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Chromogranin A ELISA Assay Kit

Catalog Number:

CHR31-K01 (1 x 96 wells)

For Research Use Only. Not for use in diagnostic procedures.

V. 2.0 (04 MAR 24)

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INTENDED USE

The Eagle Biosciences Human Chromogranin A ELISA Assay Kit (enzyme-linked immunoassay 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. The Eagle Biosciences Human Chromogranin A ELISA Assay Kit is for research use only and not to be used in diagnostic procedures.

INTRODUCTION

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 neoplastic endocrine tissues. It is demonstrated that an elevated level of circulating Chromogranin A level 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 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.

PRINCIPLE OF THE ASSAY

The Eagle Biosciences Human Chromogranin A ELISA Assay Kit 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 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 proteins 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

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 Chromogranin A ELISA Assay Kit box.

2. CgA Tracer Antibody

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 Chromogranin A ELISA Assay Kit box.

3. CgA Assay Buffer

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 Chromogranin A ELISA Assay Kit box.

4. ELISA Wash Concentrate

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 Chromogranin A ELISA Assay Kit box.

5. ELISA HRP Substrate

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 Chromogranin A ELISA Assay Kit box.

6. ELISA Stop Solution

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 Chromogranin A ELISA Assay Kit box.

7. Chromogranin A Standards

Five vials each containing human chromogranin A in a lyophilized bovine serum based matrix with a non-azide, non-mercury preservative. Refer to vial for exact concentration for each standard. These reagents should be stored at 2 – 8°C and are stable until the expiration date on the Chromogranin A ELISA Assay Kit box.

8. Chromogranin A Controls

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 Chromogranin A ELISA Assay Kit box.

SAFETY PRECAUTIONS

The Human Chromogranin A ELISA Assay Kit reagents must be used in a professional laboratory environment and is for Research Use Only and is not to be used in diagnostic procedures. Only source material from which reagents of bovine serum was derived in the contiguous 48 United States. It was obtained only from donor health 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

- Precision single channel pipettes capable of delivering 25 µL, 50 µL, 100 µL, and 1000 µL etc.
- Repeating dispenser suitable for delivering 100 µL.
- Disposable pipette tips suitable for above volume dispensing.
- Disposable 12 x 75 mm or 13 x 100 glass or plastic tubes.
- Disposable plastic 100 mL and 1000 mL bottle with caps.
- Aluminum foil.
- Deionized water.
- Plastic microtiter well cover or polyethylene film.
- ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
- 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 and 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 – 15°C 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 –8°C 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.

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 must be diluted to working solution prior use. Please see REAGENTS section for details.
3. Reconstitute all assay standards and controls by adding 0.5 mL of demineralized 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.

ASSAY PROCEDURE

1. Place a sufficient number of antibody coated microwell strips in a holder to run human chromogranin A standards, controls and unknown samples in duplicate.
2. Test Configuration for Chromogranin A ELISA Assay Kit:

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 to each well
5. Cover the plate with one plate sealer 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 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 to each of the wells.
8. Cover the plate with the plate sealer 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 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 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 into each of the wells. Mix gently.
14. Read the absorbance at dual wave length 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 onto the system to run human chromogranin A standards, controls and unknown samples in duplicate.
2. Load sufficient Chromogranin A Tracer antibody
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 into each of the wells.
12. Incubate plate at room temperature for 15 - 20 minutes
13. Add **100 µL** of ELISA Stop Solution 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 unsatisfied 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 are used for pipetting, it is recommended by adding 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.



- Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
- Incubation times or temperatures other than those stated in this insert may affect the results.
- Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading
- All reagents should be mixed gently and thoroughly prior use. Avoid foaming.
- 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-Lenear, 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 500 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 sample.

EXAMPLE DATA AND STANDARD CURVE

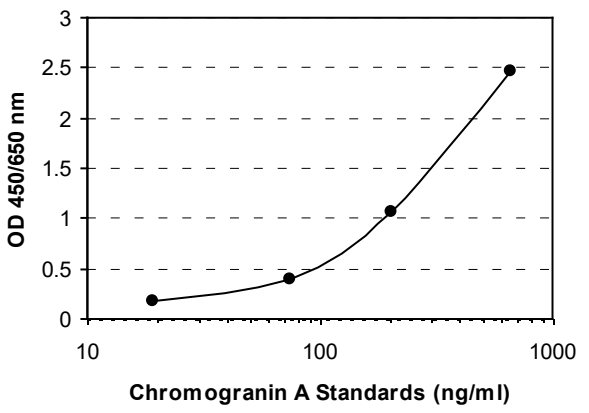
A typical absorbance data and the resulting standard curve from Human Chromogranin A ELISA Assay Kit are represented. This curve should not be used in lieu of standard curve run with each assay.

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	2.442	2.465	2.355	



ng/mL	2.488			
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 subjects 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 recommend that each laboratory should establish its own normal cut off level. Paired EDTA-Plasma and Serum samples give almost the same values.

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 highest standard or 90% value of the highest standard, it is recommend to re-assay samples with dilution.
3. Store 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 sample are not as stable as EDTA-plasma sample. 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 Assay Kit is approximately 2 ng/mL, which is determined by 2 standard deviation 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

The Eagle Biosciences Chromogranin A ELISA Assay Kit has showed that it did not exhibit 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	7.3
82.74	3.1

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	Sample A	717.5	-	-
	50%	357.4	358.8	100
	25%	171.1	179.4	95
	12.5%	74.6	89.7	83
2	Sample B	932.7	-	-



	50%	495.1	466.4	106
	25%	229.3	233.2	98
	12.5%	112.2	116.6	96
	6.25%	54.5	58.3	93
3	Sample C	467.8	-	-
	50%	234.6	233.9	100
	25%	117.8	117.0	100
	12.5%	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	Sample A	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	Sample B	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
2	Sample C	73.00	
	Control Sample 2	185.74	
	25% S2 + 75% L4	149.27	95
	50% S2 + 50% L4	125.61	97
	75% S2 + 25% L4	91.72	91



INTERFERENCE

Bilirubin, hemoglobin and lipid triglycerides were tested as potential interferents to the human chromogranin A ELISA kit. Randomly selected samples were spiked with the potential interferents at the concentrations listed in the table below:

It is indicated that hemolyzed sample may not be suitable for chromogranin A measurement.

Interferent (Concentration tested, mg/mL)	Test (ng/mL)	Control (ng/mL)	Bias (d_{obs} , %)	
Bilirubin (EP07 recommended concentration: 0.05 mg/mL)	2.0	228.6	259.7	12.0%
	0.2	235.8	259.7	92%
	0.04	244.3	259.7	5.9%
Hemoglobin (EP07 recommended concentration: 2 mg/mL)	20.0	184.0	259.7	29.1%
	2.0	240.8	259.7	7.3%
	0.4	260.8	259.7	0.4%
Lipids (EP07 recommended concentration: 5 mg/mL)	400.0	55.6	58.4	4.8%
	40.0	59.0	58.4	1.0%
	20.0	56.2	58.4	3.8%
	6.0	258.9	256.7	0.3%

SHORT ASSAY PROCEDURE

Manual Procedure

1. Add **15 μ L** of the calibrators, controls, and samples into the designated microwells.
2. Add **200 μ L** of the assay buffer to each well.
3. Mix, cover, and incubate at **room temperature (20-25 °C)** and shaking at **350 - 450 rpm** for **60 minutes**.
4. Wash each well five times.
5. Add **100 μ L** of the tracer antibody to each well.
6. Cover and incubate at **room temperature (20-25 °C)** with shaking at **350 or 450 rpm** for **60 minutes**.
7. Wash each well five times
8. Add **100 μ L** of substrate to each well.
9. Cover and incubate at **room temperature (20-25 °C)** for **20 minutes**.
10. Add **100 μ L** of the stop solution to each well.
11. Read the absorbance at **450/650 nm** or **450/620 nm**



Automated Assay Procedure

1. Prepare and load reagents
2. Add **200 μL** of assay buffer to each well.
3. Add **15 μL** of calibrators, controls and patient samples into the designated microwells.
4. Incubate plate with shaking for **1 minute** and then **at room temperature (20-25 °C)** for **60 minutes**.
5. Wash each well **4 - 5 times**.
6. Add **100 μL** of tracer antibody to each well.
7. Incubate **at room temperature (20-25 °C)** for **60 minutes**.
8. Wash each **well 4 - 5 times**.
9. Add **100 μL** of ELISA HRP Substrate into each of the wells.
10. Incubate plate at **room temperature (20-25 °C)** for **15 - 20 minutes**.
11. Add **100 μL** of stop solution to each well.
12. Read the absorbance at **450/650 nm** or **450/620 nm**.



REFERENCES

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Warranty Information

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.