invitron
high sensitivity diagnostics

IV5-101E

English

Invitron Chromogranin A (CgA) ELISA Kit

For in-vitro diagnostic use only

CE

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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 ELISA Kit

Intended Use

The Invitron Intact Chromogranin A (CgA) ELISA 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 ELISA is a two-site immunoassay, employing a specific solid phase antibody immobilised on microtitre wells and a soluble antibody labelled with horseradish peroxidase (HRP). The sample is incubated in the microtitre well together with a buffer and, after a wash step, the HRP-antibody conjugate is added. A second incubation is followed by a further wash step to remove unbound antibody conjugate. Tetramethylbenzidine (TMB) substrate is added and the resulting colour is allowed to develop for 15 minutes. The enzyme reaction is terminated by addition of a stop solution and absorbance is measured in a 96 well microplate reader.

Materials Provided

- **Coated Microtitre Plate**
  (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.
- **Antibody Conjugate Concentrate**
  (1.0ml) HRP-labelled antibody in a protein matrix including preservatives.
- **Antibody Conjugate Diluent**
  (1 x 11.0ml) Ready to use for diluting the antibody conjugate to its working strength. Protein matrix including preservatives.
- **Standards**
  (5 x 0.5ml lyophilized) of 5 concentrations – (typically) 0, 8, 40, 200, 900 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**
  (1 x 12ml) Ready to use for sample dilution. Protein matrix including preservatives.
- **Substrate**
  (1 x 15ml) Tertramethylbenzidine (TMB) substrate, ready to use.
- **Stop Solution**
  (1 x 15ml) Dilute sulphuric acid, ready to use.
- **Wash Buffer Concentrate**
  (1 x 50ml) phosphate buffered saline containing detergent and preservative.
- **Product Insert**

Materials Required But Not Provided

- Deionised water
- Precision pipettes and disposable tips to deliver 10-1000 µl
- Plate sealers
- A multi-channel dispenser or repeating dispenser
- Vortex-Mixer
- Automatic plate washer
- Standard laboratory glass or plastic vials, cups, etc.
- Microtitre plate reader.

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.

Antibody Conjugate Concentrate

Transfer the entire contents of the vial containing Antibody Conjugate Concentrate into the bottle of Conjugate Diluent and mix thoroughly. Diluted Antibody Conjugate 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.

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

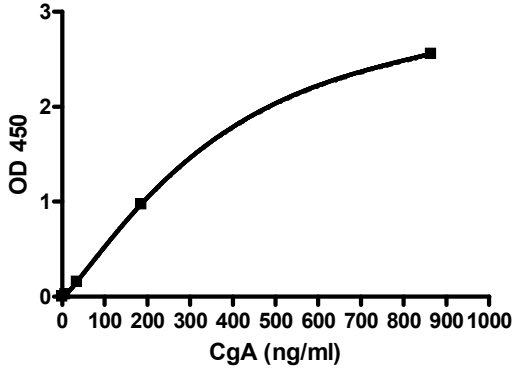
Specimens should be stored frozen at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

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 a 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 working strength antibody conjugate** into each well.
8. Attach a 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 (300 µl each cycle) using an automatic plate washer.
10. Add **100 µl TMB substrate** to each well and incubate at **room temperature for 15 minutes**.
11. Add **100 µl Stop Solution** to each well.
12. **Measure the absorbance at 450 nm** in a plate reader normalised by subtraction of absorbance at 620/650 nm.

Typical Standard Curve

This curve is for illustration only and must not be used for result calculation.



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.

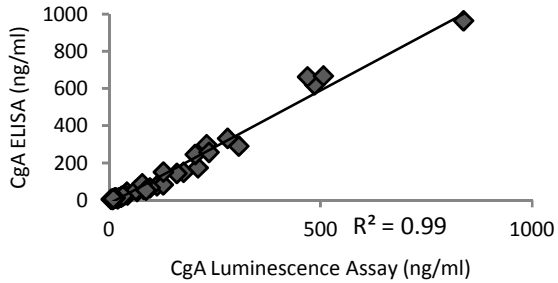
Performance Characteristics

Precision

A precision profile was created from results of duplicate measurements of 43 patient samples. Over the range 4.6-1000 ng/ml the mean CV was 5.8%

Correlation

43 specimens obtained from patients with known or suspected neuroendocrine tumours were assayed in the CgA ELISA and results were compared with those previously obtained with the Invitron CgA chemiluminescence assay.A correlation coefficient (R^2) of 0.99 was obtained, indicating close agreement between the two methods.



High Dose Hook Effect

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

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.

For additional information and product support please contact:

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