CHROMOGRANIN A - ELISA

CGARUO

DIAsource ImmunoAssays S.A. - Rue du Bosquet, 2 - B - 1348 Louvain-la-Neuve – Belgium

History

Summary of change:

<table>
<tr><th>Previous Version:
210215</th><th>Current Version:
211008</th></tr>
<tr><td>V. REAGENTS PROVIDED

<table><tr><td>CONJ</td><td>BUF</td></tr></table>
Conjugate buffer</td><td>V. REAGENTS PROVIDED

<table><tr><td>CONJ</td><td>BUF</td></tr></table>
Conjugate buffer + 0.01% gentamycine</td></tr>
<tr><td>VIII. SPECIMEN COLLECTION</td><td>IX. SPECIMEN COLLECTION

The CHROMOGRANIN A - ELISA is recommended for serum and heparin plasma samples.</td></tr>
<tr><td>XII. PERFORMANCE

A. Sensitivity

The LOB was calculated to be 2.28 ng/ml.
The LOD was calculated to be 6.55 ng/ml.
The LOQ was calculated to be 61.1 ng/ml.

B. Precision

<table><tr><td colspan="4">INTRA ASSAY</td><td colspan="4">INTER ASSAY</td></tr><tr><td>Sample</td><td>N</td><td><X> ± SD (ng/ml)</td><td>CV (%)</td><td>Sample</td><td>N</td><td><X> ± SD (ng/ml)</td><td>CV (%)</td></tr><tr><td>1</td><td>24</td><td>141.6 ± 4.1</td><td>2.9</td><td>1</td><td>10</td><td>120.0 ± 3.4</td><td>2.8</td></tr><tr><td>2</td><td>24</td><td>626.8 ± 30.1</td><td>4.8</td><td>2</td><td>10</td><td>632.3 ± 33.4</td><td>5.3</td></tr></table></td><td>XII. PERFORMANCE

A. Sensitivity

The LOB was calculated to be 2.24 ng/ml.
The LOD was calculated to be 5.34 ng/ml for serum samples.
The LOD was calculated to be 4.42 ng/ml for heparin plasma.
The LOQ was calculated to be 53.26 ng/ml.

B. Precision

<table><tr><td colspan="4">INTRA ASSAY</td><td colspan="4">INTER ASSAY</td></tr><tr><td>Sample</td><td>N</td><td><X> ± SD (ng/ml)</td><td>CV (%)</td><td>Sample</td><td>N</td><td><X> ± SD (ng/ml)</td><td>CV (%)</td></tr><tr><td>1</td><td>24</td><td>90.3 ± 6.6</td><td>7.3</td><td>1</td><td>10</td><td>130.84 ± 9.87</td><td>7.5</td></tr><tr><td>2</td><td>24</td><td>333.8 ± 13.9</td><td>4.2</td><td>2</td><td>10</td><td>318.14 ± 24.6</td><td>7.7</td></tr><tr><td>3</td><td>24</td><td>620 ± 18.8</td><td>3</td><td>3</td><td>10</td><td>645.73 ± 42.33</td><td>6.6</td></tr><tr><td>4</td><td>24</td><td>906.9 ± 59.9</td><td>6.6</td><td>4</td><td>10</td><td>977.7 ± 34.03</td><td>3.5</td></tr><tr><td>-</td><td>-</td><td>-</td><td>-</td><td>5</td><td>10</td><td>125.81 ± 8.90</td><td>7.1</td></tr><tr><td>-</td><td>-</td><td>-</td><td>-</td><td>6</td><td>10</td><td>991.67 ± 68.48</td><td>6.9</td></tr></table></td></tr>
</table>

C. Accuracy

RECOVERY TEST

Sample	Observed (ng/ml)	Expected (ng/ml)	Recovery (O/E) (%)
1	123,0	-	-
	392,2	374,6	104,7
	541,5	567,5	95,4
	693,9	724,8	95,7
2	314,1	-	-
	592,2	565,7	104,7
	691,9	758,6	91,2
	866,4	915,9	94,6

C. Accuracy

RECOVERY TEST

Sample	Reagent Addition	Observed (ng/ml)	Expected (ng/ml)	Recovery (O/E) (%)
1	-	82.0	-	-
	232.9	317.6	314.9	1.0
	426.3	536.4	508.3	1.1
	536.6	702.9	618.6	1.1
2	-	207.8	-	-
	232.9	447.7	440.7	1.0
	426.3	663.1	634.1	1.0
	536.6	846.6	744.4	1.1
3	-	149.1	-	-
	212.9	370.7	362.0	1.0
	393.2	558.0	542.3	1.0
	570.5	752.1	719.6	1.0
4	-	367.3	-	-
	212.9	613.8	580.2	1.1
	393.2	815.8	760.5	1.1
	570.5	999.2	937.8	1.1

DILUTION TEST

Sample	Dilution	Observed (ng/ml)	Expected (ng/ml)	Recovery (O/E) (%)
1	1/1	926,9	-	-
	1/2	420,8	463,4	90,8
	1/4	220,3	231,7	95,1
	1/8	118,8	115,9	102,6
2	1/1	835,3	-	-
	1/2	384,0	417,6	92,0
	1/4	201,6	208,8	96,5
	1/8	108,6	104,4	104,0

DILUTION TEST

DILUTION TEST – serum samples				
Sample	**Dilution**	**Observed (ng/ml)**	**Expected (ng/ml)**	**Recovery (O/E) (%)**
1	1/1	1194.6	-	-
	1/2	641.2	597.3	107
	1/4	290.4	298.7	97
	1/8	130.9	149.3	88
2	1/1	967	-	-
	1/2	472.3	483.5	98
	1/4	236.6	241.8	98
	1/8	125.3	120.9	104
3	1/1	1119.9	-	-
	1/2	562.3	560.0	100
	1/4	272.6	280.0	97
	1/8	130.3	140.0	93
4	1/1	993.6	-	-
	1/2	498.5	496.8	100
	1/4	241.1	248.4	97
	1/8	117.7	124.2	

DILUTION TEST – heparin plasma samples				
Sample	**Dilution**	**Observed (ng/ml)**	**Expected (ng/ml)**	**Recovery (O/E) (%)**
1	1/1	699.2	-	-
	1/2	326.3	349.6	93
	1/4	163.7	174.8	94
	1/8	83.8	87.4	96
2	1/1	701.6	-	-
	1/2	328.8	350.8	94
	1/4	154.7	175.4	88
	1/8	72.5	87.7	83

3	1/1	740.8	-	-
	1/2	321.0	370.4	87
	1/4	158.5	185.2	86
	1/8	82.9	92.6	90
4	1/1	676.5	-	-
	1/2	314.3	338.3	93
	1/4	154.6	169.1	91
	1/8	78.0	84.6	92

F. Interference

No interference was detected when samples were spiked with bilirubin unconjugated (208 mg/L) , bilirubin conjugated (200 mg/L), hemoglobin (4.84 g/L) or triglyceride (20 mg/ml).
Individuals receiving mouse anti-human antibodies for treatment or diagnosis, or those patients who have been otherwise exposed to mouse immunoglobulin, may produce human anti-mouse antibodies (HAMA). These antibodies can interfere with assays using mouse monoclonal antibodies and may cause falsely elevated levels.
A high intake of biotin (in dietary supplements or drugs) may interfere with the assay and cause falsely low results. Intake of the daily recommended allowance of biotin (0.03 mg/day, serum level 0.5-1.0 ng/mL) does not interfere with the assay. Interference in Chromogranin A has been assessed to > 10 ng/mL biotin in patient samples.

F. Interference

INTERFERENCES				
Sample	**Interferant addition (ng/ml)**	**Without interferant (ng/ml)**	**Ratio (%)**	**Deviation (%)**
Hemoglobin + 4.84 g/l				
1	124.6	136.6	91	9
2	301.4	319.8	94	6
3	527.8	540.1	98	2
4	719.3	768.2	94	6
Bilirubin unconjugated + 208 mg/l				
1	124.8	136.6	91	9
2	305.8	319.8	96	4
3	497.9	540.1	92	8
4	715.4	768.2	93	7
Triglyceride + 20 mg/ml				
1	124.2	136.6	91	9
2	307.5	319.8	96	4
3	489.5	540.1	91	9
4	735.1	768.2	96	4

Bilirubin conjugated + 200 mg/l				
1	124.6	136.6	91	9
2	311	319.8	94	6
3	510.3	540.1	98	2
4	752.4	768.2	94	6
Biotin + 10 ng/ml				
1	127.7	136.6	93	7
2	309.3	319.8	97	3
3	498.4	540.1	92	8
4	726.3	768.2	95	6

No detectable interference was confirmed with hemoglobin (4.84 g/l), bilirubin unconjugated (208 mg/l), triglycerides (20 mg/ml), bilirubin conjugated (200 mg/l) and biotin (10 ng/ml).
A high intake of biotin (in dietary supplements or drugs) may interfere with the assay and cause falsely low results.

en

Read entire protocol before use.

CHROMOGRANIN A – ELISA

I. INTENDED USE

The CHROMOGRANIN A - ELISA test kit is an enzyme-linked immunosorbent assay (ELISA) for detection and quantitation of chromogranin A in human plasma or serum.

The analysis should be performed by trained laboratory professionals. For Research Use Only. Not for Use in Diagnostic Procedures.

II. GENERAL INFORMATION

A. Proprietary name : DIAsource CHROMOGRANIN A - ELISA

B. Catalog number : CGARUO : 96 tests

C. Manufactured by : DIAsource ImmunoAssays S.A.
Rue du Bosquet, 2, B-1348 Louvain-la-Neuve, Belgium.

For technical assistance or ordering information contact :
Tel: +32 (0) 10 84.99.11 Fax: +32 (0) 10 84.99.91

III. BACKGROUND

1. Biological activities

Chromogranins and secretogranins constitute a family of uniquely acidic proteins that are co-stored with neurotransmitters and peptide hormones in the brain and the diffuse neuroendocrine system (Winkler, H. & Fischer-Colbrie, R.1992). Structurally these proteins are products of different genes but share some overall properties such as an abundance of acidic amino acid residues and several pairs of basic amino acids as potential positions for post-translational cleavage. Chromogranins are co-stored and co-released with neuropeptides and hormones in the neuroendocrine cells throughout the body. A role for chromogranins in the generation of hormonal granules and package of hormones has been suggested. Furthermore, chromogranins can be cleaved into smaller fragments, which can display biological activities such as inhibition of hormonal release, vasodilatation and anti-microbiological effects (Stridsberg M, 2000).

Tumours of neuroendocrine origin usually present with increased serum/plasma levels of chromogranin A (O'Connor, DT, Deftos LJ, 1986). The neuroendocrine tumours are derived from the neuroendocrine cells and typical neuroendocrine tumours are carcinoid tumours, pheochromocytomas, neuroblastomas, small cell lung cancers, hyperparathyroid adenomas, pituitary tumours and pancreatic islet tumours and including the MEN1 and MEN2 syndromes.

IV. PRINCIPLES OF THE METHOD

In a separate dilution plate samples, calibrators and controls are diluted 5x in Diluent. The diluted material is transferred to the microtiter wells and incubated at room temperature for 60 minutes. During this first incubation, a monoclonal antibody captures the chromogranin A to surface of the well. After washing to remove unbound material, a second horseradish peroxidase (HRP) labelled monoclonal antibody is added to detect the chromogranin A bound to the well. After incubation for 30 minutes, the wells are washed again and a colour substrate is added and incubated. The colour development is stopped after 15 minutes and the colour measured in a spectrophotometer. The colour is directly proportional to the amount of chromogranin A bound to the well. The amount of chromogranin is determined by comparison with the colour development of the calibrator samples. The calibrator in the kit is a synthetic peptide corresponding to chromogranin A. The peptide calibrator is set to give a response equal to a purified, native fragment of chromogranin A (Stridsberg, M et al. 1993, Stridsberg et al. 1995).

V. REAGENTS PROVIDED

Reagents	96 tests Kit	Color Code	Reconstitution
Microtiterplate with 96 anti - Chromogranin A coated wells.	96 wells	blue	**Ready** for use
Ab | **HRP** | **CONC** Conjugate containing HRP-labelled antibodies to Chromogranin A. 100x concentrated	1 vial 150 µl	red	Dilute 100x with conjugate buffer
CONJ | **BUF** Conjugate buffer + 0.01% gentamycine	1 vial 15 ml	red	**Ready** for use
DIL | **BUF** Diluent containing biotin-labelled antibodies to Chromogranin A	1 vial 36 ml	black	**Ready** for use
CAL | **N** Calibrators 1 to 6 containing human Chromogranin A in diluent. CAL 1 = 0 ng/mL (diluent), CAL 2 = 36 ng/mL, CAL 3 = 180 ng/mL, CAL 4 = 540 ng/mL, CAL 5 = 1080 ng/mL, CAL 6 = 1800 ng/mL.	6 vials lyophilized	yellow	**Add** 0.6 ml distilled water
WASH | **SOLN** | **CONC** Wash Solution 20x concentrated.	1 vial 70 ml	brown	Dilute 20x with distilled water
CONTROL | **N** Controls - N = 1 or 2	2 vials lyophilized	silver	**Add** 0.6 ml distilled water
CHROM | **TMB** Chromogenic TMB Solution (Tetramethylbenzydine)	1 vial 15 ml	brown	**Ready** for use
STOP | **SOLN** Stop solution: 0.5M H_2SO_4	1 vial 15 ml	white	**Ready** for use

VI. SUPPLIES NOT PROVIDED

The following material is required but not provided in the kit

1. Microplate reader with 450 nm filter. Reference wavelength is 620 nm.
2. 300 µL/well dilution plate for dilution of calibrator, samples and controls.
3. Precision pipettes with disposable tips.
4. Automatic microtiter plate washer, absorbent tissue, tubes and a timer.

Before use

-Lyophilised calibrators and controls should be carefully dissolved with 600 µL distilled water. After use the reconstituted calibrators and controls should be stored at -20 °C or lower. Calibrators and controls may go through three freeze/thaw cycles without deterioration.

-Other reagents in the kit should be stored at 2-8 °C.

-Reconstituted calibrators and controls should be diluted 5x in diluent at each test occasion.

-The amount of conjugate needed for each analysis should be diluted 100x before use (10 µL conjugate + 990 µL conjugate buffer per strip). Remaining diluted conjugate shall be discarded.

-Wash solution should be diluted 20x before use. Dilute 10 mL of the 20x concentrated wash solution in 190 mL distilled water. When stored at 2-8 °C, the diluted wash solution is stable until the date of expiration of the kit.

-The rest of the reagents in the kit are ready for use.

-Remove only the number of wells needed for testing, reseal the aluminium packaging carefully.

VIII. SPECIMEN COLLECTION

The CHROMOGRANIN A - ELISA is recommended for serum and heparin plasma samples.

Handle as if capable of transmitting infectious agents.

The samples are separated by centrifugation and can be stored at 2-8 °C up to 7 days. If samples are to be kept for longer periods, store at -20 °C or colder. Samples may go through three freeze/thaw cycles without deterioration.

Do not use a frost-free freezer because it may allow the specimens to go through freeze-thaw cycles and degrade antibody. Samples that are improperly stored or are subjected to multiple freeze-thaw cycles may yield spurious results.

IX. PROCEDURE

A. Handling notes

All solutions should be used at room temperature. Incubate all steps at room temperature (20-30 °C).

B. Procedure

Sample dilution and incubation

Dilute all samples 5x in a separate dilution plate before transferring to test plate. Calibrators, Low control, High control and plasma should all be diluted 50 µL plasma + 200 µL diluent. Mix thoroughly by pipetting up and down before transferring 100µL in duplicate to the test plate according to the diagram below.

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL1	CAL5	P1									
B	CAL1	CAL5	P1									
C	CAL2	CAL6	P2									
D	CAL2	CAL6	P2									
E	CAL3	H	etc									
F	CAL3	H										
G	CAL4	L										
H	CAL4	L										

Incubate for 60 minutes.

After sample incubation

Aspirate and wash three (3) times with 300 µL washing solution/well, filling and emptying the wells each time. After the last wash, empty the wells by tapping the microtiter plate on an absorbent tissue.

Adding conjugate

Add 100 µL conjugate to each well.

Incubate for 30 minutes.

After conjugate incubation

Wash as before.

Adding substrate solution
Add 100 µL substrate TMB to each well, incubate in the dark for 15 minutes. The incubation time may be shortened to 10 minutes if maximum Optical Density (OD) exceeds 3.0 at high temperatures or if an automated method is applied.

Adding stop solution
Add 100 µL stop solution to each well. Read the absorbance at 450 nm within 2h on a microplate reader. Read at 620 nm as a reference wavelength.

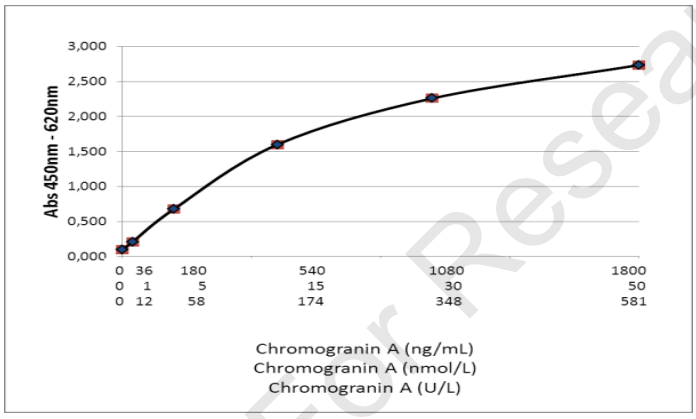
XI. CALCULATION OF RESULTS

Construct a calibrator curve by plotting the OD against the ng/mL values of the six calibrators. The six calibrators provided have values of 0 ng/mL for calibrator 1, 36 ng/mL for calibrator 2, 180 ng/mL for calibrator 3, 540 ng/mL for calibrator 4, 1080 ng/mL for calibrator 5, 1800 ng/mL for calibrator 6 respectively. Read the values of Low and High controls and the samples from the curve.

Values greater than the highest calibrator value should be reported as >1800 ng/mL, or diluted further with assay diluent and re-assayed. In this case the compensation for the dilution must be made in the calculation of the chromogranin A concentration. Please note, that in the event of calibrator 1 or calibrator 6 values out of range the test should be considered invalid and repeated.

For convenience concentrations can be calculated in ng/mL, nmol/L or U/L. All units are given in the table below. The molecular weight of the native fragment of chromogranin A (36kDa) was used for the conversion (Stridsberg, M et al. 1993, Stridsberg et al. 1995).

Calibrator	Concentration			Absorbance
	ng/mL	nmol/L	U/L	
1	0	0	0	0.094
2	36	1	12	0.209
3	180	5	58	0.678
4	540	15	174	1.599
5	1080	30	348	2.259
6	1800	50	581	2.735



A sample with an absorbance value of 1.272 will read on the X-axis as having 367 ng/mL (or 10.2 nmol/L or 118 U/L) of chromogranin A. In this example a four parameter logistic curve fit has been applied.
Important: The curve is an example and should not be used for actual sample interpretation.

XII. PERFORMANCE

A. Sensitivity

The LOB (Limit of blank) was calculated by measuring the blank twenty-four times and was calculated as the mean + 3 standard deviations of the distribution of the test values. The LOB was calculated to be 2.24 ng/ml.
The LOD for serum samples (Limit of detection) was calculated as the LOB + 1.65 standard deviation of a low concentration sample tested in 10 different runs. The LOD was calculated to be 5.34 ng/ml for serum samples.
The LOD was calculated to be 4.42 ng/ml for heparin plasma.
The LOQ for serum samples (Limit of quantitation) was calculated by testing 5 samples of low values, 10 times. The LOQ was calculated to be 53.26 ng/ml.

B. Precision

INTRA ASSAY				INTER ASSAY			
Sample	**N**	**<X> ± SD (ng/ml)**	**CV (%)**	**Sample**	**N**	**<X> ± SD (ng/ml)**	**CV (%)**
1	24	90.3 ± 6.6	7.3	1	10	130.84 ± 9.87	7.5
2	24	333.8 ± 13.9	4.2	2	10	318.14 ± 24.6	7.7
3	24	620 ± 18.8	3	3	10	645.73 ± 42.33	6.6
4	24	906.9 ± 59.9	6.6	4	10	977.7 ± 34.03	3.5
-	-	-	-	5	10	125.81 ± 8.90	7.1
-	-	-	-	6	10	991.67 ± 68.48	6.9

SD : Standard Deviation; CV: Coefficient of variation

C. Accuracy

RECOVERY TEST

Sample	Reagent Addition	Observed (ng/ml)	Expected (ng/ml)	Recovery (O/E) (%)
1	-	82.0	-	-
	232.9	317.6	314.9	1.0
	426.3	536.4	508.3	1.1
	536.6	702.9	618.6	1.1
2	-	207.8	-	-
	232.9	447.7	440.7	1.0
	426.3	663.1	634.1	1.0
	536.6	846.6	744.4	1.1
3	-	149.1	-	-
	212.9	370.7	362.0	1.0
	393.2	558.0	542.3	1.0
	570.5	752.1	719.6	1.0
4	-	367.3	-	-
	212.9	613.8	580.2	1.1
	393.2	815.8	760.5	1.1
	570.5	999.2	937.8	1.1

DILUTION TEST

DILUTION TEST – serum samples				
Sample	**Dilution**	**Observed (ng/ml)**	**Expected (ng/ml)**	**Recovery (O/E) (%)**
1	1/1	1194.6	-	-
	1/2	641.2	597.3	107
	1/4	290.4	298.7	97
	1/8	130.9	149.3	88
2	1/1	967	-	-
	1/2	472.3	483.5	98
	1/4	236.6	241.8	98
	1/8	125.3	120.9	104
3	1/1	1119.9	-	-
	1/2	562.3	560.0	100
	1/4	272.6	280.0	97
	1/8	130.3	140.0	93
4	1/1	993.6	-	-
	1/2	498.5	496.8	100
	1/4	241.1	248.4	97
	1/8	117.7	124.2	

DILUTION TEST – heparin plasma samples				
Sample	**Dilution**	**Observed (ng/ml)**	**Expected (ng/ml)**	**Recovery (O/E) (%)**
1	1/1	699.2	-	-
	1/2	326.3	349.6	93
	1/4	163.7	174.8	94
	1/8	83.8	87.4	96
2	1/1	701.6	-	-
	1/2	328.8	350.8	94
	1/4	154.7	175.4	88
	1/8	72.5	87.7	83
3	1/1	740.8	-	-
	1/2	321.0	370.4	87
	1/4	158.5	185.2	86
	1/8	82.9	92.6	90
4	1/1	676.5	-	-
	1/2	314.3	338.3	93
	1/4	154.6	169.1	91
	1/8	78.0	84.6	92

Samples were diluted with calibrator 1.

D. Hook effect.

No hook-effect is observed when testing chromogranin A concentrations up to 360 000 ng/mL (or 10 000 nmol/L or 116 000 U/L).

E. Specificity

No cross reactivity was detected when seven markers of neuroendocrine tumors, i.e 5-Hydroxyindoleacetic acid (5-HIAA), Neuron-specific enolase, Glucagon, Gastrin, Chromogranin B, Pancreatic polypeptide and Vasoactive intestinal polypeptide were analysed in the DIAsource Chromogranin A assay.

F. Interference

INTERFERENCES				
Sample	**Interferant addition (ng/ml)**	**Without interferant (ng/ml)**	**Ratio (%)**	**Deviation (%)**
Hemoglobin + 4.84 g/l				
1	124.6	136.6	91	9
2	301.4	319.8	94	6
3	527.8	540.1	98	2
4	719.3	768.2	94	6
Bilirubin unconjugated + 208 mg/l				
1	124.8	136.6	91	9
2	305.8	319.8	96	4
3	497.9	540.1	92	8
4	715.4	768.2	93	7
Triglyceride + 20 mg/ml				
1	124.2	136.6	91	9
2	307.5	319.8	96	4
3	489.5	540.1	91	9
4	735.1	768.2	96	4
Bilirubin conjugated + 200 mg/l				
1	124.6	136.6	91	9
2	311	319.8	94	6
3	510.3	540.1	98	2
4	752.4	768.2	94	6
Biotin + 10 ng/ml				
1	127.7	136.6	93	7
2	309.3	319.8	97	3
3	498.4	540.1	92	8
4	726.3	768.2	95	6

No detectable interference was confirmed with hemoglobin (4.84 g/l), bilirubin unconjugated (208 mg/l), triglycerides (20 mg/ml), bilirubin conjugated (200 mg/l) and biotin (10 ng/ml).
A high intake of biotin (in dietary supplements or drugs) may interfere with the assay and cause falsely low results.

XIII. INTERNAL QUALITY CONTROL

The OD for calibrator 1 should be < 0.15
The OD for calibrator 6 should be > 1.0
The values of Low and High controls, see lot certificate.
The controls are intended to monitor for substantial reagent failure. If any of the control values are not within their respective ranges, the test should be considered invalid and should be repeated. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organisations. Refer to NCCLS C24-A for guidance on appropriate QC practices.

XIV. REFERENCE INTERVALS

It is recommended that each laboratory establishes a reference range with samples commonly used since variations in sample handling may affect results. A strict sample handling routine as recommended above should be followed. The values given below are an indication of the expected reference range and calculated as the 97.5 percentile of 256 blood donor heparin plasma samples.
Reference range Heparin-plasma level of chromogranin A: 108 ng/mL (or 3.0 nmol/L or 35 U/L).

XV. PRECAUTIONS AND WARNINGS

For in vitro diagnostic use.
The assay reagents contain no human serum components but the patient plasmas analysed should be handled as if capable of transmitting infectious agents.
The Centres for Disease Control and Prevention and National Institutes of Health recommend that potentially infectious agents be handled at the Biosafety Level 2.
Most of kit solutions contain ProClin 300 as a preservative. Never pipette by mouth or allow reagents or patient sample to come into contact with skin. Reagents containing ProClin 300 may be irritating. Avoid contact with skin and eyes. In case of contact, flush with plenty of water.
The stop solution contains 0.5 M sulphuric acid. Do not allow the reagent to get into contact with the skin.
The concentrations of chromogranin A in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity.
All the waste should be handled as hazardous wastes.

XVI. BIBLIOGRAPHY

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XVII. SUMMARY OF THE PROTOCOL

	CALIBRATORS (µl)	**SAMPLE(S) CONTROLS (µl)**
Calibrators (1-6) Samples, Controls	100 -	- 100
Incubate for 60 min at room temperature. Aspirate the contents of each well. Wash 3 times with 300 µl of Wash Solution and aspirate.		
Conjugate	100	100
Incubate for 30 min at room temperature. Aspirate the contents of each well. Wash 3 times with 300 µl of Wash Solution and aspirate.		
Chromogenic Solution	100	100
Incubate for 15 min at room temperature.		
Stop Solution	100	100
Read on a microtiterplate reader and record the absorbance of each well at 450 nm (versus 630 or 650 nm)		

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