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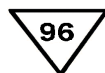


Instructions for use

Chromogranin A ELISA

REF

TM E-9000



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 biotinylated CgA antibodies. The wells are washed thoroughly and incubated with horseradish peroxidase conjugated streptavidin. After another washing step 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. **Advice on handling the test**

2.1 **Reliability of the test results**

In order to assure a reliable evaluation of the test results it must be conducted according to the instructions included and in accordance with current rules and guidelines (GLP, RILIBÅK, etc.). Special attention must be paid to control checks for precision and correctness during the test; the results of these control checks have to be within the norm range. In case of significant discrepancies between the pre-set assay characteristics of this test and the actual results please contact the manufacturer of the test kit for further instructions.

It is recommended that each laboratory establishes its own reference intervals. The values reported in this test instruction are only indicative.

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.

2.2 **Complaints**

In case of complaints please submit to the manufacturer a written report containing all data as to how the test was conducted, the results received and a copy of the original test printout. Please contact the manufacturer to obtain a reclamation form and return it completely filled in to the manufacturer.

2.3 **Warranty**

This test kit was produced according to the latest developments in technology and subjected to stringent internal and external quality control checks. Any alteration of the test kit or the test procedure as well as the usage of reagents from different charges may have a negative influence on the test results and are therefore not covered by warranty. The manufacturer is not liable for damages incurred in transit.

2.4 **Disposal**

Residual substances and/or all remaining chemicals, reagents and ready for use solutions, are special refuse. The disposal is subject to the laws and regulations of the federation and the countries. About the removal of special refuse the responsible authorities or refuse disposal enterprises inform. The disposal of the kit must be made according to the national official regulations. Legal basis for the disposal of special refuse is the cycle economic- and waste law.

The appropriate safety data sheets of the individual products are available on the homepage. The safety data sheets correspond to the standard: ISO 11014-1.

2.5 **Interference**

Do not mix reagents and solutions from different lots. Consider different transport and storage conditions. Inappropriate handling of test samples or deviations from the test regulation can the results affect. Use no kit components beyond the expiration date. Avoid microbiological contamination of the reagents and the washing water. Consider incubation periods and wash references.

2.6 **Precautions**

Observe the incubation periods and washing instructions. Never pipette by mouth and avoid contact of reagents and specimens with skin. No smoking, eating or drinking in areas where samples or kit test tubes are handled. When working with kit components or samples, always wear protective gloves and wash your hand thoroughly as soon as you have finished the work. Avoid spraying of any kind. Avoid any skin contact with reagents. Use protective clothing and disposable gloves. All steps have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes. Sodium azide could react with lead and copper tubes and may form highly explosive metal azide. When clearing up, rinse thoroughly with large volumes of water to prevent such formation.

All reagents of this testkit which contain human or animal 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.

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 ₂ SO 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-9013	ASSAY-BUFF	Assay-Buffer	1 x 50 mL	ready for use
TM E-9031	TM 96	Chromogranin A Microtiter Strips	1 plate	12 strips, 8 wells each, break apart, pre-coated with CgA- antibody
TM E-9040	CONJUGATE	Conjugate	1 x 12 mL	ready for use, streptavidin conjugated with peroxidase
TM E-9041	BIOTIN-AB	Biotin-Antibody	1 x 12 mL	ready for use, Biotin-CgA- antibody
TM E-9050	ADJUST-BUFF	Adjustment Buffer	1 x 4 mL	ready for use
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 especially 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 distilled 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+10 with assay buffer (e.g. 25 µL of sample + 250 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 25 µL of the standards, controls and diluted samples into the wells of the Chromogranin A Microtiter Strips .
2.	Pipette 25 µL of the Adjustment Buffer into all wells.
3.	Pipette 100 µL of the Biotin-Antibody into all wells.
4.	Incubate 60 min at RT (20-25°C) on a shaker (approx. 600 rpm).
5.	Discard or aspirate the contents of the wells and wash each well 3 times thoroughly with 300 µL Wash Buffer . Blot dry by tapping the inverted plate on absorbent material.
6.	Pipette 100 µL of the Conjugate into all wells.
7.	Incubate for 15 min at RT (20-25°C) on a shaker (approx. 600 rpm).
8.	Discard or aspirate the contents of the wells and wash each well 3 times thoroughly with 300 µL Wash Buffer . Blot dry by tapping the inverted plate on absorbent material.
9.	Pipette 100 µL of the Substrate into all wells.
10.	Incubate for 15 ± 2 min at RT (20-25°C) on a shaker (approx. 600 rpm).  Avoid exposure to direct sun light!
11.	Add 100 µL of the Stop Solution to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
12.	Read the absorbance of the solution in the wells within 10 minutes, using a microtiter plate reader set to 450 nm and a reference wavelength between 620 nm and 650 nm.

7. Calculation of results

Standard	Concentration of the standards				
	A	B	C	D	E
Chromogranin A [µg/l]	0	50	150	500	1500
Conversion:	[µg/l] = [ng/mL]				

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 which have been found off-curve should be diluted accordingly with assay buffer and re-assayed.

7.1 Quality control

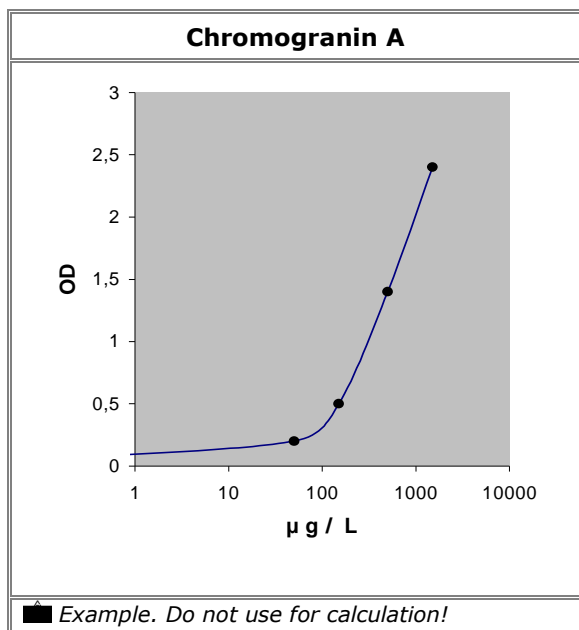
It is recommended to use control samples according to state and federal regulations. Use controls at both normal and pathological levels. The kit, or other commercially available controls should fall within established confidence limits. The confidence limits of the kit controls are printed on the QC-Report.

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



8. Assay characteristics

Expected Reference Values	< 100 µg/l
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Analytical Sensitivity (Limit of Detection)	10 µg/l	Mean signal (Zero-Standard) - 2SD
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Precision					
Inter-Assay Variation, n = 13			Intra-Assay Variation, n = 39		
Sample	Mean ± SD (µg/l)	CV (%)	Sample	Mean ± SD (µg/l)	CV (%)
1	76 ± 6.2	8.2	1	96 ± 8.9	9.3
2	674 ± 65	9.6	2	464 ± 45	9.7

Linearity		Range (µg/l)	Range (%)	Mean (%)
	Chromogranin A	50 - 1600	84 - 116	100

Recovery		Serial dilution up to	Range (%)	Mean (%)
	Chromogranin A	1:16	92 - 120	106







High-dose hook effect	No high-dose hook effect up to 2 000 000 µg/l
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Method comparison versus RIA*	RIA = 0.86 ELISA + 28; r = 0.999; n = 128
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* commercially available RIA

 **For actual 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	IVD	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content	CE	CE labelled
	Caution	REF	Catalogue number	RUO	For research use only!