EPITOPE DIAGNOSTICS, INC.

EDI™ Human Chromogranin A CLIA Kit

Chemiluminescence Immunoassay for the quantitative determination of a Human Chromogranin A in serum

REF CL0820 CE IVD 50, 100, 250

INTENDED USE

The EDI™ Human Chromogranin A CLIA Kit is a Chemiluminescence Immunoassay (CLIA) intended for the quantitative determination of human chromogranin A (CgA) levels in serum using the ECL100 or ECL25 Immunoassay analyzer. Together with clinical information and other diagnostic procedures, this test aids in monitoring disease progression during the course of disease and treatment in patients with gastro-entero-pancreatic neuroendocrine tumors such as pheochromocytoma, carcinoid, etc. It may be used to differentiate diagnosis in patients of hypertension.

For in-vitro diagnostics purposes only

SUMMARY OF PHYSIOLOGY

Chromogranin A (CgA) is a 49 kDa acidic protein that consists of 439 amino acids encoded on chromosome 14[2, 3]. The CgA acts as a precursor to several functional peptides that negatively modulate the neuroendocrine function of autocrine or paracrine[4]. Additionally, the CgA is responsible for inducing and promoting the generation of secretory granules[6].The clinical applications may include aiding in the diagnosis of phenochromocytoma[1], neuroendocrine tumors[5], and carcinoid tumors[7, 8].

ASSAY PRINCIPLE

The EDI™ Human Chromogranin A Chemiluminescence Immunoassay (CLIA)kit is designed, developed, and produced for the quantitative measurement of human CgA level in serum samples. The assay utilizes a two-site "sandwich" technique with two antibodies that bind to different epitopes of CgA.

Assay calibrators, controls, or patient serum samples are added directly to a reaction vessel together with streptavidin coated magnetic particles and biotinylated anti-CgA polyclonal antibody. After an incubation period, a wash step is introduced and an acridinium ester conjugated anti-CgA monoclonal antibody is added to each reaction vessel. The magnetic particles capture the biotin antibody as well as an immuno complex in the form of "magnetic particles–biotin CgA antibody–CgA–acridinium ester CgA antibody". Materials bound to the solid beads are held in a magnetic field while unbound materials are washed away. Then,trigger solutions are added to the reaction vessel and light emission is measured with the ECL100 or ECL25 Immunoassay analyzer. The relative light units (RLU) are *proportional* to the concentration of a CgA in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve and reported in serum CgA concentration.

REAGENTS: PREPARATION AND STORAGE

The kit must be stored at 2 – 8°C upon receipt. All components are stable until the expiration date stated over the label on the kit box. Reagents from different kit lot numbers should not be combined or interchanged.

Standard Batch Quantity: 100/kit

The following reagents are preloaded in the reagent cartridge:

1. CgA Magnetic Particle Solution (L0512)

Qty:	1 x 2.3 mL (50/kit), 1 x 2.8 mL (100/kit), 1 x 7.5 mL (250/kit)
Storage:	2 – 8°C
Preparation:	Ready to use

2. Biotin CgA Antibody (L0513)

Qty:	1 x 4.5 mL (50/kit), 1 x 8.5 mL (100/kit), 1 x 20 mL (250/kit)
Storage:	2 – 8°C
Preparation:	Ready to use

3. Acridinium Ester CgA Antibody (L0514)

Qty:	1 x 4.5 mL (50/kit), 1 x 8.5 mL (100/kit), 1 x 20 mL (250/kit)
Storage:	2 – 8°C
Preparation:	Ready to use

4. Chromogranin A Calibrators (L0517 – L0518)

Lyophilized human CgA in a bovine serum albumin-based matrix with a non-azide preservative. Refer to vials for exact concentration.

Qty:	2 x vials
Storage:	2 – 8°C before reconstitution, <-20°C after reconstitution
Preparation:	Must be reconstituted with 0.5 mL of demineralized water and then mixed by inversions or gentle vortexing. Make sure that all solids are dissolved completely and there is no air bubbles prior to use.

5. Chromogranin A Controls (L0519 – L0520)

Lyophilized human Chromogranin A in a bovine serum albumin-based matrix with a non-azide preservative. Refer to vials for exact concentration.

Qty:	2 x vials
Storage:	2 – 8°C before reconstitution <-20°C after reconstitution
Preparation:	Must be reconstituted with 0.5 mL of demineralized water and then mixed by inversions or gentle vortexing. Make sure that all solids are dissolved completely and there is no air bubbles prior to use.

SAFETY PRECAUTIONS

The reagents must be used in a professional laboratory environment and are for in vitro diagnostic use. Source material which contains reagents of bovine serum albumin was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they were potentially infectious. Avoid contact with reagents containing hydrogen peroxide. Do not get in eyes, on skin, or on clothing.

Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Exercise Good Laboratory Practices.

MATERIALS REQUIRED BUT NOT PROVIDED

1. ECL100 Immunoassay Analyzer or ECL25 Immunoassay Analyzer
2. CL011 Cuvettes (for ECL100) or CL010 Cuvettes (for ECL25)
3. Wash Reagent (P-594)
4. Trigger Solutions A and B (P-595)

The instrument must operate using materials supplied by Epitope Diagnostics, Inc. When materials are sourced from a third-party suppliers are being used, Epitope Diagnostics, Inc. takes no responsibility of the validity for obtained results. Materials are available to purchase from Epitope Diagnostics, Inc. Please contact your distributor for more information.

SPECIMEN COLLECTION AND PREPARATION

No special preparation of individual is necessary prior to specimen collection. Whole blood should be collected with red-top Vacutainer. Allow clotting at room temperature and separate serum from the cells by centrifugation (850 – 1500xg for 10 – 15 minutes). Please follow Vacutainer's instruction. The serum should be separated from the cells within one hour of blood collection.

After centrifugation and separation of serum from the blood collection tube, the sample should be stored at room temperature if serum will be packed and shipped to the central laboratory within the same day. Otherwise store it at - 20ºC until packed and shipped to the central laboratory for testing.

The serum can be transported at room temperature (packed in a foam box with no ice packs) if serum will be delivered to the lab within 3 days; or transported at - 20ºC or on dry ice if serum will be delivered to the lab more than 3 days.

When the central laboratory receives the serum, the serum should be stored at room temperature, if serum will be measured within 4 hours. Otherwise, store at -20 ºC if not measured within 4 hours. **Don't store the serum in a refrigerator!** Avoid more than three freeze-thaw cycles of serum samples.

Some substances in the samples will interfere with the test results. The common interfering substances and maximum allowable concentrations are as follows:

- bilirubin 60 mg/dL
- triglycerides 1500 mg/dL
- hemoglobin 900 mg/dL
- biotin 200 nmol/L
- For patients receiving high-dose biotin therapy (5 mg/ day), samples must be collected 8 hours after taking the last dose of biotin
- For patients receiving Proton Pump Inhibitors (PPIs), samples must be collected 5 days after taking the last dose of PPIs

A single assay of this item requires 20 µL sample. This quantity does not include the amount of dead volume in the sample container, the capacity required for retesting, and other measurement items. For the definition of minimum required sample size, refer to the equipment manual.

CALIBRATION

An active calibration curve is required for all tests. For the assay, calibration must be performed when a reagent lot is used for the first time and remains valid for 28 days. After this period, recalibration is required. Additionally, we recommend performing calibration if control results fall outside the acceptable range.

QUALITY CONTROL

The use of controls is left to the discretion of the user, based on good laboratory practices, requirements, and applicable laws. It is strongly recommended to perform a control test before running patient samples. If no patient samples are tested, a control test is not necessary. Quality control results that fall outside the acceptable range may indicate invalid test results. Please refer to the Certificate of Analysis for the correct control range.

ASSAY PROCEDURE

1. Reagents from different kit lot numbers should not be combined or interchanged. Make sure that there are no air bubbles in any reagents, calibrator and control vials.
2. Reagent Preparation

2.1 Remove reagent cartridges from packaging and replace the solid caps with the provided soft caps for ECL100. For ECL25, carefully remove the aluminum foil seal on each container on the cartridges.

2.2 For the ECL100, take out the Magnetic Particle bottle make sure to roll between hands and gently but thoroughly mix until the magnetic particle solution is homogenous. The solution should be uniform with no clumps of magnetic particles visible; this step is vital for assay performance.

- Note: For ECL 100, if the Magnetic Particle Solution volume is over 2.8 mL, it will be supplied in a glass bottle. It will need to be transferred from the glass bottle to the plastic vial in the cartridge with the rest of the reagents. Please note: The maximum transfer volume of the Magnetic Particle Solution is 2.8 mL. Make sure the Magnetic Particle Solution is mixed thoroughly before transferring.

2.3 For ECL25, mix the magnetic beads by moving back and forth the bottom part of the cartridge at upright position. Make sure to look inside the cartridge until the solution is uniform with no clumps of magnetic particles visible and no air bubbles. Recap the bottle. Open the top soft cap of all reagent bottles, leaving only the hollow soft rubber.

2.4 The reagents are now ready to be loaded into the ECL100 or ECL 25 for calibration.

3. Assay Program

The following table illustrates the protocol used by the ECL100 or ECL25 for instrument operation.

Components	Quality Control Hole (µL)	Sample Hole (µL)
CgA Controls (L0519, L0520)	20 µL	-
Patient Serum Samples	-	20 µL
Biotin CgA Antibody (L0513)	75 µL	75 µL
CgA Magnetic Particle Solution (L0512)	25 µL	25 µL
Incubation Period 1		
Wash the reaction cup 3 times with the wash solution		
Acridinium Ester CgA Antibody (L0514)	75 µL	75 µL
Incubation Period 2		
Wash the reaction cup 3 times with the wash solution		
Trigger Solution A (P-595A)	200µL	200µL
Trigger Solution B (P-595B)	200µL	200µL

The assay total incubation time is less than 30 minutes.

INTERPRETATION OF RESULTS

The chemiluminescence analyzer calculates the concentration values of the sample and control by a standard curve (fitting method: four parameters or point-to-point) and the measured RLU. Values are compared with the range of the marked value. If it exceeds indicated quality control range, it indicates that the test is unqualified and should be re-tested.

Due to methodological differences or antibody specificity, there may be deviations between the test results of reagents from different manufacturers. Therefore, direct comparisons should not be made to avoid false interpretation.

EXPECTED VALUES

CgA concentrations were established by measuring in one hundred sixty adult serum samples of normal healthy the USA population. The average CgA concentration of this group was found to be 29.06 ng/mL. The suggested normal high cut off is **100 ng/mL.** EDI recommends laboratories to establish their own normal range for CgA based on the local population.

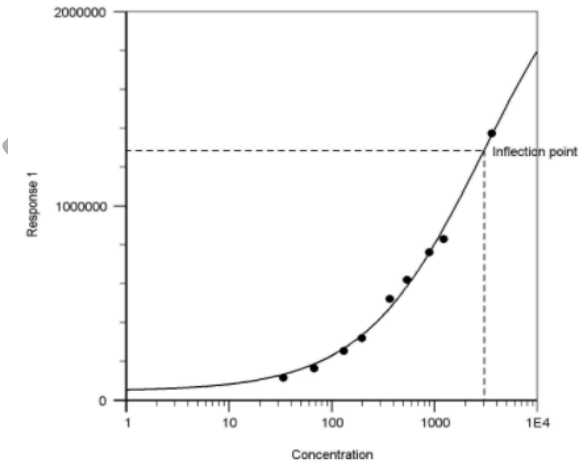
LIMITATIONS OF THE PROCEDURE

1. This product is for use on the ECL100 or ECL25 Immunoanalyzer only. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, operation, system performance, instructions, calibration, precautions, hazards, maintenance, and troubleshooting.
2. Reagents from different kit lot numbers should not be combined or interchanged
3. Test results obtained from the proposed kit should not be served as a sole basis for clinical diagnosis or patient management.
4. If the test sample result is higher than the upper limit of the calibration curve, it is recommended to re-measure after dilution according to a certain ratio. The measured value is recalculated according to the dilution ratio to ensure the accuracy of the result.

PERFORMANCE CHARACTERISTICS

Example of Calibration Curve

The calibration curve is built in the calibration card (L0516). This curve is lot dependent. Here is an example of the 10-point calibration curve.



Hook Effect

The hook effect was determined with highly concentrated CgA specimens. The test results indicated no hook effect up to 20,000 ng/mL.

Limit of Blank

The limit of blank (LoB) was determined by 60 replicates in three assays of blank samples to be 1.02 ng/mL.

Limit of Detection

The limit of detection (LoD) was determined using five low level patient samples in total 60 replicates in three assays and was found to be 2.27 ng/mL.

Limit of Quantification

The limit of Quantification (LoQ) was determined using five low level patient samples in total 60 replicates in three assays and was found to be 3.53 ng/mL.

Linearity

Linearity was determined by an assay with a diluted specimen of high CgA concentration. The linearity of this test is up to 10,000 ng/mL.

Diluted Calibrator	Average Concentration (ng/mL)	Theoretical Concentration (ng/mL)	Linear Recovery (%)	R^2
1	2.12	0.00	-	0.984
2	1467.50	1199.60	122%	
3	2804.00	2399.19	117%	
4	3150.00	3198.92	98%	
5	4859.50	4798.38	101%	
6	6223.00	6397.84	97%	
7	6186.50	7197.57	86%	
8	7374.00	8397.17	88%	
9	10199.64	9596.76	106%	

Intra-assay Precision

Intra-assay precision was determined by measuring eight replicates of two samples and one assay control. The results are as follows:

Sample	Average Concentration(ng/mL)	SD	CV (%)
1	12.6	1.1	8.5
2	52.1	3.2	6.2
Control	1949.4	139.6	7.2

Inter-assay Precision

Inter-assay precision was determined by measuring three samples in twenty-four replicates. The results are summarized below:

Sample	Average Concentration (ng/mL)	SD	CV (%)
1	13.2	1.70	12.9
2	54.2	4.95	9.1
3	2007.5	196.64	9.8

WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Diagnostics, Inc. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

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TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

For technical assistance or to place an order, please contact Epitope Diagnostics, Inc. at +1 (858) 693-7877 or fax to +1 (858) 693-7678 or email atcs@epitopediagnostic.com

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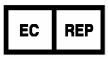

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GLOSSARY OF SYMBOLS (EN 980/ISO 15223)