



Human GLP-2 ELISA

Cat. No.: RSCYK141R

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.

RSCYK141R Human GLP-2 EIA Kit	Contents
▼ The assay kit can measure human GLP-2 within the range of 0.412 - 100 ng/mL	1) Antibody coated plate
▼ The assay completes within 16-18 hr. + 1.5 hr.	2) Human GLP-2 standard
▼ With one assay kit, 41 samples can be measured in duplicate	3) Labeled antigen
▼ Test sample: mouse serum or plasma Sample volume: 25 µL	4) GLP-2 antibody
▼ The 96-well plate in kit consists of 12 8-wells strips, so that divided use by the strips is possible at user's option..	5) SA-HRP solution
▼ Precision and reproducibility	6) Substrate buffer
Intra-assay CV (%) serum 3.7-4.8	7) OPD tablet
Inter-assay CV (%) serum 13.0-16.4	8) Stopping solution
Intra-assay CV (%) plasma 3.0-5.5	9) Buffer solution
Inter-assay CV (%) plasma 14.3-17.5	10) Washing solution (concentrated)
▼ Stability and Storage	11) Adhesive foil
Store all of the components at 2-8 °C.	
The kit is stable under the condition for 9 months	
From the date of manufacturing..	
The expiry date is described on the label of kit.	

2. Characteristics

This EIA kit is used for quantitative determination of human GLP-2 in serum or plasma samples. The kit is characterized by its sensitive quantification and high specificity. In addition, it is not influenced by other constituents in samples. Standard antigen, human GLP-2, is highly purified synthetic product. (purity: higher than 98%)

Specificity

The EIA kit has high specificity to human GLP-2 and shows cross reactivity to neither glucagon (rat/mouse/human) nor GLP-1 even in the concentration of 300 pmol/mL.

Test Principle

This EIA kit for determination of human GLP-2 in serum or plasma samples is based on a competitive enzyme immunoassay using combination of highly specific antibody to human GLP-2 and biotin-avidin affinity system. To the wells of the plate coated with goat anti rabbit IgG, standard antigen or samples, biotinylated human GLP-2, and rabbit anti GLP-2 antibody are added for competitive immunoreaction. After incubation and plate washing, horse radish peroxidase (HRP) labeled streptoavidin (SA) is added to form HRP labeled SA - biotinylated 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 human GLP-2 is calculated.

3. Composition

Component	Form	Quantity	Main Ingredient
① Antibody coated plate	Microtiter plate	1 plate (96 wells)	Goat anti rabbit IgG
② Human GLP-2 standard	Lyophilized	1 vial	Synthetic human GLP-2
③ Labeled antigen	Lyophilized	1 vial	Biotinylated human GLP-2
④ GLP-2 antibody	Liquid	1 bottle (6 mL)	Rabbit anti human GLP-2 antibody
⑤ SA-HRP solution	Liquid	1 bottle (12 mL)	HRP labeled SAn
⑥ Substrate buffer	Liquid	1 bottle (26 mL)	Citrate buffer containing 0.015% hydrogen peroxide
⑦ OPD tablet	Tablet	2 tablets	o-Phenylenediamine dihydrochloride
⑧ Stopping solution	Liquid	1 bottle (12 mL)	1M H ₂ SO ₄

⑨	Buffer solution	Liquid	1 bottle (25 mL)	Phosphate buffer
⑩	Washing solution (concentrated)	Liquid	1 bottle (50 mL)	Concentrated saline
⑪	Adhesive foil		3 sheets	

4. Method

Equipment required

1. Photometer for microtitration plate (Plate reader), which can read extinction 2.5 at 492 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. Glass 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 human GLP-2 standard (lyophilized, 50 ng/vial) with 0.5 mL of buffer solution, which affords 100 ng/mL standard solution. Dilute 0.1 mL of the standard solution with 0.2 mL of buffer solution, which yields 33.33 ng/mL standard solution. Repeat the dilution procedure to make each standard of 11.11, 3.704, 1.235, 0.412 ng/mL standard solutions. Buffer solution itself 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 to room temperature (20-30 °C) at least 1 hour before starting assay.
2. Add 0.35 mL/well of washing solution into the wells of the plate, and then aspirate the solution. Repeat this washing procedure further twice (total 3 times). Finally, invert the plate and tap it on to an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution
3. Fill 40 μ L of labeled antigen solution into the wells first, then introduce 25 each of standard solutions (0, 0.412, 3.704, 11.11, 33.33 and 100 ng/mL) or samples and finally add 50 μ L of GLP-2 antibody into the wells.
4. Cover the plate with adhesive foil and incubate it at 4°C for 16 - 18 hours.(Not shaken)
5. After incubation, take off the adhesive foil, aspirate the solution in the wells and wash the wells 3 times with approximately 0.35 mL/well each of washing solution. Finally, invert the plate and tap it on to an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.
6. Pipette 100 μ L of SA-HRP solution into each of the wells.
7. Cover the plate with adhesive foil and incubate it at room temperature (20-30°C) for 1 hour. During the incubation, the plate should be shaken with a microtiter plate shaker.
8. Resolve OPD tablet with 12 mL of substrate buffer. It should be prepared immediately before use.
9. Take off the adhesive foil, aspirate the solution in the wells and wash the wells 5 times with approximately 0.35 mL/well each of washing solution. Finally, invert the plate and tap it on to an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.
10. Add 100 μ L of substrate solution into each the wells, cover the plate with adhesive foil and incubate it for 30 minutes at room temperature.

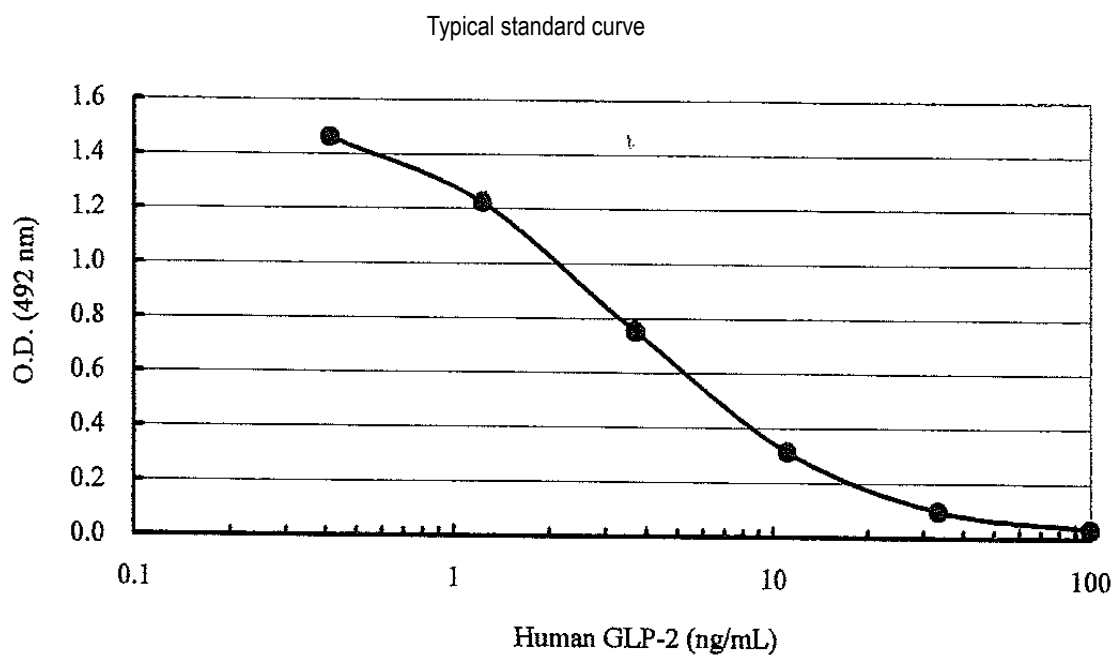
11. Add 100 μ L of stopping solution into each of the wells to stop color reaction.
12. Read optical absorbance of the solution in the wells at 492 nm. Calculate mean absorbance values of standard solutions and plot a standard curve on semi logarithmic graph paper (abscissa: concentration of standard solution; ordinate: absorbance value). Use the average absorbance of each sample to determine the corresponding value by simple interpolation from this standard curve.

5. Notes

1. EDTA-2Na additive blood collection tube is recommended for plasma sample collection. It is strongly recommended that plasma and serum samples should 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. Human GLP-2 standard, labeled antigen and substrate solution should be prepared immediately before use.
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. Pipetting operations may affect the precision of the assay. Pipette standard solution or sample into each well of the plate precisely. Use clean test tubes or vessels in assay, and new tip must be used for each standard solution or sample to avoid cross contamination.
5. When concentration of GLP-2 in a sample is expected to exceed 100 ng/mL, the sample needs to be diluted with buffer solution to appropriate concentration.
6. During incubation except the case at 4 °C and color reaction, the plate should be shaken gently with a microtiter plate shaker to promote immunoreaction.
7. Perform all the determination in duplicate.
8. Read optical absorbance of reaction solution in the wells immediately after stopping color reaction.
9. For accurate quantification, plot a standard curve for each assay.

10. Protect reagents from strong light (e.g. direct sunlight) during storage and assay.
11. Satisfactory performance of assay is guaranteed only when reagents in combination pack with identical lot number are used.

6. Performance Characteristics



Analytical recovery

Human plasma 1

Added human GLP-2 (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0	4.82		
2	6.10	6.82	89.4
5	7.60	9.82	77.4
10	14.77	14.82	99.7

Human plasma 2

Added human GLP-2 (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
	4.03		
2	5.19	6.03	86.1
5	6.96	9.03	77.1
10	13.85	14.03	98.7

Human serum 1

Added human GLP-2 (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0	3.16		
2	4.90	5.16	95.0
5	6.89	8.16	84.4
10	14.58	13.16	110.8

Human serum 2

Added human GLP-2 (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0	4.31		
2	5.21	6.31	82.6
5	7.14	9.31	76.7
10	14.07	14.31	98.3

Precision and reproducibility

	Human plasma	Human serum
Intra-assay CV (%)	3.7 – 4.8	3.0 – 5.5
Inter assay CV (%)	13.0 – 16.4	14.3 – 17.5

7. Stability and Storage

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

Shelf life The kit is stable under the condition for 9 months from the date of manufacturing
The expiry date is stated on the package.

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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