COSMO BIO CO., LTD.
Inspiration for Life Science

Rat Glicentin EIA

Cat. No. YII-YK111-EX

FOR LABORATORY USE ONLY

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Contents

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

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I. Introduction

Glicentin is a 69-amino-acid peptide containing glucagon and oxyntomodulin sequences in the molecule. It is suggested that glicentin and oxyntomodulin are produced in the intestinal L-cells and glucagon in A-cells in the pancreas, these peptides are derived from a common precursor by two different tissue-specific processing pathways. In 1983, the amino acid sequence of human glicentin was deduced by Bell et al. from the genomic sequence of human preproglucagon. Glicentin is a major form of gut glucagon-like immunoreactants (Gut GLIs).

In mammalian small intestine, proglucagon is processed into glicentin, oxyntomodulin, and glucagon -like peptide 1(GLP-1) and glucagon -like peptide 2 (GLP-2). GLP-1(7-37) and GLP-1(7-36)amide have been isolated from the intestine and pancreas. It has been known that the GLP-1 sequence is well conserved between species in all mammals studied. Using synthetic peptides, several investigators have demonstrated that in contrast to GLP-1 (1-37), truncated GLP-1(7-36)amide and GLP-1(7-37) have several physiological effects. However, the physiological role of glicentin, a major gut glucagon, is still unclear. It has been known that the circulating level of plasma glicentin-like peptides increases significantly nutrient ingestion.

Yanaihara institute Inc. has succeeded in developing a specific and convenient EIA kit for determination of rat glicentin in plasma.

YK111 Rat Glicentin EIA Kit

- ▼ The assay kit can measure Rat glicentin in the range of 0.206 - 50 pmol/mL.
- ▼ The assay completes within 16-18 hr.＋1.5 hr.
- ▼ With one assay kit, 41 samples can be measured in duplicate.
- ▼ Test sample: rat plasma
  Sample volume: 30 µL
- ▼ The 96-well plate in kit was consisted by 8-wells strips. The kit can be used separately.
- ▼ Precision and reproducibility
  Intra-assay CV (%) 4.56 - 7.82
  Inter-assay CV (%) 3.16 - 7.59
- ▼ Stability and Storage
  Store all of the components at 2-8°C.
  12 months from the date of manufacturing.
  The expiry date is described on the label of kit.

Contents

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

II. Characteristics

This EIA kit is used for quantitative determination of rat glicentin in plasma sample. The kit is characterized for sensitive quantification, high specificity and no influence with other components in plasma and needlessness of sample pre-treatment. Glicentin standard used in the kit is a highly purified synthetic product.

＜Specificity＞

The EIA kit is specificity for rat glicentin. It does not exhibit cross-reactions with human glicentin, glucagon (rat, mouse and human), GLP-1 (rat, mouse & human) and rat GLP-2.

< Test Principle >

This EIA kit for determination of rat glicentin in plasma samples is based on a competitive enzyme immunoassay using combination of highly specific antibody to rat glicentin and biotin-avidin affinity system. The 96-wells plate is coated with goat anti rabbit IgG antibody. Rat glicentin standard or samples, biotinylated rat glicentin and rabbit anti rat glicentin 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-biotinylated rat glicentin-antibody complex on the surface on the wells. Finally, HRP enzyme activity is determined by o-Phenylenediamine dihydrochloride (OPD) and the concentration of rat glicentin is calculated.

III. Composition

Component	Form	Quantity	Main Ingredient
1. Antibody coated plate	MTP[*1]	1 plate (96 wells)	Goat anti rabbit IgG
2. Glicentin standard	lyophilized	1 vial	Synthetic rat glicentin (50 pmol)
3. Labeled antigen	lyophilized	1 vial	Biotinylated rat glicentin
4. Glicentin antibody	liquid	1 bottle (6 mL)	Rabbit anti rat glicentin
5. SA-HRP solution	liquid	1 bottle (12 mL)	HRP labeled streptoavidin
6. Substrate buffer	liquid	1 bottle (26 mL)	0.015% Hydrogen Peroxide
7. OPD tablet	tablet	2 tablets	o-Phenylenediamine dihydrochloride
8. Stopping solution	liquid	1 bottle (12 mL)	1M H_2SO_4
9. Buffer solution	liquid	1 bottle (10 mL)	Phosphate buffer
10. Washing solution (concentrated)	liquid	1 bottle (50 mL)	Concentrated saline
11. Adhesive foil		3 sheets	

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

IV. Method

< Equipment required >

1) Photometer for microtiter plate (Plate reader), which can read extinction 2.5 at 492nm

2) Shaker for microtiter plate

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) Test tubes for preparation of standard solution

6) Graduated cylinder (1,000 mL)

7) Distilled water or deionized water

< Preparatory work >

1) Preparation of standard solution:
Reconstitute the Glicentin standard (lyophilized rat glicentin 50pmol/vial) with 1mL of buffer solution, which affords 50pmol/mL standard solution. The 0.1ml of the reconstituted standard solution is diluted with 0.2 mL of buffer solution that yields 16.67pmol/mL standard solution. The 0.1mL of 16.67pmol/mL standard solution is diluted with 0.2 mL of the buffer solution, that makes 5.556 pmol/mL standard solution. Repeat the dilution to make each standard of 1.852, 0.617, 0.206pmol/mL. Buffer solution is used as 0pmol/mL.

2) Preparation of labeled antigen:
Reconstitute labeled antigen with 8mL of distilled water or deionized water.

3) Preparation of substrate solution:
Resolve one OPD tablet with 12 mL of substrate buffer. It should be prepared immediately before use.

4) Preparation of washing solution:
Dilute 50 mL of washing solution (concentrated) to 1000 mL with distilled or deionized water.

5) Other reagents are ready for use.

< Procedure >

1. Bring all the reagents to room temperature (20-30°C) before beginning the test.

2. Fill 70µL of labeled antigen into wells first, then add 30µL of each of standard solutions (0, 0.206, 0.617, 1.852, 5.556, 16.667, 50pmol/mL) or samples and finally introduce 50µL of Glicentin antibody into the wells.

3. Cover the plate with adhesive foil and incubate it at room temperature for 16-18 hours.
   During the incubation, the plate should be shake with a plate shaker.

4. Take off the adhesive foil, aspirate the solution in the wells and wash the wells four 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 for 1 hour.
   During the incubation, the plate should be shake with a plate shaker.

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

9. Add 100µL of substrate solution into the wells, cover the plate with adhesive foil and incubate it for 30 minutes at room temperature.

10. Add 100µL of stopping solution into the wells to stop reaction.

11. Read the optical absorbance of the wells at 492nm. 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 rat glicentin concentrations in samples from the corresponding absorbance values.

V. Notes

1. Plasma 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 amount and frozen at or below -30°C. Avoid repeated freezing and thawing of plasma samples. EDTA-2Na additive blood collection tube is recommended for the plasma collection.

2. During storage of washing solution (concentrated) at 2-8°C, precipitates may be observed, however they will be dissolved when diluted.

3. Pipetting operations may affect the precision of the assay, pipette precisely standard solutions or samples into each well of plate. Use new tip for each sample to avoid cross contamination.

4. When sample value exceeds 50pmol/mL, it needs to be diluted with buffer solution to proper concentration.

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

6. Perform all the determination in duplicate.

7. Read optical absorbance of reaction solution in wells as soon as possible after stopping the color reaction.

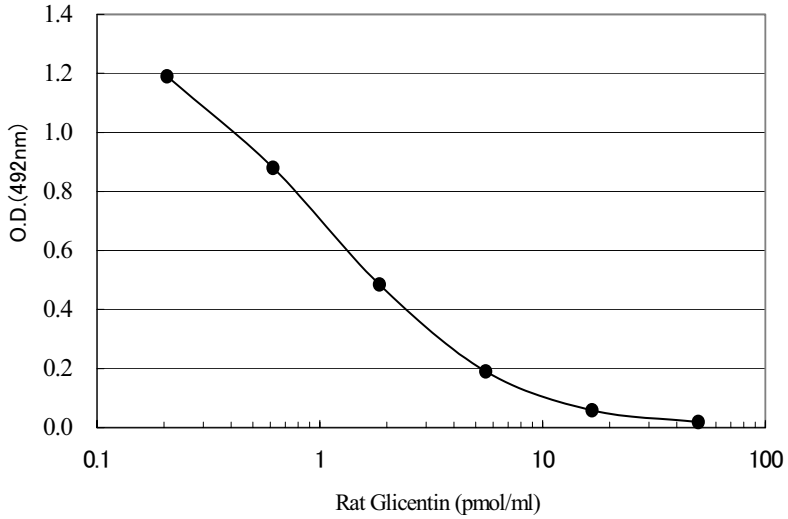
8. To quantitate accurately, always run a standard curve when testing samples.

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

10. 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



< Analytical recovery >

Rat plasma A

Rat Glicentin added (pmol/mL)	Observed (pmol/mL)	Expected (pmol/mL)	Recovery (%)
10.0	10.24	10.70	95.72
5.0	5.37	5.70	94.25
2.5	3.28	3.20	102.36
0	0.70		

Rat plasma B

Rat Glicentin added (pmol/mL)	Observed (pmol/mL)	Expected (pmol/mL)	Recovery (%)
10.0	9.20	10.85	84.80
5.0	5.51	5.85	94.13
2.5	3.09	3.35	92.34
0	0.85		

< Precision and reproducibility >

- Intra-assay CV (%): 4.56 ~ 7.82
- Inter-assay CV (%): 3.16 ~ 7.59

< Assay range >

0.206 - 50pmol/mL

VII. Stability and Storage

< Storage >	Store all of the components at 2-8°C.
< Shelf life >	12 month from the date of manufacturing
	The expiry date is described on the label of kit.
< Package >	For 96 tests per one kit including standards

VIII. References

1. Ohneda, A. et al.: Effect of glicentin-related peptides on glucagon secretion in anaesthetized dogs. DIABETOLOGIA 29: 397-401, 1986

2. Ohneda, A. et al.: Effect of intraluminal administration of amino acids upon plasma glicentin. DIABETES RESEARCH AND CLINICAL PRACTICE 5: 265-270, 1988

3. Ohneda, A. et al.: Insulinotropic action of human glicentin in dogs. METABOLISM, CLINICAL AND EXPERIMENTAL 44: 47-51, 1995

4. Ishihara, S. et al.: Helicobacter pylori infection accelerates gene expression of glicentin in gastric mucosa. Its association with intestinal metaplasia of the stomach. SCANDINAVIAN JOURNAL OF GASTROENTEROLOGY 32: 460-464, 1997

5. Shibata, C. et al.: Effect of glucagon, glicentin, glucagon-like peptide-1 and -2 on interdigestive gastroduodenal motility in dogs with a vagally denervated gastric pouch. SCANDINAVIAN JOURNAL OF GASTROENTEROLOGY 36: 1049-1055, 2001

Manufactured by Yanaihara Institute Inc.

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