

## YK111 Rat Glicentin EIA

### 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.

<b>YK111 Rat Glicentin EIA Kit</b>	<b>Contents</b>
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	4) Glicentin antibody
Sample volume: 30 µL	5) SA-HRP solution
The 96-well plate in kit was consisted by 8-wells strips. The kit can be used separately.	6) Substrate buffer
Precision and reproducibility	7) OPD tablet
Intra-assay CV (%) 4.56 - 7.82	8) Stopping solution
Inter-assay CV (%) 3.16 - 7.59	9) Buffer solution
Stability and Storage	10) Washing solution (concentrated)
Store all of the components at 2-8 °C .	11) Adhesive foil
12 months from the date of manufacturing.	
The expiry date is described on the label of kit.	

## 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 >

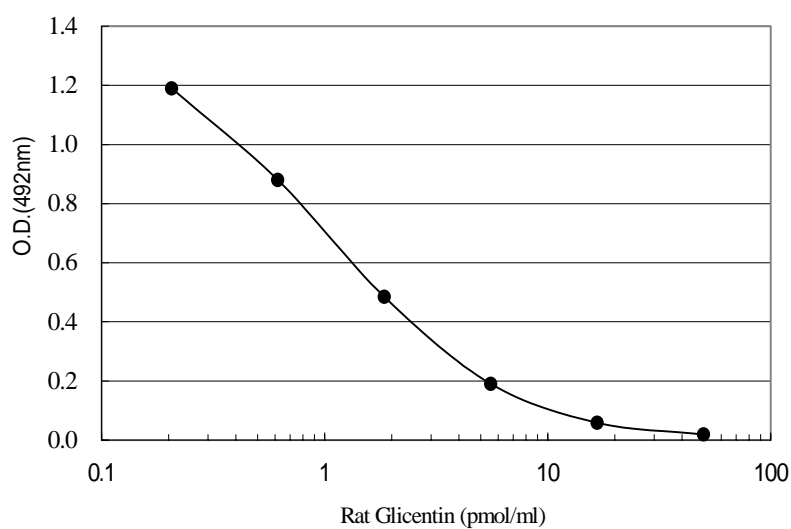
The EIA kit is specificity for rat glicentin. It does not exhibit cross-reactions with human glicentin, glucagon (rat, mouse and human), GLP-1 (rat, mouse & human) and rat GLP-2.

### < 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. 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

#### **IV. 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

#### **V. 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