



Rat Glicentin ELISA

Cat. No.: RSCYK111R

1. 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.

RSCYK111R Rat Glicentin EIA Kit	Contents
▼ The assay kit can measure rat glicentin in the range of 0.206 - 50 pmol/mL.	1) Antibody coated plate
▼ The assay completes within 16-18 hr. + 1.5 hr.	2) Glicentin standard
▼ With one assay kit, 41 samples can be measured in duplicate.	3) Labeled antigen
▼ Test sample: rat plasma Sample volume: 30 µL	4) Glicentin antibody
▼ The 96-well plate in kit was consisted by 8-wells strips. The kit can be used separately.	5) SA-HRP solution
▼ Precision and reproducibility Intra-assay CV (%) 4.56 – 7.82 Inter-assay CV (%) 3.16 – 7.59	6) Substrate buffer
▼ 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.	7) OPD tablet
	8) Stopping solution
	9) Buffer solution
	10) Washing solution (concentrated)
	11) Adhesive foil

2. Characteristics

This EIA kit is used for quantitative determination of rat glicentin in plasma sample. It has a lot of advantage to perform the assay, such as good quantification, no influence with other body fluid factors or physiological active substances and needlessness of sample pre-treatment. Glicentin standard used in the kit is a highly purified synthetic product

Specificity

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

Test Principle

This EIA kit for determination of rat glicentin in plasma is based on a competitive enzyme immunoassay using combination with highly specific antibody to rat glicentin and biotin – avidin affinity system. The 96 wells plate is coated with goat anti rabbit IgG and 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 streptoavidins are added to bind to the antigen-antibody complex so that HRP labeled streptoavidin - biotinylated rat glicentin – antibody complexes are formed on the surface on the wells. Finally, excess HRP labeled streptoavidins are rinsed out and HRP enzyme activity is determined and the concentration of rat glicentin is calculated.

3. 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 hydrochloride
⑧ Stopping solution	liquid	1 bottle (12 mL)	1M-H ₂ SO 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.....Microtitration plate			

4. Method

Equipment required

1. Photometer for microtitration plate (Plate reader), which can read extinction 2.5 at 492 nm
2. Shaker for microtitration 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 50 pmol/mL standard solution. The 0.1ml of the reconstituted standard solution is diluted with 0.2 mL of buffer solution, that yields 16.67 pmol/mL standard solution. The 0.1mL of 16.67 pmol/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, 0.206 pmol/mL. Buffer solution is used as 0 pmol/mL.

2. Preparation of labeled antigen:

Reconstitute labeled antigen with 8 mL of distilled water or deionized water.

3. Preparation of substrate solution:

Resolve 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. Warm up the reagents and samples to room temperature 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, 50 pmol/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 rotated 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.

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

6. Cover the plate with adhesive foil and incubate it at room temperature for 1 hour.

7. During the incubation, the plate should be rotated 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 100 μ L of substrate solution into the wells, cover the plate with adhesive foil and incubate it for 30 minutes at room temperature.
10. Add 100 μ L of stopping solution into the wells to stop reaction.
11. Read the optical absorbance of the wells at 492nm.
12. Calculate mean absorbance values of wells containing standards and plot a standard curve on semilogarithmic graph paper (abscissa: concentration of standard; ordinate: absorbance values.).
13. Use the standard curve to read rat glicentin concentrations in samples from the corresponding absorbance values.

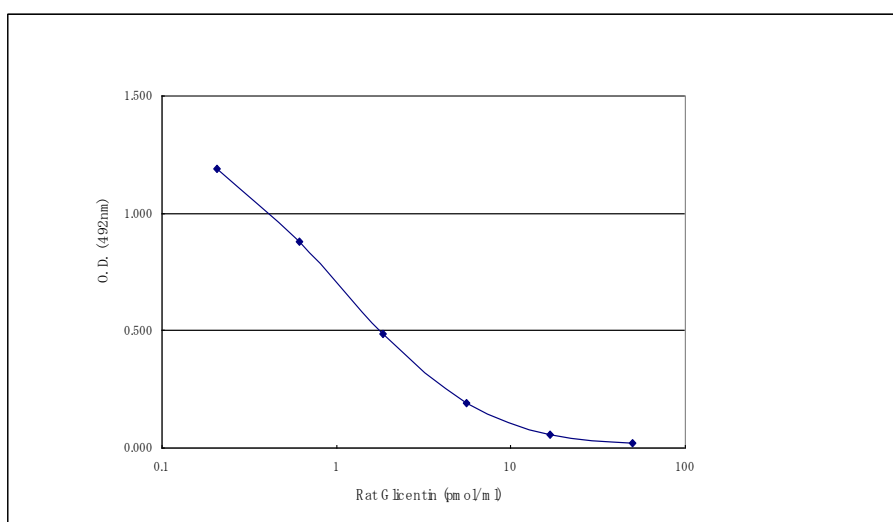
5. Notes

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 plasma is recommended to use for the determination.
2. During storage of washing solution (concentrated) at 2 to 8°C, precipitates may be observed, however they will be dissolved when diluted.
3. As pipetting operations may affect with the precision of the assay, pipette precisely standard solutions or samples into each well of plate. And use new tip for each sample to avoid cross contamination.
4. When sample value exceeds 50 pmol/mL, it needs to be diluted with buffer solution within the assay range.
5. During incubation except color reaction, the test plate should be rotated gently by plate shaker to promote immunoreaction.

6. Read optical absorbance of reaction solution in wells as soon as possible after stopping the color reaction.
7. Perform all the determination in duplicate.
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.

6. Performance Characteristics

Typical standard curve



Analytical recovery

Sample No.	Rat Glicentin added (pmol/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
Plasma 1	0.0	0.70		
	2.5	3.28	3.20	102.4
	5.0	5.37	5.70	94.3
	10.0	10.24	10.70	95.7
Plasma 2	0.00	0.85		
	2.5	3.09	3.35	92.3
	5.0	5.51	5.85	94.1
	10.0	9.20	10.85	84.8

Precision and reproducibility

- Intra-assay CV (%) 4.6 – 7.8
- Inter-assay CV (%) 3.2 – 7.6

Assay range

0.206 – 50 pmol/mL

7. Stability and Storage

Storage Store all of the components at 2-8°C.

Shelf life 6 months from the date of manufacturing
The expiry date is described on the label of kit.

Package For 96 tests per 1 kit including standards

8. References

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

**Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium
<http://www.gentaur-worldwide.com>**