Human Chromogranin A ELISA Kit

Enzyme Linked ImmunoSorbent Assay (ELISA) for the measurement of Human Chromogranin A Levels in Serum

OKDA00058

FOR RESEARCH USE ONLY

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INTENDED USE

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

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 neoplastic endocrine tissues. It was demonstrated that an elevated circulating chromogranin A level would be a marker of tumors of neuroendocrine origin. However, the most significant clinical use of chromogranin A is related to procedure in samples of pheochromocytoma. The following is a short summary of potential use of chromogranin A.

1. A very sensitive (83%) and highly specific (96%) marker in the evaluation of actual or suspected pheochromocytoma. Drugs commonly used in the treatment of pheochromocytoma have little effect on the plasma chromogranin A level. This means that it is a great advantage to measure chromogranin A in stead of 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 serum samples. The assay utilizes the two-site "sandwich" technique with two selected antibodies that bind to different epitopes of human chromogranin A.

Assay calibrators, controls and samples are directly added to the microtiter wells of a microplate coated with a polyclonal anti-chromogranin A antibody. After the first incubation period, the antibody fixed on the wall of the microtiter well captures human chromogranin A in the sample. Remaining unbound proteins in the microtiter wells are 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. The well is incubated with a substrate solution in a timed interval, the reaction is stopped and the developed colour is 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 calibration curve is generated by plotting the absorbance versus the respective human chromogranin A concentration for each calibrator on point-to-point or cubical scales or 4 parameter curve fit. The concentration of human chromogranin A in test samples is determined directly from this calibration 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 this expiration date.

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

1. **Anti-CgA Antibody Coated Microplate**

One microplate with 12x8 strips (96 wells total) coated with antibody to human chromogranin A. The plate is framed and sealed in a foil Ziploc 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. Ab | HRP | CONC **Detecting Antibody**

One vial containing 0.6 mL HRP labeled anti-human chromogranin A antibody in a stabilized protein matrix. This reagent must be diluted with dilution buffer before use. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

3. DIL | BUF **Dilution buffer**

One vial containing 12 mL ready to use buffer. It should be only used for detecting antibody dilution according to the assay procedures. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

4. INC | BUF **Incubation buffer**

One bottle containing 30 mL of ready to use phosphate buffered saline based incubation buffer with bovine serum albumin. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

5. WASH | SOLN | CONC **Washing buffer**

One bottle containing 20 mL of a 30 fold concentrate. Before use the contents must be diluted with 580 mL of distilled water and mixed well. Upon dilution this yields a working wash solution containing a surfactant in phosphate buffered saline with a non-azide preservative. The diluted solution should be stored at room temperature and is stable until the expiration date on the kit box.

6. CHROM | TMB **TMB-Substrate solution**

One bottle containing 12 mL of tetramethylbenzidine (TMB) with hydrogen peroxide. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

7. STOP | SOLN **Stop Solution**

One bottle containing 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.

8. CAL | N **Calibrators 0 - 4**

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

9. CONTROL | N **Controls 1 - 2**

Two vials, each containing human chromogranin A in a lyophilized bovine serum based matrix with a non-azide preservative. **Refer to vials for exact concentration range for each control.** Both controls should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

SAFETY PRECAUTIONS

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 are potential 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 sever 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 lease 15 minutes. Use Good Laboratory Practices.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Precision single channel pipettes capable of delivering 10 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 tubes.
5. Disposable plastic 100 mL and 1000 mL bottle with caps.
6. Aluminum foil.
7. Deionized or distilled 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 nm.

SPECIMEN COLLECTION

Only 50 µL of human serum is required for human chromogranin A measurement in duplicate. No special preparation of the individual is necessary prior to specimen collection. Whole blood should be collected and must be allowed to clot for minimum 30 minutes at room temperature before the serum is separated by centrifugation (850 – 1500xg for 10 minutes). The serum should be separated from the clot within three hours of blood collection and transferred to a clean test tube. Serum samples should be stored at –20ºC or below until measurement. Avoid repeated (more than three times) freezing and thawing of specimen.

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) Washing buffer must be diluted to working wash solution prior to use. Please see REAGENTS section for details.
(3) Reconstitute all assay calibrators and controls by adding **0.5 mL** of demineralized water to each vial. Allow the calibrators and controls to sit undisturbed for 10 minutes, and then mix well by inversion or gentle vortexing. One must make sure that all solid is dissolved completely prior to use. These reconstituted calibrators and controls must be stored at - 20°C or below. Do not exceed 3 freeze-thaw cycles.

3. Assay Procedure

(1) Place a sufficient number of antibody coated microwell strips in a holder to run calibrators, controls and unknown samples in duplicate.
(2) Test Configuration

ROW	STRIP 1	STRIP 2	STRIP 3
A	CAL 0	CAL 4	SAMPLE 2
B	CAL 0	CAL 4	SAMPLE 2
C	CAL 1	C 1	SAMPLE 3
D	CAL 1	C 1	SAMPLE 3
E	CAL 2	C 2	SAMPLE 4
F	CAL 2	C 2	SAMPLE 4
G	CAL 3	SAMPLE 1	
H	CAL 3	SAMPLE 1	

(3) Add 25 µL of calibrators, controls and samples into the designated microwell.
(4) Add 100 µL of incubation buffer to each well
(5) Cover the plate with a plate sealer and incubate the plate with orbital shaking (170 rpm) at room temperature for 2 hours.
(6) Prepare Detecting antibody working solution by diluting the detecting antibody 1:21 with the dilution buffer. For each strip, 1 mL of the dilution buffer with 50 µL of the antibody is required, in a clean test tube or vial.
The table below shows the amount of antibody working solution that is required for a number of strips.

Strip no.	Dilution buffer	Detecting Antibody
1	1 mL	50 µL
2	2 mL	100 µL
3	3 mL	150 µL
4	4 mL	200 µL
5	5 mL	250 µL
6	6 mL	300 µL
7	7 mL	350 µL
8	8 mL	400 µL
9	9 mL	450 µL
10	10 mL	500 µL
11	11 mL	550 µL
12	12 mL	600 µL

Note: this detecting antibody working solution should be freshly prepared right before running the assay.

(7) 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 aspirate the contents. Alternatively, an automated microplate washer can be used.
(8) Add 100 µL of above diluted Detecting Antibody working solution to each well.
(9) Cover the plate with the plate sealer and incubate plate with orbital shaking (170 rpm) at room temperature for 1 hour.
(10) 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 aspirate the contents. Alternatively, an automated microplate washer can be used.
(11) Add 100 µL of TMB-Substrate solution into each well.
(12) Cover the plate with a plate sealer and with an aluminum foil to avoid exposure to light.
(13) Incubate plate at room temperature for 20 minutes
(14) Remove the aluminum foil and plate sealer. Add 100 µL of Stop Solution into each well. Mix gently.
(15) Read the absorbance at 450 nm within 10 minutes in a microplate reader
NOTE: to reduce the background, one can set the instrument to dual wavelength measurement at 450 nm with background wavelength correction set at 595 nm or 620 nm or 630 nm.

PROCEDURAL NOTES

1. It is recommended that all calibrators, controls and unknown samples are assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of the results.
2. Keep light sensitive reagents in the original amber bottles.
3. Store any unused antibody coated strips in the foil Ziploc bag with desiccant to protect from moisture.
4. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
5. Incubation times or temperatures other than those stated in this insert may affect the results.
6. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading
7. All reagents should be mixed gently and thoroughly prior to use. Avoid foaming.

INTERPRETATION OF RESULTS

1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of the CAL 0 (0 ng/mL) from the average absorbance of all other readings to obtain the corrected absorbance.
3. The calibration curve is generated by the corrected absorbance of all calibrators on the ordinate against the calibrator concentration on the abscissa using point-to-point or log-log paper. Appropriate computer assisted data reduction programs may also be used for the calculation of the results.

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

REPORTING TEST RESULTS

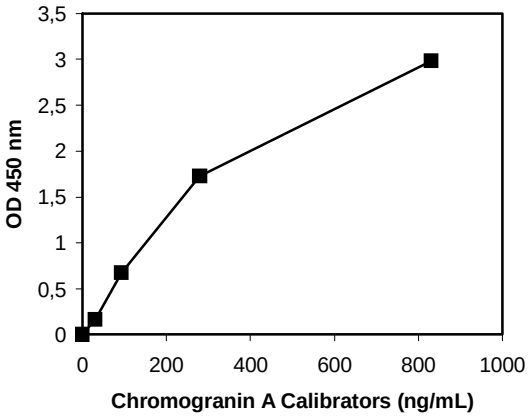
Laboratory should report test results directly derived from the assay. For samples showing high values, it is strongly recommended to dilute the sample 1:100 with assay buffer and re-assay the diluted sample for a more accurate test result. If the 1:100 diluted sample still shows a higher value than 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.

EXAMPLE DATA AND CALIBRATION CURVE

A typical absorbance data and the resulting calibration curve from human chromogranin A ELISA are shown below. **This curve should never be used instead of the real time calibration curve.**

Well I.D.	OD 450 nm Absorbance			Results ng/mL
	Readings	Average	Corrected	
0 ng/mL	0.078 0.074	0.076	0.000	
31 ng/mL	0.242 0.239	0.240	0.164	
93 ng/mL	0.757 0.740	0.748	0.672	
280 ng/mL	1.840 1.763	1.802	1.726	
830 ng/mL	3.139 2.980	3.059	2.983	
Control 1	0.444 0.451	0.447	0.371	56.27 ng/mL
Control 2	1.300 1.252	1.276	1.200	186.70 ng/mL

Human Chromogranin A ELISA



EXPECTED VALUES

Seventy-two normal adult sera were measured with this human chromogranin A ELISA. The normal values were below 100 ng/mL. Five samples with pheochromocytoma showed a chromogranin A level of over 100 ng/ml and one of them reached 330,000 ng/mL. It is highly recommend that each laboratory establishes its own normal cut off level.

LIMITATION OF THE PROCEDURE

1. Since there is no Gold Calibrator available for human chromogranin A measurement, the values of the calibrators were established by correlation to a highly purified chromogranin A calibrator.
2. If a sample value is higher than the highest calibrator, it is recommended to measure a further diluted sample.
3. Bacterial or fungal contamination of serum specimens or reagents, or cross contamination between reagents may cause erroneous results.
4. Water deionized with polyester resins may inactivate 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 of the human chromogranin A ELISA, as determined by the 95% confidence limit on 20 duplicate determinations of the zero calibrator, is approximately 5 ng/mL.

High Dose "hook" effect

This assay has showed that it did not have any high dose "hook" effect up to 1,000,000 ng/mL. This is important because some samples 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 20-replicate determinations.

Mean Chromogranin A Value (ng/mL)	CV (%)
63.5	4.2
209.	3.6

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

Mean Chromogranin A Value (ng/mL)	CV (%)
61.9	6.7
213.3	5.6

Linearity

Two human serum samples were diluted with incubation buffer and assayed. The results expressed in ng/mL are as follows:

#	DILUTION	OBSERVED VALUE	EXPECTED VALUE	RECOVERY %
1	Neat	286	-	-
	1:2	138	143	96
	1:4	75	72	104
	1:8	37.9	36	105
	1:16	19.5	18	108
2	Neat	61.8	-	-
	1:2	32.1	30.9	104
	1:4	15.9	15.5	103
	1:8	7.2	7.7	94

Recovery

Two sample serum samples were spiked with various amounts of human chromogranin A (1 vol. + 1 vol. mixture) and assayed. The results expressed in ng/mL are as follows:

#	Orig. Value	Amount Spiked	Observed Value	Expected Value	Recovery %
1	62.8	31	45.2	46.9	96
		93	75.6	77.9	97
		280	152.8	171.4	89
2	289	31	152.2	160	95
		93	176	191	92
		280	288.2	284.5	101

REFERENCES

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2. Kimura N, Miura W, Noshiro T, Mizunashi K, Hanew K, Shimizu K, et al. Plasma chromogranin A in pheochromocytoma, primary hyperparathyroidism and pituitary adenoma in comparison with catecholamine, parathyroid hormone and pituitary hormones. Endocr J 1997;44:319-27.
3. Hendy GN, Bevan S, Mattei MG, Mouland AJ. Chromogranin A. Clin Invest Med 1995;18:47-65.
4. Deftos LJ. Chromogranin A: its role in endocrine function and as an endocrine and neuroendocrine tumor marker. Endocrine Reviews: 1991;12:181-7
5. Sobol RE, Memoli V, Deftos LJ. Hormone-negative, chromogranin A-positive endocrine tumors. N Engl J Med 1989;320:444-7.

Short Assay Procedure:

1. Add 25 µL of calibrators, controls and serum samples into the designated microwell.
2. Add 100 µL of incubation buffer to each well.
3. Mix, cover and incubate the plate at room temperature and shaking (170 rpm) for 2 hours.
4. Wash each well 5 times.
5. Add 100 µL of Diluted Detecting Antibody into each well
6. Incubate 1 hour at RT and shaking (170 rpm)
7. Wash each well 5 times.
8. Add 100 µL of TMB-Substrate solution into each well.
9. Cover and incubate plate at room temperature for 20 minutes.
10. Add 100 µL of Stop Solution into each well.
11. Read the absorbance at 450 nm.

Revision date : 2009-06-03

	Used symbols	Symboles utilisés
	Consult instructions for use	Consulter les instructions d'utilisation
	Storage temperature	Température de conservation
	Use by	Utiliser jusque
LOT	Batch code	Numéro de lot
REF	Catalogue number	Référence de catalogue
CONTROL	Control	Contrôle
IVD	In vitro diagnostic medical device	Dispositif médical de diagnostic in vitro
	Manufacturer	Fabricant
	Contains sufficient for <n> tests	Contenu suffisant pour <n> tests
WASH SOLN CONC	Wash solution concentrated	Solution de lavage concentrée
CAL 0	Zero calibrator	Calibrateur zéro
CAL N	Calibrator #	Calibrateur #
CONTROL N	Control #	Contrôle #
Ag 125I	Tracer	Traceur
Ab 125I	Tracer	Traceur
Ag 125I CONC	Tracer concentrated	Traceur concentré
Ab 125I CONC	Tracer concentrated	Traceur concentré
	Tubes	Tubes
INC BUF	Incubation buffer	Tampon d'incubation
ACETONITRILE	Acetonitrile	Acétonitrile
SERUM	Serum	Sérum
DIL SPE	Specimen diluent	Diluant du spécimen
DIL BUF	Dilution buffer	Tampon de dilution
ANTISERUM	Antiserum	Antisérum
IMMUNOADSORBENT	Immunoadsorbent	Immunoadsorbant
DIL CAL	Calibrator diluent	Diluant de calibrateur
REC SOLN	Reconstitution solution	Solution de reconstitution
PEG	Polyethylene glycol	Glycol Polyéthylène
EXTR SOLN	Extraction solution	Solution d'extraction
ELU SOLN	Elution solution	Solution d'elution
GEL	Bond Elut Silica cartridges	Cartouches Bond Elut Silica
PRE SOLN	Pre-treatment solution	Solution de pré-traitement
NEUTR SOLN	Neutralization solution	Solution de neutralisation
TRACEUR BUF	Tracer buffer	Tampon traceur
	Microtiterplate	Microplaque de titration
Ab HRP	HRP Conjugate	HRP Conjugué
Ag HRP	HRP Conjugate	HRP Conjugué
Ab HRP CONC	HRP Conjugate concentrate	HRP Conjugué concentré
Ag HRP CONC	HRP Conjugate concentrate	HRP Conjugué concentré
CONJ BUF	Conjugate buffer	Tampon conjugué
CHROM TMB CONC	Chromogenic TMB concentrate	Chromogène TMB concentré
CHROM TMB	Chromogenic TMB solution	Solution chromogène TMB
SUB BUF	Substrate buffer	Tampon substrat
STOP SOLN	Stop solution	Solution d'arrêt
INC SER	Incubation serum	Sérum d'incubation
BUF	Buffer	Tampon
Ab AP	AP Conjugate	AP Conjugué
SUB PNPP	Substrate PNPP	Tampon PNPP
BIOT CONJ CONC	Biotin conjugate concentrate	Biotine conjugué concentré
AVID HRP CONC	Avidine HRP concentrate	Avidine HRP concentré
ASS BUF	Assay buffer	Tampon de test
Ab BIOT	Biotin conjugate	Biotine conjugué
Ab	Specific Antibody	Anticorps spécifique
SAV HRP CONC	Streptavidin HRP concentrate	Concentré streptavidine HRP
NSB	Non-specific binding	Liant non spécifique
2nd Ab	2nd Antibody	Second anticorps
ACID BUF	Acidification Buffer	Tampon d'acidification