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Not for use in diagnostic procedures

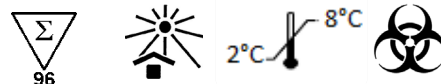
REF

CGA-EL-US-NG



www.cisbio.com/ivd-pi

Instructions for Use - ENG



Document reference: 04 – November 2022

1. NAME AND INTENDED USE

CGA-EL-US-NG is an ELISA assay for the quantitative determination of chromogranin A (CGA) in serum or EDTA plasma. For research use only. Not for use in diagnostic procedures.

2. INTRODUCTION

CGA is a hydrophilic and acidic protein of 439 aa (49 kD) presents in the chromaffin granules of neuroendocrine cells. It is part of the granin family. CGA acts as a pro-hormone. Its proteolysis is a key component of its physiology. This degradation releases biologically active peptides (vasostatins, chromostatin, pancreastatin, parastatin), which possess different paracrine and autocrine functions. This proteolysis is tissue-specific and the fragmentation of the protein will be different depending on its location. It primarily takes place in the cell, inside chromaffin granules.

3. PRINCIPLE

The CHROMOGRANIN A kit is an ELISA-type immunoassay. A first monoclonal antibody, immobilized on the microplate, captures the CGA proteins contained in the calibrators and samples. After washing, the captured proteins are then recognized by a second monoclonal antibody conjugated to HRP (Horse-Radish-Peroxidase). After a second incubation, the unbound reagents are eliminated by washing. Then the colorimetric reaction is started by the addition of an HRP substrate, TMB (3, 3', 5, 5' Tetramethyl benzidine). Once the reaction is stopped, the optical density (OD) of each well is read at 450 nm. The OD values measured are proportional to the CGA concentration contained in the calibrators and samples.

4. REAGENTS

Each kit contains enough reagents for 96 tests (including the generation of the calibration curve). The expiry date is indicated on the external label.

REAGENTS	SYMBOLS	QUANTITY	STORAGE
MICROPLATE: Ready for use. Anti-CGA monoclonal mouse antibody fixed to the bottom of the well, Bovine albumin.	MICROPLATE	1 plate with 96 wells	Before opening: 2-8°C until the expiry date. After opening: unused strips may be stored for 6 weeks in the plastic bag supplied, with a desiccant, properly sealed, within the limits of the expiry date.
CONJUGATE: Ready for use Anti-CGA monoclonal mouse antibody coupled to HRP, Non-immunised mouse immunoglobulins, stabilizers and preservative.	CONJ	1 vial 22mL	Before opening: 2-8°C until the expiry date. After opening: the conjugate can be stored at 2-8°C for a period of 6 weeks, within the limits of the expiry date.

CALIBRATORS: Lyophilized. Human recombinant CGA, human serum, EDTA, preservative. 75 – 140 – 300 – 600 – 1000 ng /mL * Reconstitute with 0.25 mL of distilled water.	CAL	5 vials	Before opening: 2-8°C until the expiry date. After reconstitution: do not store for more than one hour at room temperature, divide into aliquots and freeze at - 20°C for a period of 6 weeks, within the limits of the expiry date.
CONTROLS: Lyophilized. Human recombinant CGA, human serum, EDTA, preservative. 90 – 720 ng/mL ** Reconstitute with 0.25 mL of distilled water.	CONTROL	2 vials	Before opening: 2-8°C until the expiry date. After reconstitution: do not store for more than one hour at room temperature, divide into aliquots and freeze at - 20°C for a period of 6 weeks, within the limits of the expiry date.
DIL/CAL0 : Ready for use. This reagent is used as an incubation buffer, diluent and calibrator0. Buffer, beef serum, sodium azide, EDTA.	DIL CAL 0	1 vial 80 mL	Before opening: 2-8°C until the expiry date. After opening: the diluent/CAL0 can be stored at 2-8°C for a period of 6 weeks, within the limits of the expiry date.
TWEEN 20: Concentrated washing solution Dilute 9 mL of Tween 20 in 3 L of distilled water. Shake gently.	TWEEN 20	1 vial 10 mL	Before opening: 2-8°C until the expiry date. After opening: the Tween 20 can be stored at 2-8°C for a period of 6 weeks, within the limits of the expiry date.
SUBSTRATE: Ready for use 3, 3', 5, 5' Tetramethyl benzidine: TMB	SUBS TMB	1 vial 15 mL	Before opening: 2-8°C until the expiry date. After opening: the TMB can be stored at 2-8°C for a period of 6 weeks, within the limits of the expiry date.
STOP SOLUTION: Ready for use 0.5 M sulphuric acid.	STOP SOLN	1 vial 22 mL	Before opening: 2-8°C until the expiry date. After opening: the stop solution can be stored at 2-8°C for a period of 6 weeks, within the limits of the expiry date.
ADHESIVE FILM FOR MICROPLATE		3	
PLASTIC BAG		1	

(*)The values indicated above are target values only, the actual values are indicated on the vial labels.

(**) The actual acceptance limit values are indicated on the vial labels.

5. PRECAUTIONS FOR USE

5.1. Safety measures

- The raw materials of human origin contained in the reagents of this kit have been tested with licensed kits and have been found to be negative for anti-HIV 1, anti-HIV 2 and anti-HCV antibodies and the HBs antigen. However, as it is impossible to strictly guarantee that such products are incapable of transmitting 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 samples or kit reagents are handled. Wear disposable gloves while handling kit reagents or samples and wash hands thoroughly afterwards. Avoid splashing.
- Decontaminate and dispose of samples and all potentially contaminated materials as if they contained infectious agents. The best decontamination method 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.
- When disposing of waste, dilute thoroughly to prevent the formation of such products.



CAL CONTROL CONJ

WARNING
H317: May cause an allergic skin reaction

STOP SOLN

Not classified as dangerous but solution with acid PH

DIL CAL 0

Contains sodium azide (<0.1%)

5.2. Handling precautions

- Do not use kit components beyond their expiry date.
- Do not mix reagents from different batches.
- Avoid any microbial contamination of the reagents and water. Comply with the incubation times.

6. SAMPLE COLLECTION AND PREPARATION

6.1 Pre-analytical

The assay is typically performed directly on serum or EDTA plasma. For an assay performed within 4 hours, the samples should be stored at room temperature (18-25°C). For an assay performed within 48 hours, the samples should be stored at 2-8°C following specimen collection. For an assay beyond 48 hours, samples should be divided into aliquots which must be stored frozen (-20°C) up to 10 months.

Dilution: If high CGA levels are suspected, dilution should be performed using the diluent buffer supplied with the kit. It is recommended that dilutions be performed in disposable plastic tubes.

6.2 Pre-dilution of samples, controls and calibrators (1/51)

- **All the samples, the controls and the calibrators must be pre-diluted 51 times** in the diluent

DIL	CAL
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 0 provided in the kit before being tested. Gently mix the mixture using a Vortex mixer.

7. ASSAY PROCEDURE

7.1 Equipment required

- Precision micropipettes or similar equipment with disposable tips for distribution of 20, 50, 100, 200 and 1000 µL. Calibration of these must be regularly checked.
- Distilled water.
- Disposable plastic tubes.
- Vortex mixer.
- Microplate washer (optional).
- Microplate shaker.
- Microplate reader, capable of measuring absorbance at 450 nm. As an option, the reader may be equipped with a filter capable of reading the absorbance at a wavelength between 610 nm and 650 nm (620 nm recommended). This second reading allows to correct the microplate's imperfections.

7.2 Protocol

- All the reagents must be brought to room temperature (18-25°C) at least 30 minutes before their use. The reagents are pipetted and dispensed into wells at room temperature (18-25°C).
- Each calibrator, control or sample must be tested in duplicate.
- Reconstitute the vials of calibrators and controls. Carefully check that all the freeze-dried product is dissolved, and use within an hour following reconstitution.

7.2.1 Preparation of the Wash solution **TWEEN 20**

- To obtain reliable and reproducible results, it is recommended that the washing steps be performed as indicated; the residual washing solution volume must be as low as possible. The use of a microplate washer is recommended.
 - To prepare the washing solution, dilute 9mL of Tween 20 **TWEEN 20** in 3L of distilled water. Mix slowly.

7.2.2 Instructions - Follow the order for addition of reagents

See last page for laboratory protocol card. It is necessary to fully read the package insert in details before using the laboratory protocol card.

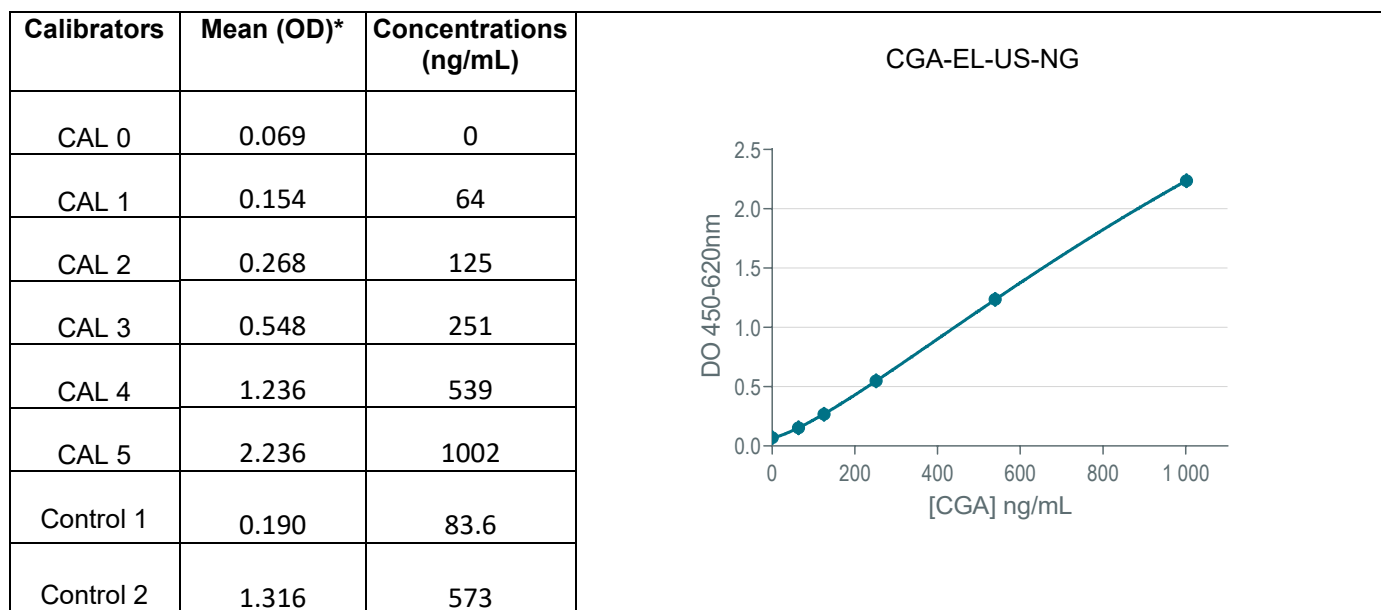
1. Prepare and identify a sufficient number of test tubes to perform a pre-dilution of samples, calibrators and controls
2. Determine the number of microtiter well strips required for the assay. Remove unused strips from the frame holder and store them at 2-8°C in the adhesive bag, properly sealed with a desiccant.
3. **Pre-dilute the calibrators, samples and controls in plastic tubes to 1:51**
 - a. Dispense 1mL of diluent **DIL CAL** 0 into the plastic tubes
 - b. Add 20 µL of each calibrator, control or sample and gently mix with a vortex-type mixer
4. Dispense 200 µL of pre-diluted calibrators **CAL** samples or controls **CONTROL** to 1/51 into the DIL/CAL0 **DIL CAL** 0 into each wells.
5. Cover with the adhesive film, agitate for **1h at 700 rpm** at room temperature (**18-25°C**).
6. Wash the wells as follows:
 - a. Remove the content of the wells
 - b. Dispense 300 µL of wash solution **TWEEN 20** prepared as described in chapter 7.2.1
 - c. Repeat steps a. and b. 2 times more for a total of 3 washing cycles.
 - d. Finish by aspirating. The residual washing solution volume must be as low as possible. It is possible to gently tap the plate upside down to remove the residual liquid.
7. Dispense **200 µL** of HRP conjugate **CONJ** in all the wells.
8. Cover with the adhesive film and **incubate for 2h +/- 5'** at room temperature (18-25°C) under **agitation at 700 rpm**.
9. Wash the wells as above then:
10. Dispense 100 µL of TMB **SUBS TMB** in all wells. Cover with the adhesive film. Incubation in darkness is not necessary
11. Allow the colorimetric reaction to develop **for exactly 10 min** at room temperature (18-25°C), **under agitation (700 rpm)**.
12. Stop the reaction by adding 50 µL of stop solution **STOP SOLN** to all wells.
 - Read off the absorbance at 450 nm. Perform a second reading (optional) of the absorbance at a wavelength between 610 nm and 650 nm.

8. RESULTS

- Optional OD correction*: subtract readings at 620 nm from the readings at 450 nm.
- For each duplicate, calculate the mean absorbance (OD) of calibrators, controls and samples.
- Build a calibration curve by plotting the (corrected*) mean OD values at 450 nm of calibrators (y-axis) against their concentration (x-axis) indicated on the vial.
- The four parameter logistic 4PL with $1/y^2$ weight. mathematical fitting model is recommended for the calibration curves. Other data reduction functions may give slightly different results.

Read the concentration of the samples from the curve. The 1:51 predilution ratio is already calculated in calibrator concentrations.

Example of assay data: for illustration only and must under no circumstances be substituted for results obtained in the laboratory.



9. LIMITATION OF THE PROCEDURE

- Samples presenting cloudiness, haemolysis, hyperlipemia or containing fibrin may give inaccurate results.
- Do not extrapolate sample values beyond the last standard. Dilute the high concentrations samples and retest.
- Chromogranin A is a calcium-binding protein and its circulating levels are affected by the Ca^{++} concentration. The values found differ depending upon whether sera sampled on dry tubes or EDTA plasmas are assayed.

10. PERFORMANCE CHARACTERISTICS

10.1 Imprecision

Samples	n	Concentration Mean (ng/mL)	Within-series (CV%)
1	34	81.6	6.43
2	36	122	4.68
3	31	182	4.13
4	35	407	3.19
5	36	445	3.98
6	35	632	4.73

Samples	n	Concentration Mean (ng/mL)	Between-series (CV%)
1	28	51.3	11.5
2	28	187	6.4
3	28	442	6.8
4	20	697	7.0

10.2 Recovery test

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

10.3 Dilution test

Samples with high concentrations were diluted. The recovery percentages obtained were between 80% and 120%.

10.4 Specificity

No interference was observed when serum samples were tested with any of the following substances:

- Glugacon (up to 3000ng/mL)
- Gastrin (up to 3000ng/mL)
- Chromogranin B (up to 3000ng/mL)
- NSE (up to 3000ng/mL)
- Pancreatic polypeptid (up to 3000ng/mL)

10.5 Measurement range

The samples must be measured in the range between the limit of quantitation and the highest concentration of the calibration range, i.e. between 30.6 and 1000 ng/mL.

10.6 Limit of detection

The limit of detection (LOD or analytical sensitivity) of the CGA-EL-US-NG kit is defined as being the lowest detectable concentration that differs from zero with a probability of 95% calculated by adding 2 standard deviations to the mean value of 30 replicate analysis of the zero calibrator (CAL0). It was measured at 16.9 ng/mL.

The functional sensitivity is defined as being the concentration measured by the imprecision profile at a CV equal to 20%. It is estimated to be 30.6 ng/mL.

10.7. Hook effect

There is no hook effect up to 1.000.000 ng/mL.

10.8. Interference

- When the assay protocol provided in the instructions for use is followed, no interference with biotin for concentration ranging from 0 to 600 ng/mL is measured.
- NOTE: Results showed that a concentration of biotin at 1200ng/mL caused a slight interference (-14% maximum bias) with the CGA-EL-US-NG kit.
- No interference with **bilirubin and hemoglobin** measured up to respective concentrations of 0.15mg/mL, 2mg/mL was observed.
- No interference was observed when serum samples were supplemented with triglycerides from hyperlipidemic human sample and tested (743.4mg/dL total TG).

CAUTION: The immunoassay is protected against potential interferences with **heterophilic antibodies** such as HAMA and rheumatoid factors (RF) by addition of a protection (non-specific mouse immunoglobulins). Nevertheless, we cannot assure that there will never be any false positive or negative result due to the presence of heterophilic antibodies and rheumatoid factors in patient samples.

CGA-EL-US-NG



LABORATORY PROTOCOL CARD

Do not use this card without having read the whole package insert.

👉 **Pre-dilute the calibrators, samples and controls in plastic tubes to 1:51.**

1:51 pre-dilution

1. DISPENSE 1mL of diluent into the plastic tubes

DIL CAL 0

2. ADD 20µL of each calibrator, control or sample and gently mix with a vortex-type mixer

CAL CONTROL

3. DISPENSE SAMPLES INTO THE MICROPLATE

CAL CONTROL

↓ Dispense 200 µL of **pre-diluted calibrators, samples or controls to 1/51** into the DIL/CAL0 into each wells (Distribution in duplicate).



4. AGITATION

↓ Cover with the adhesive film, **agitate for 1H at 700 rpm** at room temperature (18-25°C).

↻ 700 rpm 1h

5. WASH THE WELLS (see § 7.2.1)

TWEEN 20

Prepare the wash solution by dilution of 9mL of Tween 20 in 3L of distilled water.

Remove the content of the wells.

Dispense 300µL of wash solution prepared into each well

Repeat steps 2 times more for a total of 3 washing cycles

Finish by aspirating. The residual washing solution volume must be as low as possible. It is possible to gently tap the plate upside down to remove the residual liquid.



6. DISPENSE THE CONJUGATE

CONJ

Dispense 200µL of HRP conjugate in all the wells



7. INCUBATE

Cover with adhesive film and incubate for **2h +/-5'** at room temperature (18-25°C) **under agitation at 700 rpm.**

↻ 700 rpm 2h +/-5'

8. WASH THE WELLS (see § 7.2.1)

TWEEN 20

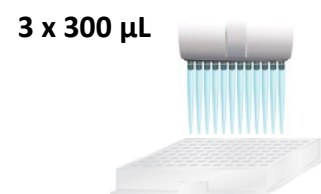
Prepare the wash solution by dilution of 9mL of Tween 20 in 3L of distilled water.

Remove the content of the wells.

Dispense 300µL of wash solution prepared into each well.

Repeat steps 2 times more for a total of 3 washing cycles.

Finish by aspirating. The residual washing solution volume must be as low as possible. It is possible to gently tap the plate upside down to remove the residual liquid.



9. DISPENSE THE SUBSTRATE

SUBS TMB

↓ Dispense 100µL of TMB in all wells. Cover with the adhesive film. Incubation in darkness is not necessary. Allow the colorimetric reaction to develop for **exactly 10 min** at room temperature (18-25°C) **under agitation at 700 rpm**

+ 100 µL
↻ 700 rpm 10 min

10. DISPENSE THE STOP SOLUTION

STOP SOLN

↓ Stop the reaction by adding 50µL of stop solution to all wells.



11. READ

🏠 Read off the absorbance at **450 nm**. Perform a second reading (optional) of the absorbance at a wavelength of 620nm (between 610 and 650 nm).

Use a **balanced 4-parameters logistic fit** for data interpolation.

450 nm



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