Chromogranin A - RIA

RB321RUO

DIAsource ImmunoAssays S.A. - Rue du Bosquet, 2 - B - 1348 Louvain-la-Neuve – Belgium

History

Summary of change:

Previous Version: 191009-1	Current Version: 200224-1
	Addition of the following sentence at the end of the English IFU: "Other translations of this Instruction for Use can be downloaded from our website: https://www.diasource-diagnostics.com/"

en

Read entire protocol before use.

Chromogranin A-RIA

I. INTENDED USE

Radioimmunoassay for the *in vitro* quantitative measurement of chromogranin A in human serum or plasma.
For Research use only. Not for use in diagnostic procedures.

II. GENERAL INFORMATION

A. Proprietary name : DIAsource Chromogranin A-RIA

B. Catalog number : RB321RUO : 100 tests

C. Manufactured by : DIAsource ImmunoAssays S.A.
Rue du Bosquet, 2, B-1348 Louvain-la-Neuve, Belgium.

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III. BACKGROUND

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.

IV. PRINCIPLES OF THE METHOD

The basic principle for determination of chromogranin A with the DIAsource chromogranin A RIA kit is the competitive radioimmunoassay using antibodies against human chromogranin A.
Chromogranin A in calibrators and samples compete with ^{125}I-labelled chromogranin A in binding to the antibodies. The ^{125}I-chromogranin A binds to the antibodies in an inverse proportion to the concentration of chromogranin A in calibrators and samples. Antibody-bound ^{125}I-chromogranin A is separated from the unbound fraction using the double antibody solid phase technique. The bound fraction of ^{125}I-chromogranin A is measured in a gamma counter.
For professional use within a laboratory. The result shall not be used for clinical diagnosis or patient management.

V. REAGENTS PROVIDED

Reagents	100 Tests Kit	Colour Code	Reconstitution
ANTISERUM Rabbit antiserum to human chromogranin A (amino acids 116-439). The antiserum is diluted and lyophilised in phosphate buffer with bovine serum albumin, NaCl, and Tween 20.	1 vial lyophilised	Blue	**Add** 11 mL distilled water
Ag ^{125}I TRACER: ^{125}Iodine labelled Chromogranin A in phosphate buffer with bovine albumin, NaCl, NaN_3 and Tween 20.	1 vial lyophilised 56 kBq	Red	**Add** 12.5 mL distilled water
DASP Double antibody solid phase : Anti-rabbit-Ig coupled to cellulose particles in phosphate buffer with bovine serum albumin, NaCl, NaN_3 and Tween 20.	1 vial 52 mL	Green	**Ready** for use
ASS BUF Assay buffer : phosphate buffer containing bovine serum albumin, sodium azide, NaCl and Tween 20. Buffer used for dilution of samples, preparation of working calibrators and for replacement of antiserum in non-specific binding controls.	1 vial 50 mL	Black	**Ready** for use
CAL Chromogranin A calibrator in phosphate buffer containing bovine serum albumin, sodium azide (<0.1%), NaCl and Tween 20.	1 vial lyophilised	Yellow	**Reconstitute** with distilled water by the volume stated on the vial label.
CONTROL N Controls - N = 1 or 2 Lyophilised controls with two different levels of chromogranin A. **The chromogranin A concentrations are given on the labels of the vials.** The controls should not be diluted after reconstitution.	2 vials lyophilised	Silver	**Add** 1 mL distilled water

VI. SUPPLIES NOT PROVIDED

The following material is required but not provided in the kit:

1. Distilled water.
2. Disposable test tubes 11-13 x 55 mm, (polystyrene).
3. Pipettes with disposable tips, 50, 100 and 500 µl.
4. Volumetric pipettes 1 mL and 5 mL
5. Vortex mixer.
6. Centrifuge, refrigerated, minimum g-force 1700 x g.
7. Gamma counter.

VII. REAGENT PREPARATION

- **A. Antiserum:** Reconstitute with 11 mL distilled water. Store at 2-8° C.
- **B. ^{125}I-Chromogranin A :** Reconstitute with 12.5 mL distilled water. Store at -20° C or lower if reused.
- **C. Double antibody solid phase :** Ready for use. Stir continuously during pipetting this reagent. Store at 2-8° C.
- **D. Assay buffer :** Ready for use. Store at 2-8° C.
- **E. Chromogranin A Calibrator :** Reconstitute with distilled water by the volume stated on vial label. Store at -20° C or lower if reused.
- **F. Controls :** Reconstitute each vial with 1 mL distilled water. Store at -20° C or lower if reused.

VIII. STORAGE AND EXPIRATION DATING OF REAGENTS

Store all reagents at 2-8° C before reconstitution and use.
The water used for reconstitution of the lyophilised 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 lyophilised reagents the expiry date is valid for the unreconstituted reagents.
Reconstituted reagents are stable for 12 weeks.

IX. SPECIMEN COLLECTION

Vein 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.

X. PROCEDURE

A. Handling notes

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 (calibrators, controls and samples) should be performed in duplicate.
A complete assay includes:
Calibrators: 7 concentrations, 0, 0.156, 0.313, 0.625, 1.25, 2.50 and 5.00 nmol/L.
Controls: Low and high.
Samples
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 buffer before assay.
Samples with chromogranin A concentrations more than 50 nmol/L can be diluted further with Assay buffer, and re-assayed.

B. Procedure

1. Reconstitute the lyophilised reagents according to the instructions and allow the reagents to reach room temperature.
2. Prepare the chromogranin A working calibrators by dilution of the chromogranin A calibrator 10.00 nmol/L with assay buffer according to the following:

a. 0.40 mL calibrator 10.00 nmol/L + 0.40 mL Assay buffer = 5.00 nmol/L
b. 0.40 mL calibrator 5.00 nmol/L + 0.40 mL Assay buffer = 2.50 nmol/L
c. 0.40 mL calibrator 2.50 nmol/L + 0.40 mL Assay buffer = 1.25 nmol/L
d. 0.40 mL calibrator 1.25 nmol/L + 0.40 mL Assay buffer = 0.625 nmol/L
e. 0.40 mL calibrator 0.625 nmol/L + 0.40 mL Assay buffer = 0.313 nmol/L
f. 0.40 mL calibrator 0.313 nmol/L + 0.40 mL Assay buffer = 0.156 nmol/L
g. Assay buffer = 0 nmol/L

Store the calibrators at –20° C or lower if reused.

3. Dilute the samples 1:10 with Assay buffer e.g. 50 µL sample and 450 µL Assay buffer. Vortex-mix carefully.
4. Pipette 100 µL of calibrators (0-5.00 nmol/L), controls and samples in their respective tubes.
5. Pipette 100 µL of zero-calibrator (assay buffer) in the NSB-tubes.
6. Pipette 100 µL ^{125}I-chromogranin A in all tubes. The TOT-tubes are sealed and kept aside.
7. Pipette 100 µL Antiserum in all tubes except the NSB-tubes and TOT-tubes.
8. Pipette 100 µL Assay buffer in the NSB-tubes.
9. Vortex-mix all tubes carefully.
10. Incubate for 20-24 hours at 2-8° C,
11. Pipette 500 µL double antibody solid phase 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° C.
13. Centrifuge for 15 minutes at +4° 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.

XI. CALCULATION OF RESULTS

1. Subtract the average count rate (CPM) of the NSB-tubes from the count rate (CPM) of the calibrators, controls and samples.
2. A calibration curve is generated by plotting the bound fraction CPM or B/TOT against the concentrations of the chromogranin A calibrators.
3. Interpolate the chromogranin A concentrations of the controls and samples from the generated calibration 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 calibration curve and the calculation of the chromogranin A concentrations in samples and controls can also be done by a computer method.

XII. TYPICAL DATA

The following data are for illustration only and should never be used instead of the real time calibration curve.

Type of tube	Average CPM	Corrected CPM	B/T %	B/B0 %
Total counts	22798			
NSB	494			
Calibrator 0 nmol/L	8430	7936	35	100
Calibrator 0.156 nmol/L	7171	6677	29	84
Calibrator 0.313 nmol/L	6384	5830	26	73
Calibrator 0.625 nmol/L	5468	4974	22	63
Calibrator 1.25 nmol/L	4359	3865	17	49
Calibrator 2.50 nmol/L	3318	2824	12	36
Calibrator 5.00 nmol/L	2485	1991	9	25

XIII. PERFORMANCE AND LIMITATIONS

A. Sensitivity

The limit of detection of this assay is 0.35 nmol/L (2 × SD 20 blanks). This is the lowest detectable concentration that differs from zero. It means that reliable measurements can be done on negative samples down to this point.

B. Precision

Intra-assay precision was determined by testing 2 different samples in 10 replicates at one occasion.

	1	2
Mean value (nmol/L)	3.12	9.63
SD	0.19	0.58
% CV	6.05	6.02

Inter-assay precision was determined by testing 2 different samples in 10 replicates at three separate occasions.

	1	2
Mean value (nmol/L)	3.47	9.80
SD	0.22	0.62
% CV	6.34	6.36

C. Specificity

92 serum samples from apparently healthy blood donors were tested, 87 out of 92 were negative, with values ≤ 3 nmol/l.

D. Dilution recovery

Dilution recovery was determined by testing five serial dilutions for three different samples.

Sample	Dilution	Mean Measured Concentration (nmol/L)	Theoretical Concentration (nmol/L)	Dilution Corrected % Recovery
1	1/1	23.4	23.4	100%
	1/2	11.8	11.7	100.8%
	1/4	5.7	5.85	97.4%
	1/8	3.0	2.93	102.5%
	1/16	1.7	1.46	116.2%
Sample	**Dilution**	**Mean Measured Concentration (nmol/L)**	**Theoretical Concentration (nmol/L)**	**Dilution Corrected % Recovery**
2	1/1	27.4	27.4	100%
	1/2	14.6	13.7	106.5%
	1/4	7.3	6.8	106.5%
	1/8	3.0	3.4	87.6%
Sample	**Dilution**	**Mean Measured Concentration (nmol/L)**	**Theoretical Concentration (nmol/L)**	**Dilution Corrected % Recovery**
3	1/1	30.1	30.1	100%
	1/2	12.4	15.0	82.3%
	1/4	6.5	7.5	86.3%
	1/8	3.5	3.7	93.0%
	1/16	2	1.8	106.3%

E. Interference

Samples displaying cloudiness, haemolysis, hyperlipemia or containing fibrin may give inaccurate results.

XIV. INTERNAL 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 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-calibrator:

$$\frac{Bo}{TOT} \times 100$$

4. Non-specific binding (NSB/TOT)
Calculate for each assay the % non-specific binding:

$$\frac{NSB}{TOT} \times 100$$

The non-specific is less than 6% if decanting is made properly.

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

XV. PRECAUTIONS AND WARNINGS

Safety

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.

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XVIII. SUMMARY OF THE PROTOCOL

	Total count	NSB	Calibrators (0-6)	Controls	Samples
Calibrators	-	-	**100 µL**	-	-
Controls	-	-	-	**100 µL**	-
Samples	-	-	-	-	**100 µL**
Assay buffer	-	**100µL**	-	-	-
^{125}I Tracer	**100 µL**				
Antiserum	-	-	**100 µL**		
Assay buffer	-	**100µL**	-		
Vortex-mix and incubate for 20-24 hours at 2-8°C.					
Double antibody solid phase	-	**500 µL**			
Vortex-mix and incubate for 30-60 min at 2-8°C.					
Centrifuge 15 min (1700 g; 4°C)					
Decant and count the radioactivity of the pellets					

Other translations of this Instruction for Use can be downloaded from our website: https://www.diasource-diagnostics.com/

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