# YK140 Rat GLP-2 EIA

**FOR LABORATORY USE ONLY**

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## Contents



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

**YK140　Rat GLP-2 EIA Kit**

## I. Introduction

The proglucagon gene is expressed in both pancreatic A cell and intestinal L cell. Tissue-specific posttranslational processing of proglucagon by the prohormone convertase produced the different proglucagon derived peptides (PGDPs) in both pancreas and intestine. The most notable pancreatic PGDP is glucagon, whereas the L cell produces several structurally related peptides, including glucagon-like peptide 1 (GLP-1) and GLP-2, as well as glicentin and oxyntomodulin, which contain glucagon sequence in their molecules. Among PGDPs, GLP-2 has recently been found to show intestinal epithelial proliferation.

**YK140 Rat GLP-2 EIA Kit**

- ▼ The assay kit can measure GLP-2 in the range of 0.137 - 100 ng/mL
- ▼ The assay completes within 16-18 hr. +1.5 hr.
- ▼ With one assay kit, 40 samples can be measured in duplicate
- ▼ Test sample: rat serum or plasma
  Sample volume: 25 μL
- ▼ The 96-well plate in kit is consisted by 8-wells strips. The kit can be used separately.
- ▼ Precision and reproducibility

  Intra-assay CV（%） serum 3.5 - 8.9
  Inter-assay CV（%） serum 7.6 - 13.0

  Intra-assay CV（%） plasma 3.1 - 7.2
  Inter-assay CV（%） plasma 6.7 - 11.5
- ▼ Stability and Storage
  Store all of the components at 2-8°C.
  The kit is stable under the condition for 19 months from the date of manufacturing.
  The expiry date is stated on the package
  .

**Contents**

1) Antibody coated plate
2) Rat GLP-2 standard
3) Labeled antigen
4) Rat GLP-2 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 GLP-2 [both GLP-2 (1-33) and GLP-2 (3-33)] in serum or plasma samples. The kit is characterized for sensitive quantification, high specificity and no influences with other components in samples. Rat GLP-2 standard is highly purified synthetic product.

< Specificity >

The EIA kit has high specificity to rat GLP-2 and shows no cross reactivity with rat glucagon and rat GLP-1 even in the concentration of 300 pmol/mL.

< Test Principle >

This EIA kit for determination of rat GLP-2 in serum or plasma samples is based on a competitive enzyme immunoassay using combination of highly specific antibody to rat GLP-2 and biotin-avidin affinity system. The 96-wells plate is coated with goat anti rabbit IgG antibody. Rat GLP-2 standard or samples, labeled antigen and anti rat GLP-2 polyclonal 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 GLP-2-antibody complex on the surface of the wells. Finally, HRP enzyme activity is determined by o-Phenylenediamine dihydrochloride (OPD) and the concentration of rat GLP-2 is calculated.

## III. Composition

| Component | Form | Quantity | Main Ingredient |
|---|---|---|---|
| 1. Antibody coated plate | MTP[*1] | 1 plate (96 wells) | Goat anti rabbit IgG |
| 2. Rat GLP-2 standard | lyophilized | 1 vial | Synthetic rat GLP-2 (50ng/vial) |
| 3. Labeled antigen | lyophilized | 1 vial | Biotinylated rat GLP-2 |
| 4. Rat GLP-2 antibody | liquid | 1 bottle (6 mL) | Rabbit anti rat GLP-2 |
| 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 (25 mL) | Tris-HCl 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 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. 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 standard (lyophilized rat GLP-2 50ng/vial) with 0.5mL of buffer solution, which affords 100ng/mL standard solutions. The 0.1ml of the reconstituted standard solution is diluted with 0.2 mL of buffer solution that yields 33.33ng/mL standard solution. Repeat the same dilution to make each standard of 11.11, 3.704, 1.235, 0.412, 0.137ng/mL. Buffer solution is used as 0 ng/mL.
2. Preparation of labeled antigen:
   Reconstitute labeled antigen with 9 mL of buffer solution.
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 and samples return to the room temperature before beginning the test.

2. Fill 75μL of labeled antigen solution into the wells first, then introduce 25μL of each standard solutions (0, 0.137, 0.412, 1.235, 3.704, 11.11, 33,33, 100 ng/mL) or samples and finally add 50μL of Rat GLP-2 antibody into the wells.

3. Cover the plate with adhesive foil and incubate it at 4ºC for 16-18 hours. (Still, plate shaker not need)

4. 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. Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual 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 (20-30ºC) for 1 hour. During the incubation, the plate should be shake with a plate shaker.

7. Take off the adhesive foil, aspirate and wash the wells 5 times with approximately 0.35 mL/well of washing solution. Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.

8. Resolve one OPD tablet with 12 mL of substrate buffer. It should be prepared immediately before use.

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 color reaction.

11. Read the optical absorbance of the wells at 490 nm. 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 GLP-2 concentrations in samples from the corresponding absorbance values.

**V. Notes**

1. Plasma or serum 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. Rat GLP-2 standard, Labeled antigen, 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 (Rat GLP-2 standard and Labeled antigen) should be stored blow -30°C.

3. During storage of washing solution (concentrated) at 2-8°C, precipitates may be observed, however they will be dissolved when diluted. Diluted washing solution is stable for 6 months at 2 - 8°C.

4. As pipetting operations may affect the precision of the assay, pipette standard solutions or samples into each well of plate precisely. Use clean test tubes or vessels in assay, and new tip must be used for each sample and standard solution to avoid cross contamination.

5. When sample value exceeds 100 ng/mL, it needs to be diluted with buffer solution to proper concentration.

6. During incubation except 4°C incubation and 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.

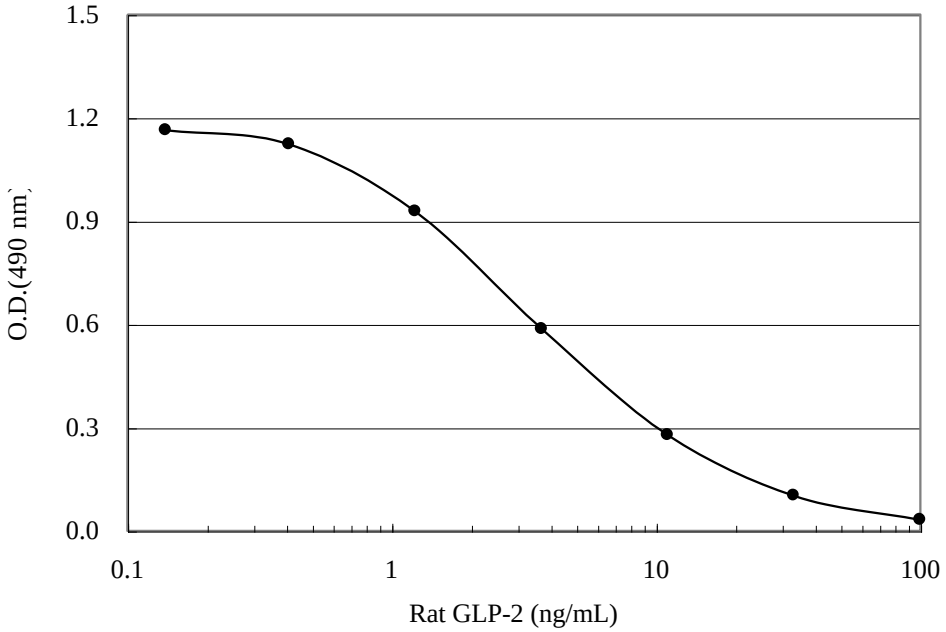
9. To quantitate accurately, always run a standard curve when testing samples.

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




Analytical recovery

< Rat serum >

| Sample No. | Rat GLP-2 added ng/mL | Observed ng/mL | Expected ng/mL | Recovery (%) |
|---|---|---|---|---|
| 1 | 0 | 2.55 | - | - |
| 2 | 1 | 3.59 | 3.29 | 109.0 |
| 3 | 5 | 8.11 | 7.29 | 111.2 |
| 4 | 20 | 27.08 | 22.29 | 122.8 |

< Rat plasma >

| Sample No. | Rat GLP-2 added ng/mL | Observed ng/mL | Expected ng/mL | Recovery (%) |
|---|---|---|---|---|
| 1 | 0 | 2.90 | - | - |
| 2 | 1 | 3.89 | 3.61 | 107.8 |
| 3 | 5 | 8.28 | 7.61 | 108.7 |
| 4 | 20 | 26.85 | 22.61 | 118.7 |

Precision and reproducibility

- Intra-assay/Rat serum CV（%） 3.5～8.9
- Inter-assay/Rat serum CV（%） 7.6～13.0
- Intra-assay/Rat plasma CV（%） 3.1～7.2
- Inter-assay/Rat plasma CV（%） 6.7～11.5

Assay range
0.137 ~ 100 ng/mL

**VII. Stability and Storage**

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

< Shelf life > The kit is stable under the condition for 19 months from the date of manufacturing. The expiry date is stated on the package

< Package > For 96 tests per one kit including standards

**VIII. References**


1. Philippe, J.: Structure and pancreatic expression of the insulin and glucagon gene. *Endocr Rev* **12**: 252 - 271, 1991

2. Mojsov S. et al: Preproglucagon gene expression in pancreas and intestine diversifies the level of post-transcriptiona processing. *J Biol Chem* **261**: pp11880 – 11889, 1986

3. Drucker, D. J. et al: Induction of intestinal epithelial proliferation by glucagon-like peptide 2. *Proc Natl Acad Sci USA* **93**: 7911 – 7916, 1996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