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LDN®

Instructions for use

Chromogranin A ELISA

REF TM E-9000

Σ 96

+2 +8 °C

IVD

CE

Chromogranin A ELISA

1. Intended use and principle of the test

Enzyme Immunoassay for the quantitative determination of 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. 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 calibration curve the CgA concentrations in the samples are determined.

2. Procedural Cautions, Guidelines and Warnings

1. This kit is 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 can not be taken liable.
3. Reagents of kits which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA 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 latex 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. When the use of water is specified for dilution or reconstitution, use deionized, or 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 and used in the frame provided.
9. It is highly recommended to determine samples in duplicate 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 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 sequences.
12. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
13. A calibrator curve must be established for every run.
14. The controls should be included in every run and fall within established confidence limits. The confidence limits are listed in the QC-report.
15. Do not mix various lot numbers of kit components 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 H_2SO_4. It may cause skin irritation and burns. In case of contact with eyes or skin, flush immediately with water.
17. Some reagents contain sodium azide (NaN3) as preservatives. In case of contact with eyes or skin, flush 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.
23. In case of complaints please submit to the manufacturer a written report (the corresponding form is available upon request) containing all data as to how the test was conducted, the results received and a copy of the original test printout.

3. Storage and stability

Store the reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Do not mix various lots of any kit component within an individual assay.

4.1 Contents of the kit

BA E-0030	WASH-CONC 50x	**Wash Buffer Concentrate**	1 x 20 mL	Concentrate. Dilute content with dist. water to a final volume of 1000 mL
BA E-0055	SUBSTRATE	**Substrate**	1 x 12 mL	ready for use, containing a solution of tetramethylbenzidine (TMB)
BA E-0080	STOP-SOLN	**Stop Solution**	1 x 12 mL	ready for use, containing 0.25 M H_2SO_4
TM E-9001	STANDARD A	**Standard A**	1 x 1 mL	ready for use
TM E-9002	STANDARD B	**Standard B**	1 x 1 mL	ready for use
TM E-9003	STANDARD C	**Standard C**	1 x 1 mL	ready for use
TM E-9004	STANDARD D	**Standard D**	1 x 1 mL	ready for use
TM E-9005	STANDARD E	**Standard E**	1 x 1 mL	ready for use
TM E-9010	CONJUGATE	**Antibody-Conjugate**	1 x 6 mL	ready for use, CgA- antibody conjugated with peroxidase
TM E-9013	ASSAY-BUFF	**Assay-Buffer**	1 x 50 mL	ready for use
TM E-9031	96	**Chromogranin A Microtiter Strips**	1 plate	12 strips, 8 wells each, break apart, pre-coated with CgA- antibody
TM E-9051	CONTROL 1	**Control 1**	1 x 1 mL	ready for use
TM E-9052	CONTROL 2	**Control 2**	1 x 1 mL	ready for use

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

- Calibrated variable precision micropipettes (e.g. 10-100 µL /100-1000 µL)
- Microtiter plate washing device
- ELISA reader capable of reading absorbance at 450 nm and 620 or 650 nm
- Shaker (shaking amplitude 3mm; approx. 600 rpm)
- Absorbent material (paper towel)
- Distilled water
- Vortex mixer

5. Sample collection and storage

Serum or EDTA plasma samples can be used with this kit.
Haemolytic and lipemic samples should not be used with this assay.
Storage: up to 1 week at 2 - 8°C; for longer periods (up to 12 months) at - 20°C.
Repeated freezing and thawing should be avoided.

6. Test procedure

Allow all reagents and samples to reach room temperature prior to use.
The measurement in duplicates is recommended.

6.1 Preparation of reagents and samples

Wash Buffer
Dilute the 20 mL Wash Buffer Concentrate with water (deionized, distilled, or ultra-pure water) to a final volume of 1000 mL.
Storage: up to 6 months 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.

6.3 Chromogranin A ELISA

1.	Pipette **50 µL** of the **standards, controls and diluted samples** into the wells of the **Chromogranin A Microtiter Strips**.
2.	Pipette **50 µL** of the **Antibody-Conjugate** into all wells.
3.	Incubate **2 hours** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
4.	Discard or aspirate the contents of the wells. **Wash** each well thoroughly **4 x** with **300 µL Wash Buffer** and blot 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 sun light!***
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** and – if available – with a reference wavelength between 620 nm and 650 nm.

7. Calculation of results

	Concentration of the standards				
Standard	**A**	**B**	**C**	**D**	**E**
Chromogranin A [µg/l]	0	50	150	500	1500

The calibration 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.

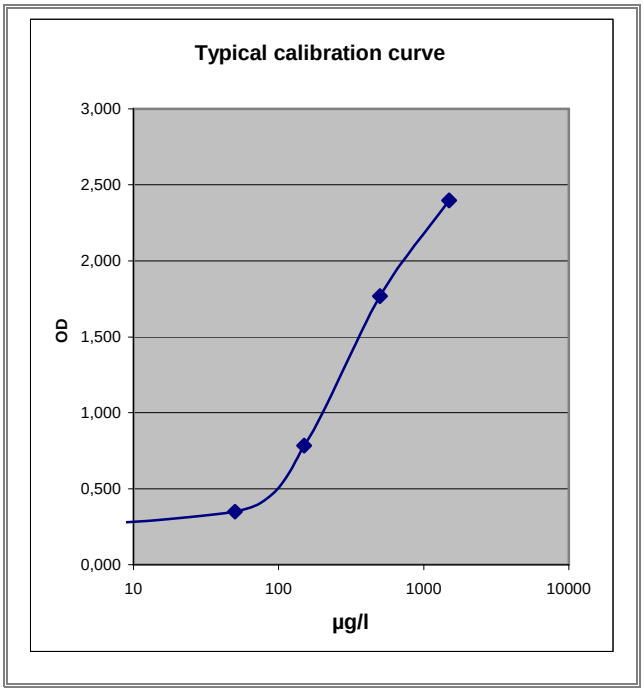
Samples found off-curve should be diluted with **Assay Buffer** and re-assayed.

7.1 Quality control
It is recommended to use control samples according to state and federal 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 printed on the QC-Report.

7.2 Calibration
The binding of the antisera and the enzyme conjugates and the activity of the enzyme used are temperature dependent, and the extinction values may vary if a thermostat is not used. The higher the temperature, the higher the extinction 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*

7.3 Typical calibration curve (*Example - do not use for calculation!)*



8. Assay characteristics

Expected Reference Values	Serum < 100 µg/l Plasma < 125 µg/l

Analytical Sensitivity (Limit of Detection = mean value of 0-standard + 3xSD)	12.4 µg/l

Precision					
Inter-Assay Variation, n = 8			**Intra-Assay Variation**, n = 78		
Sample	Mean ± SD (µg/l)	CV (%)	Sample	Mean ± SD (µg/l)	CV (%)
1	102.4 ± 7.1	6.9	1	37.5 ± 4.2	11.3
2	301 ± 12.7	4.2	2	128 ± 7.8	6.1

Recovery		Range (µg/l)	Range (%)	Mean (%)
	Chromogranin A	82 - 540	89 - 94	91

Linearity		Range (µg/l)	Range (%)	Mean (%)
	Chromogranin A	27 - 433	102 - 140	117

High-dose hook effect	Chromogranin A concentrations up to 200,000 µg/l will not show a high-dose hook effect

Method comparison versus RIA*	ELISA = 0.81x+43.9; r^2 = 0.83; n = 51

***** commercially available RIA

⚠ ***For updated literature, information about clinical significance or any other information please contact your local supplier.***

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code	I V D	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content	CE	CE labelled
	Caution	REF	Catalogue number	RUO	For research use only!