YK141　Human 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(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.

II. Characteristics

This EIA kit is used for quantitative determination of human GLP-2 in 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.
Human GLP-2 standard is highly purified synthetic product.

< Specificity >
The EIA kit has high specificity to human GLP-2 and shows no cross reactivity with glucagon and GLP-1 within the range of 300 pmol/mL.

< Test Principle >
This EIA kit for determination of human GLP-2 in plasma samples is based on a competitive enzyme immunoassay using combination with highly specific antibody to rat GLP-2(using high cross reactivity between rat and human GLP-2) and biotin – avidin affinity system. The 96 wells plate is coated with goat anti rabbit IgG antibody. Human GLP-2 standard or samples, biotinylated GLP-2 and anti rat GLP-2 polyclonal antibody are added to the wells for competitive immunoreaction. After rinsing out excess human GLP-2, HRP labeled streptoavidins are added to bind to the antigen-antibody complex so that HRP labeled streptoavidin – biotinylated 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 human 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. Human GLP-2 standard	lyophilized	1 vial	Synthetic human GLP-2(50ng/vial)
3. Labeled antigen	lyophilized	1 vial	Biotinylated rat GLP-2
4. GLP-2 Antibody	liquid	1 bottle (6 mL)	Rabbit anti rat GLP-2
5. SA-HRP solution	liquid	1 tube (0.2 mL)	HRP labeled streptoavidin
6. Diluent for SA-HRP solution	liquid	1 bottle (12 mL)	Phosphate bufffer
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 (25 mL)	Phosphate buffer
11. Washing solution (concentrated)	Liquid	1 bottle (50 mL)	Concentrated saline
12. Adhesive foil		3 sheets	

MTP*1 ….. Microtitration plate

IV. Method

< Euipment required >

1)Photometer for microtitration plate(Plate reader)which can read extinction 2.5 at 492 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 human GLP-2 50ng/vial) with 0.5mL of Buffer solution, which affords 100 ng/mL standard solution. 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 6 mL of Buffer solution.

3) Preparation of diluted SA-HRP solution
Add 120μL of SA-HRP solution into the bottle of Diluent for SA-HRP solution and mix well.

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 0.35mL/well of washing solution into the wells and aspirate the washing solution in the wells. Repeat this washing procedure further twice(total 3 times washing).

3.Fill 40μL 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 GLP-2Antibody 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 0.35 mL/well of washing solution.

6.Pipette 100μL of SA-HRP 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 0.35 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 human GLP-2 concentrations in samples from the corresponding absorbance values.

V. 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℃. Avoid repeated freezing and thawing of plasma 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℃.

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

4.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.

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 rotated gently by plate rotator 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 the determination in duplicate.

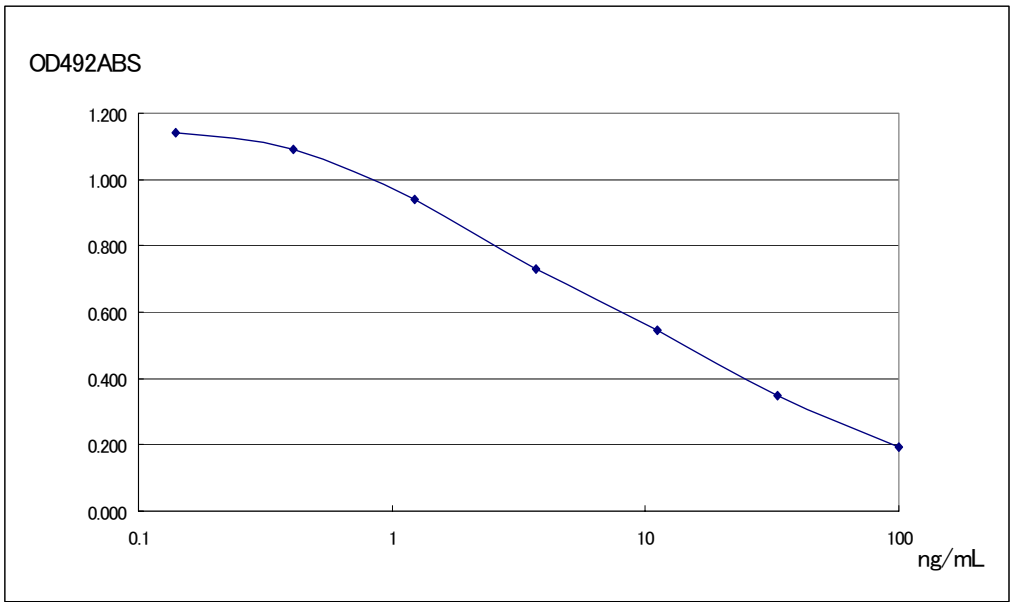
10.To quantitate accurately, always run a standard curve when testing samples.

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.

VI. Performance Characteristics

Typical standard curve



Analytical recovery

< Human plasma 1 >

Sample No.	Rat GLP-2 added ng/mL	Observed ng/mL	Expected ng/mL	Recovery %
1	0	2.02		
2	1	3.02	3.02	100.0
3	3	5.58	5.02	111.2
4	6	8.34	8.02	104.0

< Human plasma 2 >

Sample No.	Rat GLP-2 added ng/mL	Observed ng/mL	Expected ng/mL	Recovery %
1	0	1.78		
2	1	2.75	2.78	98.9
3	3	5.15	4.78	107.7
4	6	7.82	7.78	100.5

Precision and reproducibility

- Intra-assay (Human plasma) CV(%) 2.9 ～ 6.8
- Inter-assay (Human plasma) CV(%) 10.3 ～ 14.4

Assay range

0.137 ~ 100 ng/mL

VII. Stability and Storage

< Storage > Store all of the components at 2 to 8℃.

< Shelf life > 9 month from the date of manufacturing
The expiry date is described on the label of kit.

< Package > For 96 tests per 1 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