YK141　Human GLP-2 EIA

FOR LABORATORY USE ONLY

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Contents

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

YK141　Human GLP-2 EIA Kit

I. Introduction

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

YK141 Human GLP-2 EIA Kit

- ▼ The assay kit can measure human GLP-2 within the range of 0.412~100 ng/mL
- ▼ The assay is completes within 16-18 hr＋1.5 hr.
- ▼ With one assay kit, 41 samples can be measured in duplicate
- ▼ Test sample: human plasma and serum
  Sample volume: 25 μL
- ▼ The 96-well plate of this kit is consisted by 8-wells strips. The kit can be used separately.
- ▼ Precision and reproducibility
  (Human plasma)
  Intra-assay CV（%） 3.7~ 4.8
  Inter-assay CV（%） 13.0~16.4
  (Human serum)
  Intra-assay CV（%） 3.0 ~ 5.5
  Inter-assay CV（%） 14.3 ~ 17.5
- ▼ Stability and storage
  Store all the components at 2-8℃.
  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) Human GLP-2 standard
3) Labeled antigen
4) 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 human GLP-2 [both GLP-2 (1-33) and GLP-2 (3-33)] in plasma and serum 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, of the kit is a highly purified synthetic product. (purity: higher than 98%)

< Specificity >

This EIA kit is highly specific to human GLP-2 and shows crossreactivity to neither glucagon (rat/mouse/human) nor GLP-1 even at a concentration of 300 pmol/mL.

< Assay principle >

This EIA kit for determination of human GLP-2 in plasma and serum 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.

Ⅲ. Composition

	Component	Form	Quantity	Main Ingredient
1.	Antibody coated plate	Microtiter plate	1 plate (96 wells)	Goat anti rabbit IgG antibody
2.	Human GLP-2 standard	Lyophilized	1 vial (50ng)	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 SA
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_2SO_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 pieces	

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. Glass test tubes for preparation of standard solution
6. Graduated cylinder (1,000 mL)
7. Distilled or deionized water

< Preparatory work >

1. Preparation of standard solution:
   Reconstitute human GLP-2 standard (lyophilized, 50ng/vial) with 0.5mL 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 33.33ng/mL standard solution. Repeat the dilution procedure to make each of 11.11, 3.704, 1.235 and 0.412ng/mL standard solutions. Buffer solution itself is used as 0 ng/mL.
2. Preparation of labeled antigen solution:
   Reconstitute labeled antigen with 6 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 0.35mL/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 onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.

3. Fill 40μL of labeled antigen solution into the wells first, then introduce 25μL each of standard solutions (0, 0.412, 1.235, 3.704, 11.11, 33,33 and 100 ng/mL) or samples and finally add 50μL of GLP-2 antibody into the wells.

4. Cover the plate with adhesive foil and incubate it at 4ºC 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 onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.

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

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

8. Resolve one 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 onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.

10. Add 100μL of substrate solution into each the wells, cover the plate with adhesive foil and incubate it for 30 minutes at room temperature.

11. Add 100μL of stopping solution into each of the wells to stop color reaction.

12. Read optical absorbance of the solution in the wells at 490 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.

V. 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. This kit can be used dividedly in strips of the plate. In such case, the rest of reconstituted reagents (Human GLP-2 standard and Labeled antigen) should be stored below -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 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.

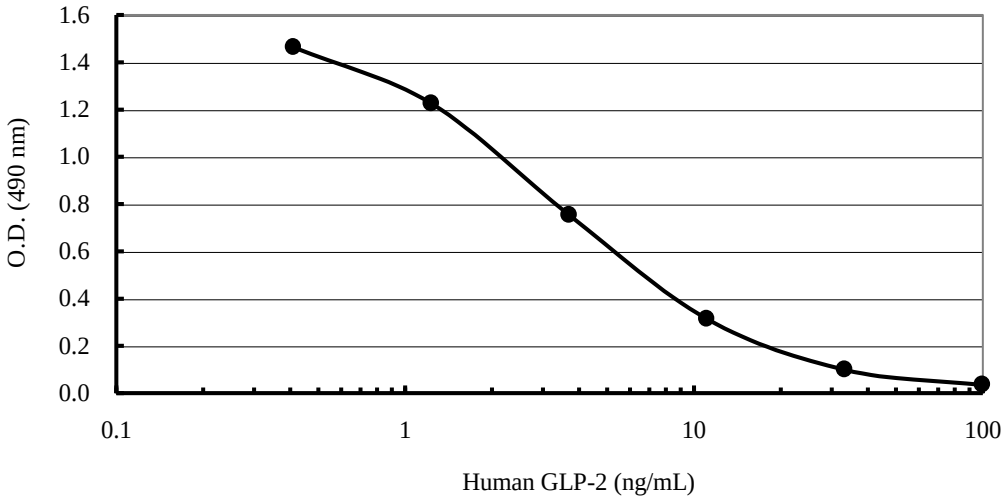
9. For accurate quantification, plot a standard curve for each assay.

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.

Ⅵ. Performance Characteristics

Typical standard curve



<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

Ⅶ. Stability and Storage

< Storage > Store all 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**: 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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