invitron
high sensitivity diagnostics

IV5-101L

English

Invitron Chromogranin A (CgA) Kit

For in-vitro diagnostic use only

For research use only

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Definitions

	Instructions for use
REF	Catalogue number
	Use by
LOT	Lot/Batch Code
	Storage temperature limitations
IVD	In vitro diagnostic medical device
	Manufactured by
	Contains sufficient for <N> tests

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Invitron Chromogranin A Kit

Intended Use

The Invitron Intact Chromogranin A (CgA) kit is an immunometric assay for the quantitative measurement of CgA in human samples. Measurements of CgA are used in the diagnosis and treatment of patients with neuroendocrine tumours (NETs).

Summary and Explanation

Chromogranin A (CgA) is a 439 amino acid protein that is present in the secretory dense core granules of neuroendocrine tissues. It is co-secreted with the amines and peptides present in the granules and is considered to be a sensitive and specific marker of neuroendocrine tumours. Immunoassays of CgA may be useful in the diagnosis and monitoring of neuroendocrine neoplasms including carcinoids, phaeochromocytomas, neuroblastomas, medullary thyroid carcinomas, some pituitary tumours, functioning and non-functioning islet cell tumours and other APUD tumours.

Principle

The Invitron CgA Assay is a two-site immunoassay, employing a specific solid phase antibody immobilised on microtitre wells and a soluble antibody labelled with a chemiluminescent acridinium ester. The sample is incubated in the microtitre well together with a buffer and, after a wash step, the labelled antibody solution is added. A second incubation is followed by a further wash step to remove unbound labelled antibody before measurement. The bound luminescence is quantified by a microtitre plate luminometer capable of *in situ* reagent addition. The luminescent reaction is a rapid flash type (>95% complete in 1 second) which permits the entire plate to be read in approximately 5 minutes.

Materials Provided

- **Coated Microtitre Plate (a)**
  (12 x 8 wells) stripwells coated with a specific monoclonal antibody. The plate is sealed inside a foil pouch with a desiccant to maintain a moisture-free environment.
- **Labelled Antibody Concentrate (b)**
  (1 x 0.9ml) chemiluminescent labelled antibody in a protein matrix including preservatives and 0.05% sodium azide.
- **Labelled Antibody Diluent (c)**
  (1 x 14.1ml) Ready to use for diluting the labelled antibody to its working strength. Protein matrix including preservatives and 0.05% sodium azide.
- **Standards (d) - (h)**
  (5 x 0.5ml lyophilized) of 5 concentrations – (typically) 0, 8, 40, 200, 1000 ng/ml – Recombinant CgA in a serum matrix, lyophilized and sealed under vacuum for stability. Refer to the Certificate of Analysis for each lot for actual concentrations.
- **Assay controls: High and Low**
  (2 x 0.5ml lyophilized) of 2 concentrations– Recombinant CgA in a serum matrix, lyophilized and sealed under vacuum for stability. Refer to the Certificate of Analysis for each lot for actual concentrations.
- **Sample Buffer (i)**
  (1 x 12ml) Ready to use for sample dilution. Protein matrix including preservatives and 0.05% sodium azide.
- **Wash Buffer Concentrate (IV1-005)**
  (1 x 50ml) phosphate buffered saline containing detergent and preservative.
- **Plate sealers – 2 each**
- **Product Insert**

Materials Required But Not Provided

- Detection reagents. Invitron Product code: IV1-001.
- Deionised water
- Precision pipettes and disposable tips to deliver 10-1000 µl
- Foil to cover the microtitre plate
- A multi-channel dispenser or repeating dispenser
- Vortex-Mixer
- Standard laboratory glass or plastic vials, cups, etc.
- Microtitre plate Luminometer capable of direct injection and of measuring flash kinetics.

Warnings and Precautions

- For *in-vitro* diagnostic use only. For professional use only.
- For information on hazardous substances included in the kit please refer to the Material Safety Data Sheet.
- Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- Wear disposable latex gloves and appropriate protective clothing when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
- Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- Do not use reagents beyond expiry date as shown on the kit labels.
- Optimal test results are only obtained when using calibrated pipettes and luminometer.
- Do not mix or use components from kits with different lot numbers.
- This kit contains no human-derived material.

Preparation, Storage & Stability of Reagents

When stored at 2-8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date. Opened reagents must be stored at 2-8°C. Microtitre wells must be stored at 2-8°C. Once the foil bag has been opened, care should be taken to close it tightly again. Opened kits retain activity for two months if stored as described above.

Standards

Reconstitute each of the standards by the addition of 0.5 ml of deionised water. Allow these to stand for 5 minutes, then mix gently to ensure all solids are dissolved. Reconstituted standards must be stored frozen at -20ºC.

Labelled Antibody Concentrate

Transfer the entire contents of the vial containing Labelled Antibody Concentrate into the bottle of Labelled Antibody Diluent and mix thoroughly. Diluted Labelled Antibody is stable for 2 weeks when stored at 2-8°C.

Wash Buffer

Make up working strength Wash Buffer by diluting 1 part of Wash Buffer concentrate with 29 parts of deionised water. The diluted Working Wash Buffer is stable for 2 weeks at room temperature.

Luminometer Set-up

The microtitre plate luminometer must be fitted with 2 injectors and it is important to check that the instrument is capable of measuring "flash" type kinetics. The measurement protocol should be set as follows:

1. Set injector 1 to deliver 100 µl of Detection Reagent 1
2. Set injector 2 to deliver 100 µl of Detection Reagent 2
3. Set a delay of 2 seconds between injection 1 and injection 2.
4. Light measurement must start at the time of the second injection (i.e. there is no delay between injection 2 and measurement).
5. Measurement time is 1 second.

Specimen Collection & Storage

Use only EDTA Plasma. Do not use severely haemolysed specimens.

Specimen Collection

Plasma: Whole blood should be collected into a tube containing EDTA anticoagulant and centrifuged immediately after collection. Plasma should be frozen at -20ºC as soon as possible after separation.

Specimen Storage

Specimens should be stored frozen at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

Assay Procedure

1. Bring all kit components and samples to room temperature before use.

2. Assemble the required number of coated strips in the plate holder. Any strips not used immediately may be stored inside a sealed polythene bag with silica gel desiccant. Make sure to fill remaining spaces in the plate holder with uncoated strips to ensure uniform heat transfer during incubation.

3. Pipette **100 µl Sample Buffer** into each well.

4. Pipette **25 µl each of Standard or sample** into the respective wells. Standards must be run in duplicate.

5. Attach the plate sealer and incubate for **2 hours at 37ºC.**

6. Remove the plate sealer and perform **3 wash cycles** with working strength Wash Buffer (300 µl each cycle) using an automatic plate washer.

7. Pipette **100 µl labelled antibody** solution into each well.

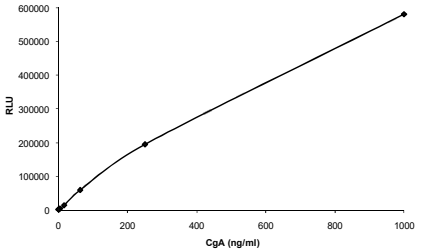
8. Attach the plate sealer and incubate for a further **1 hr at 37ºC.**

9. Remove the plate sealer and perform **3 wash cycles** with working strength Wash Buffer using an automatic plate washer.

10. Measure the light output from each well in a plate luminometer within 15 minutes.

Typical Standard Curve

This curve is for illustration only and must not be used for result calculation.
RLU = Relative Light Units.



Calculation of Results

The results may be calculated automatically using a 4-Parameter curve fit. Other data reduction functions may give slightly different results. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard should be further diluted. For the calculation of the concentrations this dilution factor has to be taken into account.

Expected Values

It is strongly recommended that each laboratory determines its own normal and abnormal values.

Quality Control

The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels. It is also recommended to make use of national or international Quality Assessment programs where possible in order to ensure the accuracy of the results. Employ appropriate statistical methods for analyzing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices; luminometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods. After checking the above mentioned items without finding any error contact Invitron directly.

Limitations

- For Research Use Only.
- Only if test instructions are rigidly followed will optimum results be achieved.
- Use fresh plasma or specimens frozen and thawed no more than twice. Specimens that are improperly stored or are subjected to multiple freeze-thaw cycles may yield spurious results.
- Reproducible results depend on careful pipetting, observation of incubation periods and temperature, as well as thorough mixing of all prepared solutions.
- While rinsing, check that all wells are filled evenly with Washing Solution, and that there are no residues in the wells.
- Instructions for using appropriate luminometers are to be observed. Check that the instrument has the correct measurement protocol installed.

Performance Characteristics

Precision

A precision profile was created from results of duplicate measurements of 117 patient samples from 3 assays. Over the range 1.5-900 ng/ml the mean CV was 6.5%

CgA (ng/ml)	Mean CV%	n
0 - 1.5	11.8	20
1.5 - 12	6.6	20
12 - 26	6.4	20
26 - 90	7.0	20
90 - 281	7.3	20
281 - 900	5.1	17

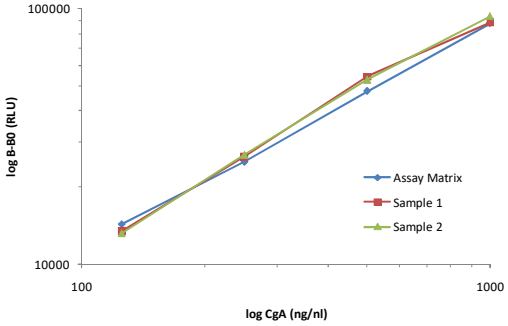
Mean CV (1.5-900ng/ml) = 6.5%

High Dose Hook Effect

No high dose hook effect has been observed at CgA concentrations up to 50,000 ng/ml.

Recovery

Plasma samples containing low endogenous CgA were spiked with recombinant CgA at 4 levels. Recoveries are shown as percentages of the expected result.



Target	Sample 1	Sample 2	Mean	%Recovery
1000	965	918	941	94
500	431	449	440	88
250	229	233	231	92
125	126	132	129	103

Mean spiking recovery was 94%.

Sensitivity

The detection limit was calculated as the dose giving a response twice that of the zero standard. Calculated in this way, the detection limit of the CgA assay is 0.33 ng/ml. The dynamic range of the assay is therefore 0.33 to 1000 ng/ml.

Linearity

Three patient samples containing elevated CgA concentrations were diluted in human plasma with negligible CgA (<2ng/ml). The following table shows the measured CgA concentrations of the undiluted and diluted specimens.

Measured CgA (ng/ml)			
Dilution Factor	Sample 1	Sample 2	Sample 3
0	1000	550	600
1:2	576	310	351
1:4	383	196	169
1:8	202	102	102
1:16	112	60	60

Cross Reactivity

Cross reactivities of related proteins were investigated at concentrations of 100 pmol/l. Results are expressed as percentages of the reactivity of an identical concentration of CgA.

Peptide	**CR (%)**
Chromogranin B	0