



1. NAME AND INTENDED USE

CGA-RIACT is a kit for the radioimmunoassay of human chromogranin A in serum or plasma.

2. INTRODUCTION

CGA is an acidic, hydrophilic protein of 439 aa (49 kD), present in chromaffin granules of the neuroendocrine cells. It is a member of the granin family.

CGA acts as a pro-hormone. Its proteolysis constitutes a key element of its physiology. This degradation releases biologically active peptides (vasostatins, chromostatin, pancreastatin, parastatin...) which have different paracrine and autocrine functions. The proteolysis is tissue-specific, and the protein's fragmentation differs depending on its location. It takes place mainly in the cell, inside the chromaffin granules. In immunohistochemistry, the presence of CGA in tumoral cells can be related to the neuroendocrine origin of the tumor.

Circulating CGA exists in healthy subjects and the values obtained are independent of age and sex.

The interest of seric CGA assay was first shown in pheochromocytoma, then rapidly extended to other endocrine cancers with particularly significant high levels in intestinal cancers and endocrine tumors of the pancreas. Unlike other biological markers, plasmatic catecholamines for example, the levels of CGA are affected neither by stress nor by the administration of drugs used in the treatment of pheochromocytomas. Recent studies have shown that the levels of circulating CGA are associated with a neuroendocrine differentiation and are linked to the tumor mass, without however substituting for more specific secretions such as NSE in small-cell lung cancer.

Some authors have also shown that the presence of CGA in prostate cancers may be the sign of an unfavorable evolution of the disease. It has been demonstrated that these pathological levels are associated with a lowered survival rate, independently of the patient's stage.

3. PRINCIPLE

CGA-RIACT is a solid-phase two site immunoradiometric assay. Two monoclonal antibodies were prepared against sterically remote sites on the CGA molecule. The first one is coated on the solid phase (coated tube), while the second, radiolabeled with iodine 125, is used as a tracer.

CGA (molecules or fragments) present in the standards or the samples to be tested are "sandwiched" between the two antibodies. Following the formation of the coated antibody/antigen/iodinated antibody sandwich, the unbound tracer is easily removed by a washing step.

The radioactivity bound to the tube is proportional to the concentration of CGA present in the sample.

4. REAGENTS

Each kit contains enough reagents for 100 tubes. The expiry date is marked on the external label.

REAGENTS	QUANTITY	STORAGE
COATED TUBES: ready for use. Anti-CGA monoclonal antibody coated on the bottom of the tube.	2 packs of 50 tubes	2-8°C until the expiry date. Unused coated tubes removed from their pack must be stored in the plastic bag supplied with the kit.
ANTI CGA¹²⁵ I: ready for use. ¹²⁵ I anti CGA monoclonal antibody, buffer, bovine serum, sodium azide, red dye, non immunized mouse immunoglobulins, EDTA. ≤ 407 kBq (≤ 11 µCi).	1 55 ml vial	2-8°C until the expiry date.
STANDARDS: lyophilized.* Recombinant human CGA, human serum, EDTA, preservative. 50-125-300-600-1200 ng/ml Reconstitute with 0.5 ml of distilled water.	5 vials qsp 0.5 ml	2-8°C until the expiry date. After reconstitution, do not keep more than 1 hour at room temperature. Store frozen in aliquots at - 20°C for 6 weeks .
CONTROL: lyophilized **. Recombinant human CGA, human serum, preservative. 180 ng/ml Reconstitute with 0.5 ml of distilled water.	1 vial qsp 0.5 ml	2-8°C until the expiry date. After reconstitution, do not keep more than 1 hour at room temperature. Store frozen in aliquots at - 20°C for 6 weeks .
BUFFER: ready for use. Used as dilution buffer, diluent and 0 standard. Buffer, bovine serum, sodium azide, EDTA.	1 60 ml vial	2-8°C until the expiry date.
WASH REAGEN : tablets. Dilute in 500 ml of distilled water. Shake gently.	5 tablets under blister	2-8°C until the expiry date. After dilution, store in a capped container for a maximum of 15 days.
PLASTIC BAG	1	

* The values shown are target values only; the true value of each standard is shown on its label.

** The acceptance range true values are printed on the vial label.

5. PRECAUTIONS FOR USE

5.1. Safety measures

Raw materials of human origin contained in the reagents of this kit have been tested with licensed kits and found negative for the anti-HIV 1, anti-HIV 2, anti-HCV antibodies and the HBs antigen. However as it is impossible to strictly guarantee that such products will not transmit hepatitis, the HIV virus, or any other viral infection, all raw materials of human origin including the samples to be assayed must be treated as potentially infectious.



Do not pipette by mouth.

Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.

Wear disposable gloves while handling kit reagents or specimens and wash hands thoroughly afterwards.

Avoid splashing. Decontaminate and dispose of specimens and all potentially contaminated materials as if they contained infectious agents. The recommended method of doing this is autoclaving for a minimum of one hour at 121.5°C.

Sodium azide may react with lead or copper piping to form highly explosive metal azides. During waste disposal, flush the drains thoroughly to prevent a build-up of these products.

5.2. Radioprotection rules

This radioactive product may only be received, purchased, stored or used by persons so authorized, and by laboratories covered by such authorization. The solution should under no circumstances be administered to humans or to animals.

The purchase, storage, use or exchange of radioactive products are subject to the laws in force in the user's country.

Enforcement of the basic radioprotection rules will ensure adequate safety.

A summary of these is given below :

Radioactive products must be stored in their original containers in a suitable area. A record of the reception and storage of radioactive products must be kept up to date. Handling of radioactive products should take place in a suitably-equipped area with restricted access (controlled zone). Do not eat, drink, smoke or apply cosmetics in a controlled zone.

Do not mouth-pipette radioactive solutions. Avoid any direct contact with all radioactive products by using laboratory coats and protective gloves. Contaminated laboratory equipment and glassware must be disposed of immediately after contamination to prevent cross-contamination of different isotopes. Any contamination or radioactive substance loss should be dealt with in accordance with the established procedures. All radioactive waste disposal must be carried out according to the regulations in force.

5.3. Handling precautions

Do not use kit components beyond their expiry date. Do not mix reagents from different batches. Do not test more than 100 tubes at the same time. Avoid any microbic contamination of the reagents or of the water. Fully respect the incubation times and the washing instructions indicated.

6. SPECIMEN COLLECTION AND PREPARATION

The assay is performed directly on serum or plasma. If the test is to be carried out within 24 hours, serum and plasma must be refrigerated at 2-8°C. Otherwise, they should be divided into aliquots and deep frozen (-20°C) until needed. If the samplings are carried out with plasma, the values will be systematically higher.

Dilutions

Should elevated CGA levels be suspected, the buffer diluent found in the kit is used for dilution.

It is recommended to carry out the dilutions using disposable plastic tubes.

7. ASSAY PROCEDURE

7.1. Material required

Precision micropipettes or similar, with disposable tips, capable of dispensing 50 µl, 500 µl, 1000 µl, and 2000 µl (± 1%). Their calibration should be checked regularly. Distilled water. Disposable plastic tubes. Vortex-type mixer. Circular horizontal shaker (400 rpm). Gamma scintillation counter calibrated for 125 iodine measurement. Equipment suitable for this assay is available from CIS bio international; further information on request.

7.2. Protocol

All reagents must be brought to room temperature (18-25°C) at least 30 minutes before their use. Dispensing of the reagents into the tubes is carried out at room temperature (18-25°C).

The assay requires the following groups of tubes:

Standard "0" group, for the determination of non specific binding.

Standard groups, to establish the standard curve. Control group for the control. Sx groups, to test serum or plasma samples.

It is recommended that the assay be performed in triplicate for the standards and in duplicate for the samples.

Strictly observe the order in which reagents are to be added:

Dispense 500 µl of buffer into each tube. Add 50 µl of standard, control or sample to be assayed to the corresponding tube.

Mix each tube gently with a Vortex-type mixer. Incubate for 18-20h at room temperature (18-25°C).

Wash the coated tubes:

Aspirate the contents of all tubes as completely as possible. Add 1.0 ml of washing solution to each tube.

Aspirate the contents of all tubes. Repeat the process once more. Aspirate the contents of the tubes as completely as possible.

There must be no residual volume in the coated tubes after washing.

To obtain reliable and reproducible results, the different washing steps have to be performed correctly: the addition of the washing solution must be carried out with sufficient speed to create turbulence in the tubes.

Dispense 500 µl ml of ¹²⁵I anti CGA monoclonal antibody into each tube, including the 3 total activity tubes.

Incubate for 2h ± 5 min at room temperature (18-25°C) under agitation (400 rpm). Wash the coated tubes as described above.

Measure the remaining radioactivity bound to the tubes with a gamma scintillation counter.

8. QUALITY CONTROL

Good laboratory practices require that quality control samples be used in each series of assays to check the quality of the results obtained. All specimens should be treated identically, and result analysis using the appropriate statistical methods is recommended.

9. RESULTS

For each group of tubes, calculate the mean counts after subtracting the background.

Draw up the standard curve by plotting the standards' cpm against their concentrations. The curve should be plotted by linear point by point smoothing. Read the sample values directly from the curve, correcting the read value for the dilution factor if necessary.



Typical standard curve (example only): these data must not be substituted for results obtained in the laboratory.

Tube groups	Mean cpm	Concentration ng/ml
Standard 0	55	0
Standard 1	2954	55
Standard 2	7110	145
Standard 3	14878	350
Standard 4	29522	700
Standard 5	60248	1370
Control	7288	165

10. PROCEDURAL LIMITATIONS

Samples which show turbidity, haemolysis, hyperlipemia or contain fibrin may give misleading results. Do not extrapolate sample values beyond the last standard. Dilute the samples concerned and re-assay.

11. EXPECTED VALUES

Each laboratory should establish its own range of normal values according to the type of sample currently used. CGA is a calcium binding protein and its measurement is influenced by Ca⁺⁺ concentration. Normal values differ depending on whether serum or EDTA plasma is used. The data below gives an example of the serum values obtained with population of 162 presumed normal individuals. 95% of the population values are between 19.4 and 98.1 ng/ml, with the median at 41.6 ng/ml. For any use on plasma, it is recommended that the normal values also be established on plasma.

12. SPECIFIC CHARACTERISTICS OF THE ASSAY

12.1. Imprecision

This has been assessed using 3 samples with different concentrations. They were tested either 15 times in the same series of assays, or in duplicate in 20 different series.

Sample	Mean ng/ml	Within-run CV %	Between-run CV %
1	29.9	6.0	8.5
2	144	3.8	5.7
3	996	2.2	5.3

12.2. Recovery test

Known quantities of human CGA were added to human sera. The recovery percentages of human CGA in the samples ranged from 90 to 110%.

12.3. Dilution test

Samples with high levels were diluted, with the recovery percentages ranging from 90 to 110%.

12.4. Specificity

The two monoclonal antibodies enable whole and fragmentary circulating CGA to be assayed.

12.5. Detection limit

The detection limit is defined as being the smallest detectable concentration different from zero with a probability of 95%. It has been assessed as being 1.5 ng/ml.

ASSAY FLOW CHART

Tubes	Buffer μl	Buffer (Standard 0) μl	Standards 1 – 5 Control Samples μl		Anti-CGA 125 I μl		
Standard 0	500	50	--	Mix gently Incubate 18-20h at 18-25°C Wash twice See Ch.7.2	500	Mix gently Incubate 2 h ± 5 min at 18-25°C under agitation	Count
Standards	500	--	50		500		
Control	500	--	50		500		
Samples	500	--	50		500	Wash twice See ch.7.2	