

EURIA-CHROMOGRANIN A

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Chromogranin A radioimmunoassay

(Cat. No. RB 321 US)

100 tubes

For Research Use Only – Not for Use in Diagnostic Procedures

Doc. no. US-E-23-0045-14

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INTRODUCTION

Chromogranins and secretogranins constitute a family of uniquely acidic proteins that are co-stored with neurotransmitters and peptide hormones in the brain and the diffuse neuroendocrine system (Winkler, H. & Fischer-Colbrie, R.1992). Structurally these proteins are products of different genes but share some overall properties such as an abundance of acidic amino acid residues and several pairs of basic amino acids as potential positions for post-translational cleavage. Chromogranins are co-stored and co-released with neuropeptides and hormones in the neuroendocrine cells throughout the body. A role for chromogranins in the generation of hormonal granules and package of hormones has been suggested. Furthermore, chromogranins can be cleaved into smaller fragments, which can display biological activities such as inhibition of hormonal release, vasodilatation and anti-microbiological effects.

Tumours of neuroendocrine origin usually present with increased serum/plasma levels of chromogranin A. The neuroendocrine tumours are derived from the neuroendocrine cells and typical neuroendocrine tumours are carcinoid tumours, pheochromocytomas, neuroblastomas, small cell lung cancers, hyperparathyroid adenomas, pituitary tumours, prostate cancers and pancreatic islet tumours and including the MEN1 and MEN2 syndromes. This also includes the different neuroendocrine tumour syndromes, namely the gastrinomas, insulinomas, glucagonomas, somatostatinomas, PPomas and the non-functioning neuroendocrine tumours (Eriksson, B. et al. 2000). For these tumours, chromogranin A has been shown to be the best circulating marker (Bajetta, E. et al. 1999).

The first radioimmunoassay for measurements of chromogranin A was introduced in 1986 (O'Connor, D.T. & Deftos, L.J. 1986). Since then other assays for measurements of intact human chromogranin A have been reported. Assays for measurements of defined regions of chromogranin A have also been established, such as specific methods for pancreastatin and other regions of chromogranin A (Stridsberg, M. 2000).

The present chromogranin A is a competitive method based on polyclonal antibodies raised in rabbits. The antibodies were raised against a purified fragment containing amino acid sequence 116-439 in the chromogranin A molecule.

PRINCIPLE OF THE METHOD

The intended use of these reagents is the determination of chromogranin A in human serum or plasma. The basic principle for determination of chromogranin A with the EURO-DIAGNOSTICA chromogranin A RIA kit is competitive radioimmunoassay using antibodies against human chromogranin A.

Chromogranin A in standards and samples compete with ¹²⁵I-labelled chromogranin A in binding to the antibodies. The ¹²⁵I-chromogranin A binds to the antibodies in an inverse proportion to the concentration of chromogranin A in standards and samples. Antibody-bound ¹²⁵I-chromogranin A is separated from the unbound fraction using the double antibody solid phase technique. The bound fraction of ¹²⁵I-chromogranin A is measured in a gamma counter. For professional use within a laboratory. For research use only – not for use in diagnostic procedures.

PHYSIOLOGICAL CONSIDERATIONS

Tumours of neuroendocrine origin usually present with increased serum/plasma levels of chromogranin A. The neuroendocrine tumours are derived from the neuroendocrine cells. Typical neuroendocrine tumours are carcinoid tumours, pheochromocytomas, neuroblastomas, small cell lung cancers, hyperparathyroid adenomas, pituitary tumours, prostate cancers and pancreatic islet tumours and including the MEN1 and MEN2 syndromes. This also includes the different neuroendocrine tumour syndromes, namely the gastrinomas, insulinomas, glucagonomas, somatostatinomas, PPomas and the non-functioning neuroendocrine tumours.

Reference range, serum: < 6.0 nmol/L.

The reference range was set by testing 122 blood donors.

The upper range < 6.0 nmol/L was set as the 97.5 percentile.

It is recommended that users establish reference ranges for the populations served by their own laboratories.

Non-tumour associated increases of chromogranin A

Increased levels of chromogranin A can be seen in individuals with decreased renal function, atrophic gastritis and with ongoing treatment with proton-pump inhibitory drugs.

PRECAUTIONS

For research use only – not for use in diagnostic procedures.

As the regulations may vary from one country to another, it is essential that the person responsible for the laboratory is familiar with current local regulations, concerning all aspects of radioactive materials of the type and quantity used in this test.

This kit contains components of human origin. They have been tested by immunoassay for hepatitis B surface antigen, antibodies to HCV and for antibodies to HIV-1 and HIV-2 and found to be negative. Nevertheless, all recommended precautions for the handling of blood derivatives should be observed.

This kit contains ^{125}I (half-life: 60 days), emitting ionizing X (28 keV) and γ (35.5 keV) radiations. Steps should be taken to ensure the proper handling of the radioactive material, according to local and/or national regulations. Only authorized personnel should have access to the reagents.

The following precautions should be observed when handling radioactive materials:

- Radioactive material should be stored in specially designated areas, not normally accessible to unauthorized personnel.
- Handling of radioactive material should be conducted in authorized areas only.
- Care should be exercised to prevent ingestion and contact with the skin and clothing. Do not pipette radioactive solutions by mouth.
- Drinking, eating or smoking should be prohibited where radioactive material is being used.
- Hands should be protected by gloves and washed after using radioactive materials.
- Work should be carried out on a surface covered by disposable absorbing material.
- Spills of radioactive material should be removed immediately, and all contaminated materials disposed as radioactive waste. Contaminated surfaces should be cleaned with a detergent.

The reagents in this kit contain sodium azide. Contact with copper or lead drainpipes may result in the cumulative formation of highly explosive azide deposits. On disposal of the reagents in the sewerage, always flush with copious amounts of water, which prevents metallic azide formation. Plumbing suspected of being contaminated with these explosive deposits should be rinsed thoroughly with 10% sodium hydroxide solution.

COMPOSITION OF THE REAGENT KIT

The reagents provided in this kit are sufficient for 100 tubes.

1. Anti-chromogranin A (Reagent A)

Rabbit antiserum to human chromogranin A (amino acids 116-439). The antiserum is diluted and lyophilized in 2.0 mL 0.25 M phosphate buffer, pH 7.4, with 1.0% bovine serum albumin, 0.375 M NaCl, 0.25% NaN₃ and 2.5% Tween 20.

Colour: Yellow. Reconstitution in 11.0 mL distilled water.

2. ¹²⁵I-Chromogranin A (Reagent B)

Activity: 56 KBq (1.5 µCi) on activity reference date. Lyophilized in 2.5 mL 0.25 M phosphate buffer, pH 7.4, with 1.0% bovine albumin, 0.375 M NaCl, 0.25% NaN₃ and 2.5% Tween 20.

Colour: Blue. Reconstitution in 12.5 mL distilled water.

3. Double antibody solid phase (Reagent C)

Anti-rabbit-Ig coupled to cellulose particles. 52 mL suspension.

4. Assay diluent (Reagent D)

50 mL of 0.05 M phosphate buffer, pH 7.4, with 0.2% bovine serum albumin, 0.075 M NaCl, 0.05% NaN₃ and 0.5% Tween 20. Buffer used for dilution of samples, preparation of working standards and for replacement of antiserum in non-specific binding controls.

5. Chromogranin A standard (Reagent E)

Concentration: 10.0 nmol/L

Volume: 2.00 mL standard after reconstitution.

Lyophilized in 5.00 mL 0.05 M phosphate buffer, pH 7.4 with 0.2% bovine serum albumin, 0.075 M NaCl, 0.05% NaN₃ and 0.5% Tween 20.

Reconstitution in 2.00 mL distilled water.

6. Controls (Reagent F-G)

Lyophilized controls with two different levels of chromogranin A. 1.00 mL of each control after reconstitution. The chromogranin A concentrations are given on the labels of the vials. The controls should not be diluted after reconstitution.

Reconstitution in 1.00 mL distilled water.

REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

Distilled water.

Disposable test tubes 11-13 x 55 mm, (polystyrene).

Pipettes with disposable tips, 50, 100 and 500 µL.

Volumetric pipettes 1.00 and 5.00 mL

Vortex mixer.

Centrifuge, refrigerated, minimum g-force 1700 x g.

Gamma counter.

REAGENT PREPARATION AND STORAGE

Store all reagents at 2-8° C before reconstitution and use. The water used for reconstitution of the lyophilized reagents should be distilled in an all-glass apparatus or be of corresponding purity. Dissolve the contents in the vials by gentle inversion and avoid foaming. The stability of the reagents is found on the labels of the vials. For lyophilized reagents the expiry date is valid for the unreconstituted reagents. Reconstituted reagents are stable for 12 weeks.

Reagent A: Anti-chromogranin A

Reconstitute with 11.0 mL distilled water.
Store at 2-8° C.

Reagent B: ¹²⁵I-Chromogranin A

Reconstitute with 12.5 mL distilled water.
Store at -20° C or lower if reused.

Reagent C: Double antibody solid phase

Ready for use. Stir continuously during pipetting this reagent.
Store at 2-8° C.

Reagent D: Assay diluent

Ready for use.
Store at 2-8° C.

Reagent E: Chromogranin A standard

Reconstitute with 2.00 mL distilled water.
Store at -20° C or lower if reused.

Reagent F-G: Controls

Reconstitute each vial with 1.00 mL distilled water.
Store at -20° C or lower if reused.

SPECIMEN COLLECTION

Veinous blood is collected in tubes without additives or in tubes containing Heparin (144 U.S.P. Heparin in a 10 mL tube), EDTA or Lithium. The samples are cooled in an ice-bath. The samples are separated by centrifugation at 2-4° C and stored at -20° C or lower. The samples should be frozen at -20° C within three hours from sample collection.

ASSAY PROCEDURE

Reconstitute the reagents as specified. The reagents should be brought to room temperature prior to use.

Accuracy in all pipetting steps is essential. All tests (standards, controls and samples) should be performed in duplicate.

For an overview see page 14.

A complete assay includes:

Standards (St-tubes): 7 different concentrations, 0, 0.156, 0.313, 0.625, 1.25, 2.50 and 5.00 nmol/L.

Controls (C-tubes): Low and high.

Samples (P-tubes)

Tubes for determination of the **non-specific binding (NSB-tubes)**

Tubes for determination of the **total radioactivity** added (**TOT-tubes**).

Dilution of samples

Samples should be diluted 1:10 with the assay diluent (Reagent D) before assay.

Samples with chromogranin A concentrations more than 50 nmol/L can be diluted further with assay diluent, and re-assayed.

PERFORMANCE

1. Reconstitute the lyophilized reagents according to the instructions on page 8 and allow the reagents to reach room temperature.
2. Prepare the chromogranin A working standards by dilution of the chromogranin A standard 10.00 nmol/L (Reagent E) with assay diluent (Reagent D) according to the following:
 - a. 0.40 mL standard 10.00 nmol/L + 0.40 mL assay diluent = 5.00 nmol/L
 - b. 0.40 mL standard 5.00 nmol/L + 0.40 mL assay diluent = 2.50 nmol/L
 - c. 0.40 mL standard 2.50 nmol/L + 0.40 mL assay diluent = 1.25 nmol/L
 - d. 0.40 mL standard 1.25 nmol/L + 0.40 mL assay diluent = 0.625 nmol/L
 - e. 0.40 mL standard 0.625 nmol/L + 0.40 mL assay diluent = 0.313 nmol/L
 - f. 0.40 mL standard 0.313 nmol/L + 0.40 mL assay diluent = 0.156 nmol/L
 - g. Assay diluent = 0 nmol/LStore the standards at -20°C or lower if reused.
3. Dilute the samples 1:10 with assay diluent e.g. 50 μL sample and 450 μL assay diluent. Vortex-mix carefully.
4. Pipette 100 μL of standards (0-5.00 nmol/L) controls and samples in their respective tubes.
5. Pipette 100 μL of zero-standard (assay diluent) in the NSB-tubes.
6. Pipette 100 μL ^{125}I -chromogranin A (Reagent B) in all tubes. The TOT-tubes are sealed and kept aside.
7. Pipette 100 μL anti-chromogranin A (Reagent A) in all tubes except the NSB-tubes and TOT-tubes.
8. Pipette 100 μL assay diluent (Reagent D) in the NSB-tubes.
9. Vortex-mix all tubes carefully.
10. Incubate for 20-24 hours at $2-8^{\circ}\text{C}$,
11. Pipette 500 μL double antibody solid phase (Reagent C) in all tubes except the TOT-tubes. This reagent should be stirred continuously with a magnetic stirrer during pipetting. Vortex-mix carefully.
12. Incubate for 30-60 minutes at $2-8^{\circ}\text{C}$.
13. Centrifuge for 15 minutes at $+4^{\circ}\text{C}$ (minimum 1700 x g).
14. Decant the supernatants.
15. Count the radioactivity of the pellet in all tubes in a gamma counter. Counting time: 1-3 minutes.

CALCULATION

1. Subtract the average count rate (CPM) of the NSB-tubes from the count rate (CPM) of the standards, controls and samples.
2. A standard curve is generated by plotting the bound fraction CPM or B/TOT against the concentrations of the chromogranin A standards. An example of a standard curve is given on page 15.
3. Interpolate the chromogranin A concentrations of the controls and samples from the generated standard curve. Multiply the found concentrations in the samples with the dilution factor 10 (or actual dilution factors if further dilution has been done).
4. The standard curve and the calculation of the chromogranin A concentrations in samples and controls can also be done by a computer method.

QUALITY CONTROL

In order to enable the laboratory to completely monitor the consistent performance of the assay, the following important factors should be checked.

1. Controls

The found concentrations of the controls (Reagent F-G) should be within the limits given on the labels of the vials.

2. Total counts

Counts obtained should approximate the expected CPM when adjusted for counter efficiency and radioactive decay. The content of ^{125}I -chromogranin A in this kit will give a total counts in the assay (TOT) of 21.000 CPM (-10, +20%) at the activity reference date (counting efficiency = 80%).

3. Maximum binding (Bo/TOT)

Calculate for each assay the % bound radioactivity in the zero-standard: $\frac{\text{Bo}}{\text{TOT}} \times 100\%$.

$\frac{\text{Bo}}{\text{TOT}} \times 100$ is generally 28-42% at the activity reference date.

$\frac{\text{Bo}}{\text{TOT}} \times 100$ may have decreased a few % at the expiry date of the kit.

4. Non-specific binding (NSB/TOT)

Calculate for each assay the non-specific binding $\frac{\text{NSB}}{\text{TOT}} \times 100$.

$\frac{\text{NSB}}{\text{TOT}} \times 100$ is less than 6% if decanting is made properly.

5. Shape of standard curve

For example, monitor the 80, 50 and 20% points of the standard curve for run to run reproducibility.

ASSAY CHARACTERISTICS**Sensitivity**

The lowest detectable concentration in the assay is 0.15 nmol/L. This figure corresponds to a decrease in binding of 2 x SD of the bound radioactivity in the zero-concentration standard. The lowest detectable concentration for a sample diluted 1:10 will be 1.5 nmol/L.

Precision

Intra assay variation:

<u>Level</u>	<u>Coefficient of variation (%CV)</u>	<u>N</u>
0.52 nmol/L	8.2%	32
2.22 nmol/L	7.4%	32

Inter assay variation (total variation):

<u>Level</u>	<u>Coefficient of variation (%CV)</u>	<u>N</u>
0.52 nmol/L	5.5%	10
1.99 nmol/L	6.4%	10

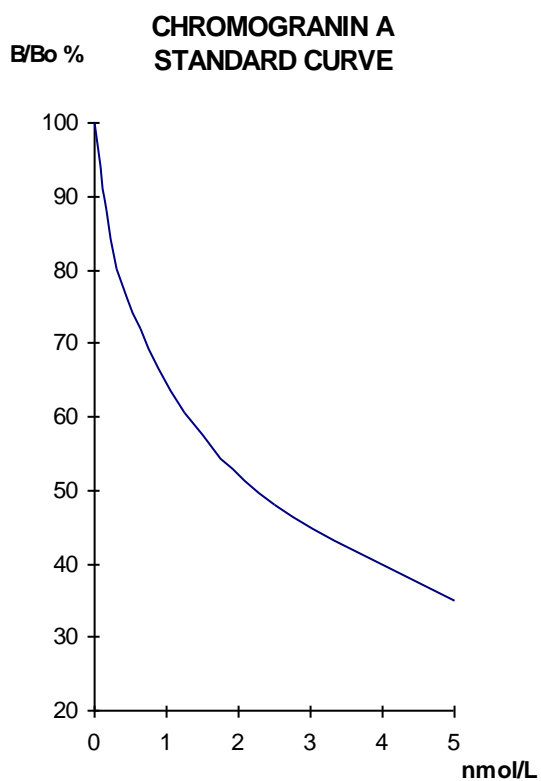
OUTLINE OF THE RIA PROCEDURE

Type of tubes	Tube no	Standard sample or control	¹²⁵ I-Chromogranin A (B)	Anti-Chromogranin A (A)	Assay diluent (D)		Double antibody Solid phase (C)	
TOT	1- 2	-	100 µL	-			-	
NSB	3- 4	100 µL	100 µL	-	100 µL	Vortex-mix and incubate for 20-24 hours at 2-8° C.	500 µL	Vortex-mix and incubate for 30-60 min. at 2-8° C. Centrifuge 15 min. at 1700 x g at +4° C. Decant and count the radio-activity of the pellets.
Stand 0	5- 6	100 µL	100 µL	100 µL	500 µL			
Stand 0.16	7- 8	100 µL	100 µL	100 µL	500 µL			
Stand 0.32	9-10	100 µL	100 µL	100 µL	500 µL			
Stand 0.63	11-12	100 µL	100 µL	100 µL	500 µL			
Stand 1.25	13-14	100 µL	100 µL	100 µL	500 µL			
Stand 2.50	15-16	100 µL	100 µL	100 µL	500 µL			
Stand 5.00	17-18	100 µL	100 µL	100 µL	500 µL			
Control F	19-20	100 µL	100 µL	100 µL	500 µL			
Control G	21-22	100 µL	100 µL	100 µL	500 µL			
Sample 1	23-24	100 µL	100 µL	100 µL	500 µL			
Sample 2	25-26	100 µL	100 µL	100 µL	500 µL			
etc.			100 µL	100 µL	500 µL			

EXAMPLE OF STANDARD CURVE

	Average cpm	Corrected cpm	B/T %	B/Bo %
Total counts	19992			
NSB	951			
Standard 0 nmol/L	8268	7317	36.7	100.0
Standard 0.156 nmol/L	7412	6461	32.3	88.0
Standard 0.313 nmol/L	6831	5880	29.4	80.1
Standard 0.625 nmol/L	6232	5281	26.4	71.9
Standard 1.25 nmol/L	5381	4430	22.2	60.5
Standard 2.50 nmol/L	4469	3518	17.6	47.9
Standard 5.00 nmol/L	3525	2574	12.9	35.1











Example of standard curve



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SYMBOLS USED ON LABELS

	<p>Lot number.</p>
	<p>Catalogue number.</p>
	<p>Use by.</p>
	<p>Temperature limitation.</p>
	<p>Radioactivity reference date.</p>
	<p>Radioactive.</p>
	<p>Biological risk.</p>
	<p>Read instructions for use.</p>
	<p>Manufacturer.</p>
	<p>Number of tests.</p>

REAG A Ab	Anti-chromogranin A.
REAG B Ag ¹²⁵ I	¹²⁵ I-chromogranin A.
REAG C DASP	Double antibody solid phase.
REAG D DIL AS	Assay diluent.
REAG E CAL 10.0	Chromogranin A standard 10.0 nmol/L.
REAG F CONTROL	Control, level 1 (normal).
REAG G CONTROL	Control, level 2 (high).

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