BioVendor
Research
and Diagnostic Products

Rat GLP-2 ELISA

Cat. No.: RSCYK140R

1. 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(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.

RSCYK140R 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: mouse serum or plasma
  Sample volume: 25 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（%） 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 ºC.
  12 months from the date of manufacturing.
  The expiry date is described on the label of kit.

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

2. Characteristics

This EIA kit is used for quantitative determination of rat GLP-2 in serum or plasma samples. It has a lot of advantage to perform the assay, such as good quantification, high specificity and no influence with other body fluid factors or physiological active substances. 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 within the range 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 with 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, biotinylated rat GLP-2 and anti rat GLP-2 polyclonal antibody are added to the wells for competitive immunoreaction. After rinsing out excess rat GLP-2, HRP labeled streptoavidins are added to bind to the antigen-antibody complex so that HRP labeled streptoavidin – biotinylated rat GLP-2 – antibody complexs are formed on the surface of the wells. Finally, excess HRP labeled streptoavidins are rinsed out and HRP enzyme activity is determined and the concentration of rat GLP-2 is calculated.

3. 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 (50 ng/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 (0.2 mL)	HRP labeled streptoavidin
⑥	Diluent for SA-HRP	liquid	1 bottle (12 mL)	Phosphate buffer
⑦	Substrate buffer	liquid	1 bottle (26 mL)	0.015% Hydrogen Peroxide

⑧	OPD tablet	tablet	2 tablets	o-Phenylenediamine hydrochloride
⑨	Stopping solution	liquid	1 bottle (12 mL)	2N-H_2SO_4
⑩	Buffer solution	liquid	1 bottle (35 mL)	Phosphate buffer
⑪	Washing solution	liquid	1 bottle (50 mL)	Concentrated saline
12.	Adhesive foil		3 sheets	

MTP*1……..Microtittration plate

4. Method

Equipment required

1. Photometer for microtitration plate (Plate reader),which can read extinction 2.5 at 490 nm

2. Rotator for microtitration plate

3. Washing device for microtitration plate and dispenser for approximately 0.3 mL 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 100 ng/mL standard solution. The 0.1 ml 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, 0.137 ng/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 SA-HRP diluted solution:

Add 0.12 mL of SA-HRP solution to a bottle of Diluent for SA-HRP (12mL) and mix them thourghly. It should be prepared immediately before use.

4. Preparation of substrate solution:

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

5. Preparation of washing solution:

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

6. Other reagents are ready for use.

Procedure

1. Warm up the reagents and samples to room temperature before beginning the test.

2. Add 300μL/well of washing solution into the wells. Aspirate the washing solution in the wells. Repeat this washing procedure twice.

3. Fill 75μL of labeled antigen solution into the wells first, then introduce 25μL of each of 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 .

4. Cover the plate with adhesive foil and incubate it at 4ºC overnight (16 - 18 hours).

5. Take off the adhesive foil, aspirate the solution in the wells and wash the wells three times with approximately 0.3 mL/well of washing solution.

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

7. Cover the plate with adhesive foil and incubate it at room temperature (20 - 30ºC) for 1 hour. During the incubation, the plate should be rotated with a plate rotator.

8. Take off the adhesive foil, aspirate and wash the wells five times with approximately 0.3 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 490nm.

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 GLP-2 concentrations in samples from the corresponding absorbance values.

5. 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 ºC. Avoid repeated freezing and thawing of plasma or serum samples.

2. Rat GLP-2 standard, labeled antigen, and substrate solution should be prepared immediately before use in assay using clean test tubes or vessels. Diluted washing solution is stable for 6 months at 2 to 8 ºC.

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

4. As pipetting operations may affect 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.

5. When sample value exceeds 100 ng/mL, it needs to be diluted with buffered solution within the assay range.

6. During incubation with SA-HRP solution at room temperature, the test plate should be shake gently by plate shaker to promote immunoreaction.

7. .During continuous rotation of test plate, the plate rotator may be heated up. It is recommended to place styrene form or plywood between the plate and the rotator

8. Read plate. optical absorbance of reaction solution in wells as soon as possible after stopping color reaction.

9. Perform all determination in duplicate..

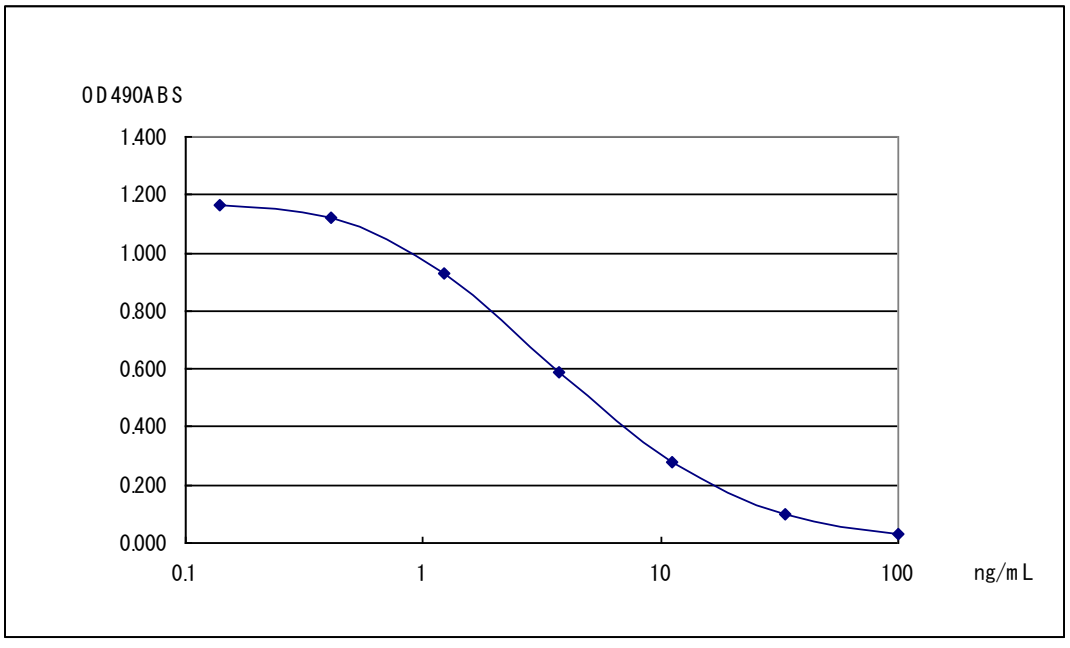
10. To quantitate accurately, always run a standard curve when testing samples.

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

12. 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

Rat serum

Sample No.	Rat GLP-2 added (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
1	0	1.96		
2	1	2.62	2.96	88.51
3	3	4.33	4.96	87.30
4	6	7.08	7.96	88.88

Rat plasma

Sample No.	Rat GLP-2 added (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
1	0	1.56		
2	1	2.17	2.56	84.74
3	3	3.63	4.56	79.58
4	6	6.00	7.56	79.42

Precision and reproducibility

- Intra-assay/Rat serum CV (%) 6.03 – 16.13
- Intra-assay/Rat plasma CV (%) 0.48 – 8.48

Assay range

0.137 - 100 ng/mL

7. Stability and Storage

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

Shelf life 12 months from the date of manufacturing

The expiry date is described on the label of kit.

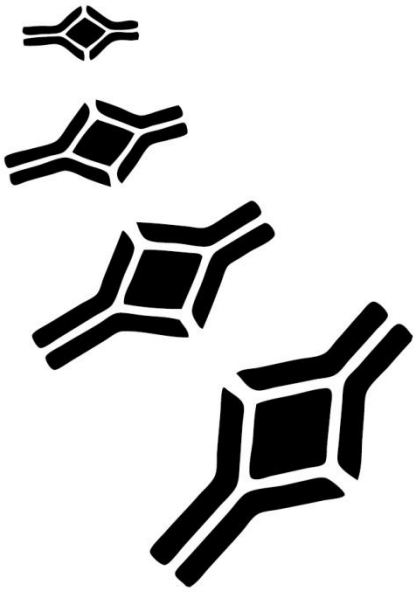
Package For 96 tests per 1 kit including standards

8. References

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

2. Mojsov S. et al: Preproglucagon gene expression in pancreas and intestine diversifies the level of post-transcriptional processing. *J Biol Chem* ***261***: 11880-11889,1986

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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