# Human Chromogranin A ELISA Kit

Enzyme Immunoassay for the quantification of Chromogranin A in Human serum.

Catalog number: ARG82803

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

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**MANUFACTURED BY:**

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

## INTRODUCTION

Chromogranin A or parathyroid secretory protein 1 (gene name CHGA) is a member of the granin family of neuroendocrine secretory proteins. As such, it is located in secretory vesicles of neurons and endocrine cells such as islet beta cell secretory granules in the pancreas. In humans, chromogranin A protein is encoded by the CHGA gene.

Chromogranin A is the precursor to several functional peptides including vasostatin-1, vasostatin-2, pancreastatin, catestatin and parastatin. These peptides negatively modulate the neuroendocrine function of the releasing cell (autocrine) or nearby cells (paracrine).

Chromogranin A induces and promotes the generation of secretory granules such as those containing insulin in pancreatic islet beta cells. It is used as an indicator for pancreas and prostate cancer and in carcinoid syndrome. It might play a role in early neoplasic progression. Chromogranin A is cleaved by an endogenous prohormone convertase to produce several peptide fragments. See chromogranin A GeneRIFs for references. In immunohistochemistry it can be used to identify a range of neuroendocrine tumours and is highly specific for both benign and malignant cells of this type. [Provide by Wikipedia: Chromogranin A]

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A capture antibody specific for Chromogranin A (CgA) has been pre-coated onto a microtiter plate. Standards, Controls or samples are pipetted into the wells and any CgA present is bound by the immobilized antibody. After incubation and wash step. Added Antibody Conjugate specific for CgA to each well and incubate. After washing away any unbound substances, the TMB substrate is added to the wells and color develops in proportion to the amount of CgA bound in the initial step. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450 nm. The concentration of CgA in the samples is then determined by comparing the O.D of samples to the standard curve.

## MATERIALS PROVIDED & STORAGE INFORMATION

Store all other components at 2-8°C. Use the kit before expiration date. Opened kits retain activity for 4 weeks if stored as described above.

| Component | Quantity | Storage information |
| --- | --- | --- |
| Antibody Coated microplate | 8 X 12 strips | 4°C |
| Standards A to E (0, 30, 110, 450, 900 µg/L ) | 5 vial, 1 mL each (ready to use) | 4°C |
| Control 1 & 2 | 2 vial, I mL each (ready to use) | 4°C |
| Diluent Buffer | 50 mL (ready to use) | 4°C |
| Antibody Conjugate (Anti-Chromogranin A Antibody conjugate HRP) | 6 mL (ready to use) | 4°C |
| 50X Wash Buffer | 20 mL | 4°C |
| TMB substrate | 12 mL (ready to use) | 4°C (protect from light) |
| STOP solution | 12 mL (ready to use) | 4°C |

## MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Deionized or distilled water
- Mixer or Ultra-Turrax
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Microtiter plate washer (recommended)

## TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature (20-25°C).
- Unused wells must be stored at 2-8 °C in the sealed foil pouch and used in the frame provided.
- Avoid air bubbles in the wells as this could result in lower binding efficiency and higher CV% of duplicate reading.
- Briefly spin down the all vials before use.
- If crystals are observed in the 50X Wash Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.

- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. If the solution is blue before use, DO NOT USE IT.
- Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates (triplicate is recommended).
- Medications like proton pump inhibitors and histamine type-2 receptor antagonists can influence CgA level in serum. People who are taking such medication should consult with their doctor before specimen collection. Sport and ingestion of a meal can also influence CgA level.

## SAMPLE COLLECTION & STORAGE INFORMATION

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes.

Note:

1. Avoid disturbing the white buffy layer when collection serum / plasma sample.
2. Samples containing sodium azide should not be used in the assay.
3. Specimens should be capped and may be stored for up to 24 hours at 2-8°C prior to assaying. Specimens stored for a longer time (up to 6 months) should be frozen only once at-20°C prior to assay. Thawed samples should be inverted several times prior to testing. Avoid freeze and thaw cycles.
4. Samples containing precipitates or fibrin strands might cause inaccurate results. Biotin (up to 600 ng/ml), hemolytic samples (up to 0.5 mg/ml hemoglobin), icteric samples (up to 12.5 mg/dl bilirubin) and lipemic samples (up to 1657 mg/dl triglycerides) have no influence on the assay results.
5. **Prior to use. The sample have to be diluted 1:8 with Diluent Buffer, E.g. 25 µL of sample + 200 µL of Diluent Buffer.**

## REAGENT PREPARATION

- **1X Wash Buffer**: Dilute 50X Wash Buffer into distilled water to yield 1X Wash Buffer. (E.g., add 20 mL of 50X Wash Buffer into 980 mL of distilled water to a final volume of 1000 mL) The 1X Wash Buffer is stable for up to 4 weeks at room temperature.

## ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-26°C) before use. Standards and samples should be assayed in duplicates.

1. Add **50 µL** of **diluted samples, Controls and Standards** to the **Antibody Coated microplate**.
2. Incubate at **RT** for **1 hour** on a microplate shaker.
3. Aspirate each well and wash, repeating the process 3 times for a total **4 washes.** Wash by filling each well with **1× Wash Buffer (300 µL)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance.

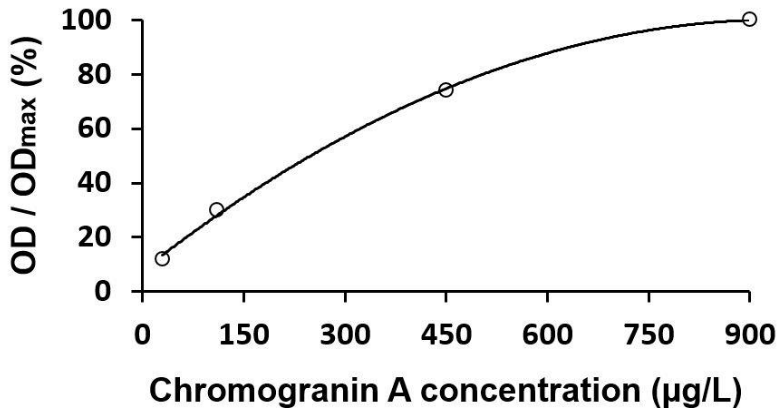
   **Note:** blotting dry each time by tapping the inverted plate on absorbent material.
4. Add **50 µL** of the **Antibody Conjugate** to each well.
5. Incubate at **RT** for **1 hour** on a microplate shaker.
6. Aspirate each well and **wash as step 3**.
7. Add **100 µL** of **TMB Substrate** to each well, including the blank wells. Incubate for **25 ± 5 minutes** at **RT** in the dark.
8. Immediately Add **100 µL** of **Stop Solution** to each well, including the blank

wells. The color of the solution should change from blue to yellow.

9. Read the OD with a microplate reader at **450 nm** immediately. (optional: read at 620 nm as reference wavelength) It is recommended reading the absorbance **within 10 minutes** after adding the stop solution.

## EXAMPLE OF TYPICAL STANDARD VALUES

The following figures demonstrate typical results with the Human Chromogranin A ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



## CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of Controls, standards and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
4. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
5. The concentration of the samples can be read directly from this standard curve. Diluted samples with concentrations higher than that of the highest standard have to be further diluted. For the calculation of the concentrations this dilution factor has to be taken into account.
6. Expected reference value: serum < 100 µg/L.

## QUALITY ASSURANCE

### Sensitivity

The limit of detection (LOD) of CgA is 6.5 µg/L.

### Intra-assay and Inter-assay precision

The CV value of intra-assay was 4.3-5.6% and inter-assay precision was 9.4-11%

### Recovery

93-96%