YK070　Human Chromogranin A EIA

FOR LABORATORY USE ONLY

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Contents

- Please read all the package insert carefully before beginning the assay -

YK070: Human Chromogranin A EIA Kit

Ⅰ. 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 be co-stored and co-released with catecholamines from adrenal medulla and sympathetic neuronal vesicles during exocytosis. O'Conner and Bernstein have first reported radioimmunological measurement of CgA in human plasma under conditions of physiologic, pharmacologic and pathologic alteration of sympathoadrenal function. Accumulated data, thereafter, have confirmed high concentrations of plasma or serum CgA measured by radioimmunoassay (CgA-like immunoreactivity: CgA-LI) in patients with neuroendocrine and endocrine tumors, especially in those with pheochromocytoma, anterior pituitary tumors and rectal and prostatic carcinoma. On the other hand, Nakane et al. 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, Kanno et al. presented an 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, Yanaihara et al. 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. Nakane et al. also used the assay system in their 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, Yanaihara Institute Inc. succeeded in developing, for the first time, a specific, sensitive, stable and easy manipulative enzyme immunoassay (EIA) system for measurement of human CgA-LI using anti-synthetic human CgA (344-374) antibody, synthetic human CgA (344-374) as standard antigen and N^{α}-biotinylglycylglycyl human CgA (344-374) as labeled antigen. The assay kit now available from the Institute can be used for measurement of CgA-LI in human biological fluid such as plasma 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

YK070 Chromogranin A (Human) EIA Kit

- ▼ The assay kit can measure CgA in the range of 33.33 -0.14 pmol/mL.
- ▼ The assay completes within 16-20 hr. + 2.5 hr.
- ▼ With one assay kit, 41 samples can be measured in duplicate
- ▼ Test sample: human plasma or saliva
  Sample volume 25 μL
- ▼ The 96-wells plate in kit was consisted by 8-wells strips. The kit can be used separately.
- ▼ Precision and reproducibility
  Intra-assay CV (%) Plasma 10.13-13.26
  Inter-assay CV (%) Plasma 11.57-15.33
  Intra-assay CV (%) Saliva 8.15-12.84
  Inter-assay CV (%) Saliva12.42-14.22
- ▼ Stability and Storage
  11 months from the date of manufacturing.
  The expiry date is described on the label of kit.
  Store all of the components at 2-8℃.

Contents

1) Antibody coated plate
2) CgA standard
3) Labeled antigen
4) Specific antibody
5) SA-HRP solution
6) Substrate buffer
7) OPD tablet
8) Stopping solution
9) Buffer solution (concentrated)
10) Washing solution (concentrated)
11) Adhesive foil

Ⅱ. Characteristics

This EIA kit is used for quantitative determination of CgA-LI in human plasma and saliva samples. The kit is characterized for sensitive quantification, high specificity and no influence with other components in samples. CgA standard is highly purified synthetic product (purity over 98%) and the content indicated is the absolute weight of the standard. N$^{\alpha}$-biotinylglycylglycyl human CgA(344-374)is used as labeled antigen which is proved stability.

＜Crossreactivity＞

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%

<Test Principle>

This EIA 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-wells plate is coated with goat anti rabbit IgG. Human CgA standard 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.

III. Composition

	Components	Form	Quantity	Main Ingredient
①	Antibody coated plate	Plate	96 wells	Goat anti-rabbit IgG
②	CgA Standard	Lyophilized	100 pmol x 1	Synthetic human CgA (344-374)
③	Labeled antigen	Lyophilized	30 ng x 1	Biotinylated human CgA (344-374)
④	Specific antibody	Lyophilized	1	Rabbit anti-human CgA (344-374) antibody
⑤	SA-HRP solution	Liquid	12 mL x1	HRP labeled streptoavidin
⑥	Substrate buffer	Liquid	25 mL x1	0.1M phosphate-citrate buffer containing 0.015% H_2O_2
⑦	OPD tablet	Tablet	2 tablets	o-Phenylenediamine dihydrochloride
⑧	Stopping solution	Liquid	12 mL x1	2N H_2SO_4
⑨	Buffer solution (Concentrated)	Liquid	12 mL x1	50 mM phosphate buffer containing 2.5% BSA, 0.125M EDTA and 0.75M NaCl
⑩	Washing solution (Concentrated)	Liquid	50 mL x1	1.8% NaCl and 1% tween20
⑪	Adhesive foil	Sheet	3	

Ⅳ. Method

<Equipment required>

1. Photometer for microtiter plate (Plate reader), which can read extinction 2.5 at 490 nm
2. Microtiter plate shaker
3. Washing device for microtiter plate and dispenser with aspiration system
4. Micropipettes, multi-channel pipettes for 8 wells or 12 wells and their tips
5. Polyethylene or polypropylene made test tube for preparing standard solution
6. Graduated cylinder (1,000 mL)
7. Distilled water or deionized water

<Preparatory work>

1. Preparation of buffer solution:
   Dilute ⑨ buffer solution (concentrated) (10 mL) to 50 mL with distilled water.
2. Reconstitute ② lyophilized CgA standard in vial (100 pmol) with 1mL of buffer solution, which affords 100 pmol/mL standard solution. The 0.1 ml of the reconstituted standard solution is diluted with 0.2 mL of buffer solution that yields initial standard solution 33.33 pmol/mL. The 0.1mL of 33.33 pmol/mL standard solution is diluted with 0.2 mL buffer solution that makes 11.11pmol/mL standard solution. Repeat the dilution to make each standard solution of 3.70, 1.23, 0.41 and 0.14 pmol/mL. Buffer solution itself is used as 0 pmol/mL.
3. Preparation of labeled antigen solution:
   Dissolve ③ lyophilized labeled antigen in vial with distilled water (6 mL).
4. Preparation of specific antibody solution:
   Dissolve ④ lyophilized specific antibody in vial with distilled water (12 mL).
5. Preparation of substrate solution:
   Resolve one ⑦ OPD tablet with 12 mL of substrate buffer. It should be prepared immediately before use.
6. Preparation of washing solution:
   Dilute ⑩ washing solution (concentrated) (50 mL) to 1,000 mL with distilled water.
7. Other reagents are ready for use.

＜Procedure＞

1. Before beginning the test bring all the reagents and samples to room temperature.

2. Add 0.35mL/well of washing solution into the wells and aspirate the washing solution in the wells. Repeat this washing procedure further twice (total 3 times).

3. Fill 50 μL of buffer solution into the wells first, then introduce 25 μL of each of CgA standard 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 adhesive foil and keep it at room temperature (20-30℃) for overnight (16 -20 hours) with gentle shaking on a microtiter plate shaker.

5. Take off the adhesive foil, aspirate the solution in the wells and wash the wells 3 times with approximately 0.35 mL/well of washing solution.

6. Pipette 100μL of SA-HRP solution into the wells.

7. Cover the plate with adhesive foil and incubate it at room temperature (20-30ºC) for 2 hour. During the incubation, the plate should be shake with a plate shaker.

8. Resolve one OPD tablet with 12 mL of substrate buffer. It should be prepared immediately before use. Take off the adhesive foil, aspirate and wash the wells 4 times with approximately 0.35 mL/well of washing solution.

9. Add 100 μL of substrate solution into each well and incubate the plate at room temperature for 30 minites.

10. Add 100 μL of stopping solution into each well to stop color reaction.

11. Read the optical absorbance of reaction mixture in each well at 490 nm with a microtiter plate reader. Calculate mean absorbance values of wells containing standards and plot a standard curve on semilogarithmic graph paper (abscissa: concentration of standard; ordinate: absorbance values). Use the standard curve to read CgA concentrations in samples from the corresponding absorbance values.

Ⅴ. Notes

1. Samples must be used as soon as possible after collection. If the samples are tested later, they should be divided into test tubes in small amount and frozen at or below -30°C. Avoid repeated freezing and thawing of samples.

2. CgA standard, labeled antigen solution, specific antibody solution and substrate solution should be prepared immediately before use. This kit can be used dividedly in strips of the plate. In such case, the rest of reconstituted reagents (standard, labeled antigen and specific antibody) should be stored below -30℃.

3. During storage of washing solution (concentrated) at 2-8℃, precipitates may be observed, however they will be dissolved when diluted. Diluted washing solution is stable for three months at 2-8℃.

4. Pipetting operations may affect the precision of the assay, pipette standard solutions or samples precisely into each well of plate. In addition, using clean test tubes or vessels in assay and use new tip for each sample to avoid cross contamination.

5. When sample value exceeds 33.33 pmol/mL, it needs to be diluted with diluted buffer solution to proper concentration.

6. During incubation except color reaction, the test plate should be shake gently by plate shaker to promote immunoreaction.

7. Perform all the determination in duplicate.

8. Read plate optical absorbance of reaction solution in wells as soon as possible after stopping color reaction.

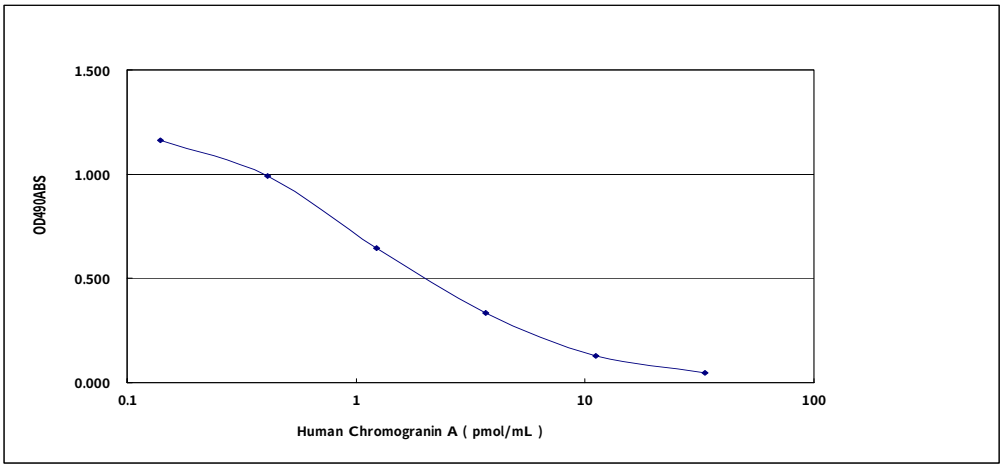
9. To quantitate accurately, always run a standard curve when testing samples.

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

11. Satisfactory performance of the test is guaranteed only when reagents are used from combination pack with identical lot number.

Ⅵ. Performance characteristics

＜Typical standard curve＞



＜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

＜Analytical recovery＞

Plasma sample

Human CgA (344-374) added (pmol/ml)	Observed (pmol/ml)	Expected (pmol/ml)	Recovery (%)
0	0.54		
0.25	0.86	0.79	108.86
1	1.93	1.54	125.32
4	6.58	4.54	144.93

Saliva sample

Human CgA (344-374) added (pmol/ml)	Observed (pmol/ml)	Expected (pmol/ml)	Recovery (%)
0	0.47		
0.25	0.81	0.72	112.50
1	1.40	1.47	95.24
4	3.66	4.47	81.88

VII. Sample Collection

Total 250 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 it at or below -30℃. When only a small quantity of saliva is available, it is should absorbed into cotton, 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°C. Avoid repeated freezing and thawing of samples.

Ⅷ. Stability and Storage

< Storage >	Store all of the components at 2-8°C.
< Shelf life >	11 months from the date of manufacturing
	The expiry date is described on the label of kit.
< Package >	For 96 tests per one kit including standards

Ⅸ. References

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