Epitope Diagnostics, Inc.

EDI™ Human Chromogranin A ELISA Kit

Enzyme Linked ImmunoSorbent Assay (ELISA) for the measurement of Human Chromogranin A Level

REF **KTR 820** Σ **96**

For Research Use Only. Not for Use in Clinical Diagnostic Procedures

INTENDED USE

This ELISA (enzyme-linked immunosorbent assay) kit is intended for the quantitative determination of human chromogranin A levels in EDTA-plasma and serum samples. This assay exclusively measures human chromogranin A without the high dose "hook" effect up to 1,000,000 ng/ml. This test may be used as an aid for detecting patients with pheochromocytoma and neuroendocrine tumors (carcinoids). This kit is for research only.

SUMMARY OF PHYSIOLOGY

Chromogranin A is a 49 kDa acidic protein that consists of 439 amino acids encoded on chromosome 14. Chromogranin A has been identified in a number of normal and neopastic endocrine tissues. It is demonstrated that an elevated level of circulating chromogranin A is a marker for tumors of neuroendocrine origin. However, the most significant clinical use of chromogranin A is related to the diagnostic procedure in patients with pheochromocytoma. The following is a short summary of the potential usages of chromogranin A.

1. A very sensitive (83%) and highly specific (96%) marker in the evaluation of actual or suspected pheochromocytoma. Drugs commonly employed in the diagnosis or treatment of pheochromocytoma have little effect on the plasma chromogranin A level, which is a great advantage of measuring chromogranin A over catecholamines.

2. To ascertain the source of a tumor. A high chromogranin A level indicates that the tumor arises from neuroendocrine tissues.
3. Endocrine tumors that do not produce their specific hormones, for example, calcitonin negative but chromogranin A positive C-cell carcinoma; zero-cell carcinoma; beta-cell carcinoma; parathyroid carcinoma.

ASSAY PRINCIPLE

This ELISA is designed, developed and produced for the quantitative measurement of human chromogranin A in EDTA-plasma or serum sample. The assay utilizes the two-site "sandwich" technique with two selected antibodies that bind to different epitopes of human chromogranin A.

Assay standards, controls and patient samples are added directly to wells of microplate that is coated with a polyclonal chromogranin A antibody. After the first incubation period, the antibody on the wall of microtiter well captures human chromogranin A in the sample and unbound protein in each microtiter well is washed away. Then a horseradish peroxidase (HRP)-labeled monoclonal anti-human chromogranin A antibody is added to each microtiter well and a "sandwich" of "monoclonal antibody - human chromogranin A – polyclonal antibody" is formed. The unbound monoclonal antibody is removed in the subsequent washing step. For the detection of this immunocomplex, the well is incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader. The enzymatic activity of the immunocomplex bound to the chromogranin A on the wall of the microtiter well is directly proportional to the amount of chromogranin A in the sample. A standard curve is generated by plotting the absorbance versus the respective human chromogranin A concentration for each standard with a 4 parameter curve fit. The concentration of human chromogranin A in test samples is determined directly from this standard curve.

REAGENTS: Preparation and Storage

This test kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until the expiration date.

Allow all reagents to come to room temperature prior to use. Reagents from different kit lot numbers should not be combined or interchanged.

1. **Anti-CgA Antibody Coated Microplate (Cat. No. 30063)**
One microplate with 12 x eight strips (96 wells total) coated with antibody to human chromogranin A. The plate is framed and sealed in a foil zipper bag with a desiccant. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

2. **CgA Tracer Antibody (Cat. No. 30431)**
One vial containing **12 mL** HRP-labeled anti-human chromogranin A monoclonal antibody in a stabilized protein matrix. **This reagent is ready to use (Caution: no further dilution required)**. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

3. **CgA Assay Buffer (Cat. No. 30074)**
One bottle containing 30 mL of ready-to-use phosphate-buffered saline based assay buffer with bovine serum albumin added. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

4. **ELISA Wash Concentrate (Cat. No. 10010)**
One bottle contains **30 mL** of 30-fold concentrate. The contents must be diluted with **870 mL** of deionized water and mix well before use. Upon dilution this yields a working wash solution containing a surfactant in phosphate-buffered saline with a non-azide, non-mercury preservative. The diluted buffer should be stored at room temperature and is stable until the expiration date on the kit box.

5. **ELISA HRP Substrate (Cat. No. 10020)**
One bottle contains 12 mL of tetramethylbenzidine (TMB) with stabilized hydrogen peroxide. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

6. ELISA Stop Solution (Cat. No. 10030)
One bottle contains 12 mL of 0.5 M sulfuric acid. This reagent should be stored at 2 – 8°C or room temperature and is stable until the expiration date on the kit box.

7. Chromogranin A Standards (Cat. No. 30064 – 30068)
Five vials each containing human chromogranin A in a lyophilized bovine serum-based matrix with a non-azide, non-mercury preservative. **Refer to vials for exact concentration for each standard.** These reagents should be stored at 2 – 8°C and are stable until the expiration date on the kit box.

8. Chromogranin A Controls (Cat. No. 30069 – 30070)
Two vials each containing human chromogranin A in a lyophilized bovine serum-based matrix with a non-azide, non-mercury preservative. **Refer to vials for exact concentration range for each control.** Both controls should be stored at 2 – 8°C and are stable until the expiration date on the kit box.

SAFETY PRECAUTIONS
The reagents must be used in a professional laboratory environment and are for research use. The source material for reagents containing bovine serum 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 TMB, hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Sulfuric acid may cause severe irritation on contact with skin. 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. Use Good Laboratory Practices.

MATERIALS REQUIRED BUT NOT PROVIDED
1. Precision single channel pipettes capable of delivering 15 µL, 50 µL, 100 µL, and 1000 µL etc.
2. Repeating dispenser suitable for delivering 100 µL.
3. Disposable pipette tips suitable for above volume dispensing.
4. Disposable 12 x 75 mm or 13 x 100 glass or plastic tubes.
5. Disposable plastic 100 mL and 1000 mL bottle with caps.
6. Aluminum foil.
7. Deionized water.
8. Plastic microtiter well cover or polyethylene film.
9. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
10. Spectrophotometric microplate reader capable of reading absorbance at 450/650 nm or 450/620 nm.

SPECIMEN COLLECTION
Only 30 µL total (15 µL each) of human EDTA-plasma or serum is required for human chromogranin A measurement in duplicate. No special preparation of individual is necessary prior to specimen collection. Whole blood should be collected with lavender-top Vacutainer. Separate the plasma from cells by centrifugation (850 – 1500xg for 10 minutes). The plasma should be separated from the cells within one hour of blood collection and transferred to a clean test tube. **Plasma samples should be stored at – 15ºC** if the assay is not to be performed within 72 hours. Otherwise, the plasma samples should be stored at room temperature for up to 72 hours. It is important that the plasma samples must not be stored at 2 –8ºC for long term storage. Avoid more than three freeze-thaw cycles of specimen.

Serum sample can also be used for chromogranin A measurement. Tests with paired EDTA-plasma and serum sample from same donor shows that serum gives almost the same chromogranin A level as EDTA-plasma by using this ELISA.

SPECIMEN SHIPMENT
Collected EDTA-plasma or serum samples should be shipped to designated laboratory in frozen condition with dry ice. In case frozen condition is not available, samples should be shipped at room temperature in an insulated container for maximum 48 hour delivery. Samples must **not** be shipped refrigerated, such as with blue ice pack.

ASSAY PROCEDURE
1. Reagent Preparation
(1) Prior to use allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.
(2) ELISA Wash Concentrate (Cat. 10010) must be diluted to working solution prior to use. Please see REAGENTS section for details.
(3) Reconstitute all assay standards and controls by adding **0.5 mL** of deminerialized water to each vial. Allow the standards and controls to sit undisturbed for 10 minutes, and then mix well by inversions or gentle vortexing. Make sure that all solids are dissolved completely prior to use. These reconstituted standards and controls must be stored at -10°C or below. Do not exceed 3 freeze-thaw cycles.

2. Assay Procedure
Assay with Manual Protocol
(1) Place a sufficient number of antibody-coated microwell strips (Cat. 30063) in a holder to run human chromogranin A standards, controls and unknown samples in duplicate.
(2) Test Configuration

ROW	STRIP 1	STRIP 2	STRIP 3
A	STD 1	STD 5	SAMPLE 2
B	STD 1	STD 5	SAMPLE 2
C	STD 2	C 1	SAMPLE 3
D	STD 2	C 1	SAMPLE 3
E	STD 3	C 2	SAMPLE 4
F	STD 3	C 2	SAMPLE 4
G	STD 4	SAMPLE 1	
H	STD 4	SAMPLE 1	

(3) Add **15 µL** of standards, controls and patient samples into the designated microwells.
(4) Add **200 µL** of assay buffer (Cat. 30074) to each well
(5) Cover the plate with one plate sealer and also with aluminum foil, and incubate plate on an ELISA plate shaker with a shaking rate at 350 rpm to 450 rpm at room temperature **for 1 hour**.
(6) Remove aluminum foil and plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
(7) Add **100 µL** of Chromogranin A Tracer Antibody (Cat. 30431) to each of the wells.
(8) Cover the plate with the plate sealer and also with aluminum foil, and incubate plate on an ELISA plate shaker with a shaking rate at 350 rpm to 450 rpm at room temperature **for 1 hour**.
(9) Remove aluminum foil and plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
(10) Add **100 µL** of ELISA HRP Substrate (Cat. 10020) into each of the wells.
(11) Cover the plate with one plate sealer and also with aluminum foil to avoid exposure to light.
(12) Incubate plate at room temperature **for 20 minutes.**
(13) Remove the aluminum foil and plate sealer. Add 100 µL of ELISA Stop Solution (Cat. 10030) into each of the wells. Mix gently.

(14) Read the absorbance at dual wavelength at **450/650 nm or 450/620 nm** within 10 minutes in a microplate reader and select a **4-parameter curve fit** for result calculation.

Assay Procedure with Dynex DS-2 Automated ELISA System:

(1) Load a sufficient number of antibody-coated microwell strips (Cat. 30063) onto the system to run human chromogranin A standards, controls and unknown samples in duplicate.
(2) Load sufficient Chromogranin A Tracer antibody (Cat. 30431).
(3) Prepare and load kit standards/controls, patient samples, TMB, Stop Solution, 1x Wash Buffer onto the system accordingly.
(4) Add 200 µL of assay buffer to each well.
(5) Add 15 µL of standards, controls and patient samples into the designated microwells.
(6) Incubate plate with initial shaking for 1 min and then at room temperature for 60 minutes.
(7) Wash each well 4 - 5 times.
(8) Add 100 µL of Chromogranin A Tracer Antibody working solution to each of the wells.
(9) Incubate plate at room temperature for 60 minutes.
(10) Wash each well 4 - 5 times.
(11) Add 100 µL of ELISA HRP Substrate (Cat. 10020) into each of the wells.
(12) Incubate plate at room temperature for 15 - 20 minutes.
(13) Add 100 µL of ELISA Stop Solution (Cat. 10030) into each of the wells. Mix gently.
(14) Read the absorbance at 450/650 nm or 450/620 nm with a 4-parameter curve fit program.

Note for DS2:
(1) Open automated ELISA system other than DS-2 can also be used.
(2) It is very important to incubate the assay 18-22°C. A change of incubation temperature would cause unsatisfactory standard curve and erroneous test results.

PROCEDURAL NOTES

1. It is recommended that all standards, controls and unknown samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
2. If a Tecan is used for pipetting, it is recommended to add 200 µL assay buffer before adding the 15 µL assay standards, controls and test samples into each designated well. This is the same as the procedure with DS-2, but a reverse with the manual procedure.
3. Keep light-sensitive reagents in the original amber bottles.
4. Store any unused antibody-coated strips in the foil zipper bag with desiccant to protect from moisture.
5. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
6. Incubation times or temperatures other than those stated in this insert may affect the results.
7. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading.
8. All reagents should be mixed gently and thoroughly prior to use. Avoid foaming.
9. We strongly recommend using 4-Parameter curve fit for control and patient sample calculation. Other curve fit programs such as Point-to-Point, Log-Log, Log-Linear, etc. may give a poor linear recovery.

INTERPRETATION OF RESULTS

The human chromogranin A concentrations for the controls and patient samples are read directly from the standard curve using their respective corrected absorbance.

REPORTING TEST RESULTS

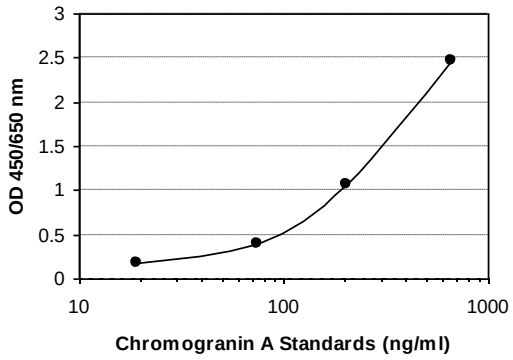
Laboratory should report test results directly derived from the assay. For samples showing a higher than 90% value of the highest assay standard, it is strongly recommended that the patient sample is diluted 1:100 with assay buffer and re-assay the diluted sample for a more accurate test result. For example, The highest assay standard is about 550 ng/mL, any sample that shows a value of greater than 495 ng/mL (90% of 550 ng/mL) should be repeated with a 1:100 diluted sample. If the 1:100 diluted sample still shows a higher value than that of the highest assay standard, one can either report the sample value as greater than the highest assay standard (e.g. > 56,000 ng/mL) or further measure a 1:10,000 diluted sample. It is preferred to obtain a diluted sample value located between standard level 2 and level 4, wherein, this measured value is multiplied by the dilution factor to obtain the report value for the patient.

EXAMPLE DATA AND STANDARD CURVE

A typical absorbance data and the resulting standard curve from human chromogranin A ELISA are represented. **This curve should not be used in lieu of standard curve run with each assay.**

Well I.D.	OD 450/650 nm Absorbance			Results ng/mL
	Readings	Average	Corrected	
0 ng/mL	0.107 0.112	0.110	0.000	
19.2 ng/mL	0.185 0.184	0.184	0.074	
75 ng/mL	0.400 0.400	0.400	0.290	
203 ng/mL	1.098 1.031	1.064	0.954	
660 ng/mL	2.442 2.488	2.465	2.355	
Control 1	0.248 0.248	0.248	0.138	40.66 ng/mL
Control 2	0.452 0.461	0.456	0.346	84.51 ng/mL

Human Chromogranin A ELISA



EXPECTED VALUES

Seventy-two normal adult sera were measured with this human chromogranin A ELISA. The normal range was found to be less than 100 ng/mL. Five patients with pheochromocytoma showed a chromogranin A level of significantly over 100 ng/mL and one of them reached 400,000 ng/mL. It is highly recommended that each laboratory should establish its own normal cut-off level. Paired EDTA-Plasma and Serum samples give almost the same values. Although a plasma or serum chromogranin A level above 100 ng/mL would be an aid in clinical diagnosis, it is recommended to establish a baseline level of chromogranin A for each patient in order to monitor cancer patients after surgery, especially if this assay is used for the monitoring of prostate cancer patients. A clear surge of chromogranin A level would indicating an increased cancer cell activity.

Some endocrine diseases such as primary hyperparathyroidism, hyperthyroidism, or secondary hyperparathyroidism caused by chronic renal failure would also give a higher than normal serum chromogranin A level. It is reported that patients with rheumatoid arthritis, Systemic LupusErythematosus would also cause higher chromogranin A level. Some therapeutic drugs that stimulate the endocrine system such as sexual hormone releasing hormone may also cause higher chromogrnain A level in patient sample.

LIMITATION OF THE PROCEDURE

1. Since there is no Gold Standard concentration available for human chromogranin A measurement, the values of assay standards were established by correlation to a highly purified chromogranin A standard.
2. For sample values reading greater than the highest standard or 90% value of the highest standard, it is recommended to re-assay samples with dilution.
3. Storing samples at refrigerated condition causes significant degradation of intact chromogranin A into small fragments. These fragments may cause interference of the assay resulting in false low test result.
4. Serum samples are not as stable as EDTA-plasma samples. Therefore, it is strongly recommended to use EDTA-plasma sample for chromogranin A measurement.
5. Bacterial or fungal contamination of plasma specimens or reagents, or cross-contamination between reagents may cause erroneous results.
6. Water deionized with polyester resins may inactive the horseradish peroxidase enzyme.

QUALITY CONTROL

To assure the validity of the results each assay should include adequate controls with known chromogranin A levels. We recommend that all assays include the laboratory's own chromogranin A controls in addition to those provided with this kit.

PERFORMANCE CHARACTERISTICS

Sensitivity

The sensitivity (LLOD or LOD) of the human chromogranin A ELISA is approximately 2 ng/mL, which is determined by 2 standard deviations above the average of zero standard in 7 replicate determination of zero standard and 7 replicate determination of standard level 2 of 19.2 ng/mL.

High Dose "hook" effect

This assay has showed that it did not exibit any high dose "hook" effect up to 1,000,000 ng/mL. This is essential because some patients with pheochromocytoma had over 300,000 ng/mL of chromogranin A level in their serum sample.

Precision

The intra-assay precision is validated by measuring two controls samples in a single assay with 8 replicate determinations.

Mean Chromogranin A Value (ng/mL)	CV (%)
28.24	2.9
59.81	3.7

The inter-assay precision is validated by measuring two control samples in duplicate in 12 individual assays.

Mean Chromogranin A Value (ng/mL)	CV (%)
41.91	7.28
82.74	3.06

Linearity

Three human serum samples were diluted with assay buffer and assayed. The results in the value of ng/mL are as follows:

#	DILUTION	OBSERVED VALUE	EXPECTED VALUE	RECOVERY %
1	Neat	717.5	-	-
	1:2	357.4	358.8	100
	1:4	171.1	179.4	95
	1:8	74.6	89.7	83
2	Neat	932.7	-	-
	1:2	495.1	466.4	106
	1:4	229.3	233.2	98
	1:8	112.2	116.6	96
	1:16	54.5	58.3	93
3	Neat	467.8	-	-
	1:2	234.6	233.9	100
	1:4	117.8	117.0	100
	1:8	52.2	58.5	89

Recovery

Three patient serum samples were spiked with various amounts of human chromogranin A control samples and assayed. The results indicate a very satisfactory spike recovery for this assay.

#	Spiked Sample	Measured CgA Value (ng/mL)	RECOVERY (%)
1	S1	12.78	
	Control Sample 1	62.43	
	25% S1 + 75% L3	46.48	93
	50% S1 + 50% L3	37.94	100
	75% S1 + 25% L3	24.27	95
2	S2	51.74	
	Control Sample 2	185.74	
	25% S2 + 75% L4	143.91	95
	50% S2 + 50% L4	112.07	121
	75% S2 + 25% L4	80.22	94
3	S3	73.00	
	Control Sample 2	185.74	
	25% S3 + 75% L4	149.27	95
	50% S3 + 50% L4	125.61	97
	75% S3 + 25% L4	91.72	91

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.

REFERENCES

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TECHNICAL ASSISTANCE AND CUSTOMER SERVICE
For technical assistance or place an order, please contact Epitope Diagnostics, Inc. at (858) 693-7877 or fax to (858) 693-7678.
www.epitopediagnostics.com

This product is developed and manufactured by
Epitope Diagnostics, Inc.
San Diego, CA 92121, USA

Manufacturer	No. of tests
REF Catalog Number	Keep away from heat and direct sun light
CONC Concentrate	Store at
LOT Lot No.	Use by

Short Assay Procedure of Human Chromogranin A:

(1) Add **15 µL** of standards, controls and patient samples into the designated microwell.

(2) Add **200 µL** of assay buffer to each well.

(3) Mix, cover and incubate the plate at room temperature and shaking 350 rpm – 450 rpm for **1 hour**.

(4) Wash each well 5 times.

(5) Add **100 µL** of Tracer Antibody into each well.

(6) Incubate **1 hour** at RT and shaking 350 rpm – 450 rpm

(7) Wash each well 5 times.

(8) Add **100 µL** of ELISA HRP Substrate into each well.

(9) Cover and incubate plate at room temperature for **20 minutes**.

(10) Add **100 µL** of ELISA Stop Solution into each of the wells.

(11) Read the absorbance at OD 450/650 nm or 450/620 nm.