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.
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 streptavidins 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 streptavidins are rinsed out and HRP enzyme activity is determined and the concentration of rat glicentin is calculated.
3. Composition

<table>
<thead>
<tr>
<th>Component</th>
<th>Form</th>
<th>Quantity</th>
<th>Main Ingredient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody coated plate</td>
<td>MTP*</td>
<td>1 plate (96 wells)</td>
<td>Goat anti rabbit IgG</td>
</tr>
<tr>
<td>Glicentin standard</td>
<td>lyophilized</td>
<td>1 vial</td>
<td>Synthetic rat glicentin (50 pmol)</td>
</tr>
<tr>
<td>Labeled antigen</td>
<td>lyophilized</td>
<td>1 vial</td>
<td>Biotinylated rat glicentin</td>
</tr>
<tr>
<td>Glicentin antibody</td>
<td>liquid</td>
<td>1 bottle (6 mL)</td>
<td>Rabbit anti rat glicentin</td>
</tr>
<tr>
<td>SA-HRP solution</td>
<td>liquid</td>
<td>1 bottle (12 mL)</td>
<td>HRP labeled streptoavidin</td>
</tr>
<tr>
<td>Substrate buffer</td>
<td>liquid</td>
<td>1 bottle (26 mL)</td>
<td>0.015% Hydrogen Peroxide</td>
</tr>
<tr>
<td>OPD tablet</td>
<td>tablet</td>
<td>2 tablets</td>
<td>o-Phenylenediamine hydrochloride</td>
</tr>
<tr>
<td>Stopping solution</td>
<td>liquid</td>
<td>1 bottle (12 mL)</td>
<td>1M-H₂SO₄</td>
</tr>
<tr>
<td>Buffer solution</td>
<td>liquid</td>
<td>1 bottle (10 mL)</td>
<td>Phosphate buffer</td>
</tr>
<tr>
<td>Washing solution</td>
<td>liquid</td>
<td>1 bottle (50 mL)</td>
<td>Concentrated saline</td>
</tr>
<tr>
<td>Adhesive foil</td>
<td></td>
<td>3 sheets</td>
<td></td>
</tr>
</tbody>
</table>

MTP*........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 50 pmol/vial) with 1 mL of buffer solution, which
   affords 50 pmol/mL standard solution. The 0.1 mL of the reconstituted standard solution is diluted with 0.2 mL
   of buffer solution, that yields 16.67 pmol/mL standard solution. The 0.1 mL 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 70 µL of labeled antigen into wells first, then add 30 µL of each of standard solutions (0, 0.206, 0.617,
   1.852, 5.556, 16.667, 50 pmol/mL) or samples and finally introduce 50 µL 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 100 µL 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 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.

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](image-url)
Analytical recovery

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

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


