ratio (LR-)	
3.3		0.53	

9.2 Metrological Traceability

The values assigned to the standards and controls of the Chromogranin A ELISA are traceable to the reference method B.R.A.H.M.S CgA II Kryptor.

Standards and Controls	Uncertainty [%]
	7.5

Chromogranin A ELISA	Expanded Uncertainty [%] k=2*
	16.5

* This defines an interval about the measured result that will include the true value with a probability of 95%

10. References/Literature

1. O'Toole, D., et al., *ENETS Consensus Guidelines for the Standards of Care in Neuroendocrine Tumors: biochemical markers.* Neuroendocrinology, 2009. **90**(2): p. 194-202.
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3. Singh, S. and C. Law, *Chromogranin A: a sensitive biomarker for the detection and post-treatment monitoring of gastroenteropancreatic neuroendocrine tumors.* Expert Rev Gastroenterol Hepatol, 2012. **6**(3): p. 313-34.
4. Louthan, O., *Chromogranin a in physiology and oncology.* Folia Biol (Praha), 2011. **57**(5): p. 173 – 81.
5. Yang, X., et al., *Diagnostic value of circulating chromogranin a for neuroendocrine tumors: a systematic review and meta-analysis.* PLoS One, 2015. **10**(4): p. e0124884.
6. Corti, A., F. Marcucci, and T. Bachetti, *Circulating chromogranin A and its fragments as diagnostic and prognostic disease markers.* Pflugers Arch, 2018. **470**(1): p. 199 – 210.
7. van Treijen, M.J.C., et al., *Blood Transcript Profiling for the Detection of Neuroendocrine Tumors: Results of a Large Independent Validation Study.* Front Endocrinol (Lausanne), 2018. **9**: p. 740.

For updated literature or any other information please contact your local supplier.
The summary of safety and performance according to article 29 of regulation (EU) 2017/746 can be downloaded from the website www.ldn.de.

11. Changes

Version	Release Date	Chapter	Change
18.0	2021-07-09	All	– Revision of the assay due to lot-change of the matched antibody pair used – The IFU was revised according to the IVDR regulation (EU) 2017/746 – Sample Dilution changed from 1+8 to 1+20 (Chapter 6.2) – Typical pathological range was added (Chapter 7.1) – Assay characteristics changed (Chapter 9.1) – Lot to Lot and diagnostic performance was added to the assay characteristics – Metrological traceability was added (Chapter 9.2) – References/Literature was updated (Chapter 10)
19.0	2022-06-27	2.2.2	– Medications that can influence chromogranin level have been updated. – Editorial changes

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Use-by date	LOT	Batch code	IVD	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content	CE	CE marking of conformity
	Caution	REF	Catalogue number		Distributor
	Date of manufacture				