KAMIYA BIOMEDICAL COMPANY

Human Chromogranin A ELISA

For the quantitative determination of CgA-LI in human plasma, urine and saliva.

Cat. No. KT-436

For Research Use Only.

PRODUCT INFORMATION

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INTENDED USE
The Human Chromogranin A ELISA is for the quantitative determination of CgA-LI in human plasma, urine and saliva. For research use only.

INTRODUCTION
Chromogranin A (CgA) is an acidic secretory protein consisting of 439 amino acids in human. The protein is found in a wide variety of hormone and neurotransmitter storage vesicles, and it is known to be co-stored and co-released with catecholamines from adrenal medulla and sympathetic neuronal vesicles during exocytosis. Radioimmunological measurement of CgA in human plasma under conditions of physiologic, pharmacologic and pathologic alteration of sympathoadrenal function has been reported. Accumulated data, thereafter, have confirmed high concentrations of plasma or serum CgA measured by radioimmunoassay (CgA-like immunoreactivity: CgA-LI) in samples with neuroendocrine and endocrine tumors, especially in those with pheochromocytoma, anterior pituitary tumors and rectal and prostatic carcinoma. On the other hand, it was recently discovered that CgA-LI exists in saliva, the concentration of which elevates rapidly under psychosomatic stress even prior to the elevation of salivary cortisol level. Subsequently, further studies presented evidence for autonomic control of submandibular CgA-LI secretion in the anaesthetized rat.

Most of the reported measurement of CgA by radioimmunoassay utilized native CgA antigens (full or partial length) and antibodies against the native proteins. On the other hand, studies provided a novel radioimmunoassay system for estimation of CgA-LI level in human plasma with use of synthetic human CgA (344-374) and antibody raised against the synthetic peptide. Studies also used the assay system in the work on human salivary CgA as mentioned above. CgA molecules contain 9-10 sites of basic amino acid pairs (Arg-Arg, Lys-Arg, etc.), which are generally accepted as the proteolytic processing sites. In fact, the sequences corresponding to CgA-derived peptides having some biological activities, such as β-granin, pancreastatin and parastatin, in CgA molecules are all preceded and followed by basic amino acid pairs. However, it is also known that in the adrenal medulla which is the major site of CgA production, CgA is found to exist predominantly in large molecular forms, supporting the least processing of CgA in adrenal chromaffin cells. In addition, it was shown that there is no rapid degradation of the protein within the blood stream.

On the basis of these findings, KAMIYA BIOMEDICAL COMPANY now offers, for the first time, a specific, sensitive, stable and easy manipulative ELISA system for measurement of human CgA-LI using anti-synthetic human CgA (344-374) antibody, synthetic human CgA (344-374) as calibrator antigen and N^{α} -biotinylglycylglycyl human CgA (344-374) as labeled antigen. The assay kit can be used for measurement of CgA-LI in human biological fluid such as plasma, urine and saliva.

The amino acid sequence of human CgA (344-374):

E-E-E-E-D-N-R-D-S-S-M-K-L-S-F-R-A-R-A-Y-G-F-R-G-P-G-P-Q-L-R-R

PRINCIPLE
This ELISA kit for determination of human CgA in sample is based on the competitive enzyme immunoassay using combination of highly specific antibody to human CgA (344-374) and biotin-avidin affinity system. The 96-well plate is coated with goat anti-rabbit IgG. Human CgA calibrator or samples, labeled antigen and specific antibody are added to the wells for competitive immunoreaction. After incubation and plate washing, HRP-labeled streptoavidin (SA-HRP) are added to form HRP-labeled streptoavidin-labeled antigen-specific antibody complex on the surface of the wells. Finally, HRP enzyme activity is determined by o-Phenylenediamine dihydrochloride (OPD) and the concentration of human CgA is calculated. The kit is characterized by sensitive quantification, high specificity and no influence with other components in samples. CgA calibrator is a highly purified synthetic product (purity over 98%) and the content indicated is the absolute weight of the calibrator. N^{α} -biotinylglycylglycyl human CgA (344-374) is used as labeled antigen which has proven stability.

COMPONENTS

Component	Form	Quantity	Main Ingredient
1. Antibody-Coated Plate	MTP[*1]	1 plate (96-well)	Goat anti-rabbit IgG
2. CgA Calibrator	Lyophilized	1 vial (100 pmol)	Synthetic human CgA (344-374)
3. Labeled Antigen	Lyophilized	1 vial (30 ng)	Biotinylated human CgA (344-374)
4. Substrate Buffer	Liquid	1 bottle (25 mL)	0.1M phosphate-citrate buffer with 0.015% H_2O_2
5. Specific Antibody	Lyophilized	1 vial	Rabbit anti-human CgA (344-374) antibody
6. OPD Tablet	Tablet	2 tablets	o-Phenylenediamine dihydrochloride
7. Stop Solution	Liquid	1 bottle (12 mL)	2N H_2SO_4
8. Buffer Solution Concentrate	Liquid	1 bottle (12 mL)	50 mM Phosphate buffer with protein stabilizer, EDTA and NaCl
9. SA-HRP Solution	Liquid	1 bottle (12 mL)	HRP-labeled streptoavidin
10. Wash Solution Concentrate	Liquid	1 bottle (50 mL)	1.8% NaCl and 1% Tween 20
11. Plate Seal		3 sheets	

MTP[*1] ..... Microtiter plate

MATERIALS REQUIRED BUT NOT PROVIDED

- Photometer for microtiter plate (plate reader), which can read absorbance up to 2.5 at 490 nm
- Rotator for microtiter plate
- Washing device for microtiter plate and dispenser with aspiration system
- Micropipettes, multi-channel pipettes for 8 wells or 12 wells and their tips
- Test tubes for preparation of Calibrator Solution (polyethylene or polypropylene)
- Graduated cylinder (1,000 mL)
- Distilled water or de-ionized water

PRECAUTIONS

Protect reagents from strong light (e.g. direct sunlight) during storage and assay.

Satisfactory performance of the test is guaranteed only when reagents are used from a kit with identical lot number.

As pipetting operations may affect the precision of the assay, precisely pipette the prepared Calibrator Solutions or samples into corresponding wells. Use a new tip for each sample and calibrator to avoid cross-contamination. Use clean test tubes or vessels.

Always run a calibration curve when testing samples.

REAGENT PREPARATION

Note: This kit can be used dividedly in strips of the plate. In such case, the rest of reconstituted reagents (calibrator, labeled antigen and specific antibody) should be stored below -30°C.

1. Preparation of Buffer solution: Dilute buffer solution (concentrated) (10 mL) to 50 mL with distilled water.

2. Preparation of Calibrator:
   Reconstitute the lyophilized CgA calibrator (100 pmol) with 1 mL of buffer solution, which affords a 100 pmol/mL calibrator solution. 0.1 mL of the reconstituted calibrator solution is diluted with 0.2 mL of buffer solution to yield an initial calibrator solution of 33.33 pmol/mL. 0.1 mL of the 33.33 pmol/mL calibrator solution is diluted with 0.2 mL buffer solution to make a 11.11 pmol/mL calibrator solution. Repeat the dilution to make each calibrator solution of 3.70, 1.23, 0.41 and 0.14 pmol/mL. Buffer solution is used as 0 pmol/mL.

   Note: Calibrator Solution must be prepared immediately before assay. Use clean test tubes or vessels.

3. Preparation of Labeled Antigen solution: Dissolve lyophilized labeled antigen with distilled water (6 mL).

   Note: Labeled Antigen solution must be prepared immediately before assay.

4. Preparation of Specific Antibody solution: Dissolve lyophilized specific antibody with distilled water (12 mL).

   Note: Specific Antibody solution must be prepared immediately before assay.

5. Preparation of Substrate Solution: Dissolve one OPD Tablet in 12 mL of Substrate Buffer.

   Note: Substrate Solution must be prepared immediately before assay. Use clean test tubes or vessels.

6. Preparation of Wash Solution: Dilute 50 mL of Wash Solution Concentrate to 1,000 mL with distilled water. Diluted Wash Solution is stable for 3 months at 4°C.

   Note: During storage of the Wash Solution Concentrate at 4°C, precipitates may be observed, however, they will dissolve when diluted.

7. Other reagents are ready for use.

STORAGE

Store kit at 4°C.

SPECIMEN COLLECTION AND HANDLING

Samples must be used as soon as possible after collection. If the samples are to be tested at a later time, they should be divided into test tubes in small amounts and frozen at or below –30℃. Avoid repeated freeze/thaw cycles.

Total 100 µL of a sample is enough for measurement of CgA-LI.
<Saliva sample>
Saliva should be collected in a tube used for measurement of salivary amylase activity [e.g. Salivette (Sarstedt, Germany)]. After centrifugation at 3,000 rpm, the supernatant is transferred into a small polypropylene tube (Eppendorf tube), then frozen at or below -30℃. When only a small quantity of saliva is available, it should be absorbed into cotton, after which the saliva is squeezed out into Salivette tube. Measured values of CgA-LI must be corrected on the basis of protein content in saliva sample.
<Plasma sample>
EDTA-2Na additive blood collection tube (1 mg/mL blood) is recommended for the plasma collection. Namely, mix blood and EDTA well and centrifuge at 3,000 rpm. The plasma should be divided into small polypropylene tube (Eppendorf tube) in small amount and frozen at or below -30℃. Avoid repeated freezing and thawing of samples.

ASSAY PROTOCOL

1. Warm the reagents and samples to room temperature (20-30°C) before beginning the test.
2. Add 0.35 mL/well of washing solution into the wells and aspirate the washing solution in the wells. Repeat this washing procedure further twice (total three times).
3. Fill 50 µL of buffer solution into wells first and then introduce 25 µL of calibrator solutions (0, 0.14, 0.41, 1.23, 3.70, 11.11, 33.33 pmol/mL) or samples and add 50 µL of labeled antigen solution, finally add 100 µL of specific antibody solution.
4. Cover the plate with a Plate Seal and incubate it at room temperature overnight for 16~20 hours with gentle shaking on a microtiter plate shaker. During incubation except color reaction, the test plate should be shaken gently by plate shaker to promote immunoreaction.
5. Take off the Plate Seal, aspirate and wash the wells three times with approximately 0.35 mL/well of washing solution.
6. Pipette 100 µL of SA-HRP solution into the wells.
7. Cover the plate with a Plate Seal and incubate it for two hours at room temperature. During the incubation, the plate should be shaken with a microtiter plate shaker.

8. Resolve OPD tablet with 12 mL of substrate buffer. It should be prepared immediately before use.
9. Take off the Plate Seal, aspirate the solution in the wells and wash the wells four times with approximately 0.35 mL/well of washing solution.
10. Pipette 100 µL of substrate solution into the wells, incubate the plate for 30 minutes at room temperature.
11. Add 100 µL of stop solution into the wells to stop color reaction.
12. Read the optical absorbance of the wells at 490 nm. Read optical absorbance of reaction solution in wells as soon as possible after stopping the color reaction.

Note: Perform all determinations in duplicate.

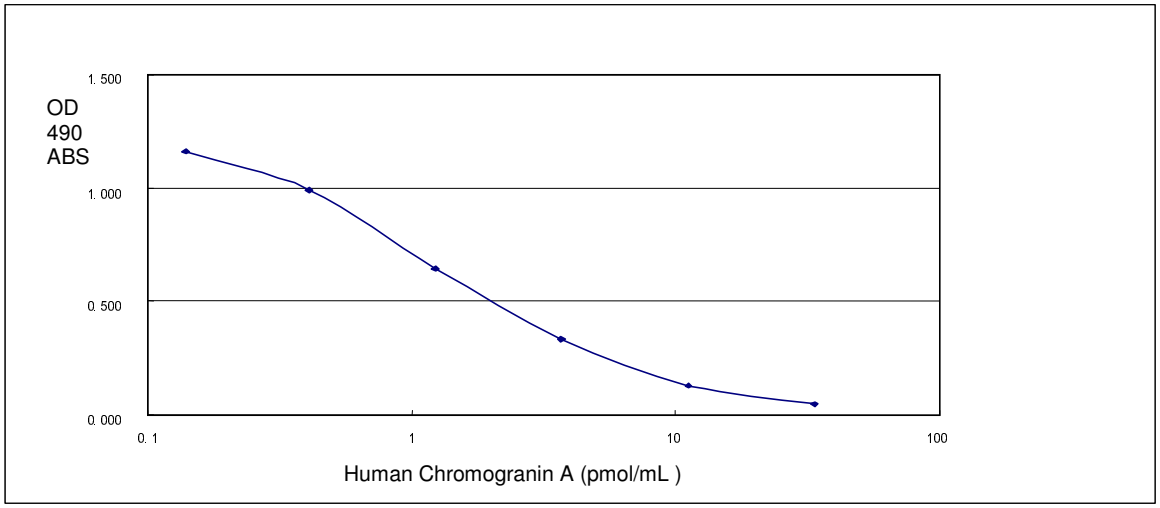
RESULTS

Calculate mean absorbance values of wells containing the Calibrators and plot a calibration curve on semilogarithmic graph paper (abscissa: concentration of Calibrators; ordinate: absorbance values of Calibrators). Use the calibration curve to read CgA concentrations in samples from the corresponding absorbance values.

When a sample value exceeds 33.33 pmol/mL, it needs to be diluted with buffer solution until the value is within the assay range.

PERFORMANCE

Typical Calibration Curve (example only, a new calibration curve for each run must be established by the end-user)



Analytical Recovery

Sample	CgA (344-374) Added (pmol/mL)	Observed (pmol/mL)	Expected (pmol/mL)	Recovery (%)
Plasma sample	0.00	0.54	-	-
	0.25	0.86	0.79	108.86
	1.00	1.93	1.54	125.32
	4.00	6.58	4.54	144.93
Saliva sample	0.00	0.47	-	-
	0.25	0.81	0.72	112.50
	1.00	1.40	1.47	95.24
	4.00	3.66	4.47	81.88

Precision and reproducibility

- Intra-assay CV (%):
  Plasma: 10.13 – 13.26
  Saliva: 8.15 – 12.84
- Inter-assay CV (%):
  Plasma: 11.57 – 15.33
  Saliva: 12.42 – 14.22

Assay Range

0.14 – 33.33 pmol/mL

Cross-Reactivity

Rat CgA	70%
Human β-granin	0%
Human pancreastatin (35-52) [Human CgA (284-301)]	0%
Human parastatin (1-19) [Human CgA (356-374)]	100%
WE-14 [Human CgA (324-337)]	0%
Porcine pancreastatin (1-49)	0%

FOR RESEARCH USE ONLY

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