YK160 GLP-1 EIA Kit

For Measurement of Rat, Mouse & Human GLP-1

FOR RESEARCH USE ONLY

＜Distributed by＞

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

YK160 **GLP-1 EIA Kit**

I. Introduction

GLP-1 is a peptide hormone from the intestinal mucosa, which is produced from its precursor, proglucagon by post transnational processing. The mammalian proglucagon [1)] is synthesized in the neuroendocrine L-cell of the intestine and the alpha-cells of the pancreas. It contains within its structure the sequences of glucagon and two glucagon-like peptides (GLP-1 and GLP-2) in tandem flanked at their amino and carboxyl termini by dibasic residues. GLP-1 is a 37 amino acids peptide and produced in the small intestine and in the pancreas in the human, in either C-terminal-amidated on glycine-extended form[2) 3)].

GLP1 (7-36) amide and its receptor are present in several brain regions and may play a role in the physiological control of feeding[4)]. Several reports have been presented as follows as to the biological activities of GLP-1. GLP-1 (7-37) and (7-36) amide is known as one of the most potent insulin secretagogues [5)].

GLP-1 (7-36) amide was supposed to improved glycemic control in patients with type 2 diabetes by increasing insulin secretion, by inhibiting glucagon secretion and by delaying gastric emptying rather than by altering extrapancreatic glucose metabolism[6)]. Intravenous GLP-1(7-37) and (7-36)amide could normalize fasting hyperglycaemia in type 2 diabetic patients[7)]. Hyperglycaemia during parenteral nutrition could be controlled by exogenous GLP-1, whereas the chronic therapy of type 2 diabetes required GLP-1 derivatives with longer duration of action [8)]. Recombinant GLP-1 (7-36) amide was recently shown to cause significant weight loss in type 2 diabetics when administered for 6 weeks as a continuous subcutaneous infusion, 5-day treatment of hereby obese human subjects with GLP-1 at high doses by prandial subcutaneous infusion promptly slowed gastric emptying as a probable mechanism of action of increased satiety, decreased hunger and reduced food intake with an ensuing weight loss[9)].

A G-protein-coupled receptor, GPR120, which is abundantly expressed in intestine, functions as a receptor for unsaturated long-chain FFAs (free fatty acids). The stimulation of GPR120 by FFAs promotes the secretion of GLP-1 *in vitro* (measured by YK160, Yanaihara Institute Inc) and *in vivo*, and increases circulation insulin, indicate that GPR120-mediated GLP-1 secretion induced by dietary FFAs is important in the treatment of diabetes[10)].

All these approaches have shown remarkable efficacy in both experimental and clinical studies. The GLP-1-based therapy of type 2 diabetes, therefore, represents a new and attractive alternative[11)].

Yanaihara Institute Inc. developed a quantitative EIA kit with high specificity and sensitivity (detection limit 0.206ng/mL) for rat/mouse/human GLP-1 (YK160) as a useful tool for these necessaries.

YK160 GLP-1 EIA Kit

- ▼ The assay kit can measure Rat, Mouse&Human GLP-1 in the range of 0.206 - 50 ng/mL.
- ▼ The assay completes within 16-18 hr.＋1.5 hr.
- ▼ With one assay kit, 41 samples can be measured in duplicate.
- ▼ Test sample: plasma (rat/mouse/human)
  Sample volume: 30 μL
- ▼ The 96-well plate in kit was consisted by 8-wells strips. The kit can be used separately.
- ▼ Rat plasma measurement
  Intra-assay CV（%） 5.36 - 6.60
  Inter-assay CV（%） 5.51 -18.87
- ▼ Human plasma measurement
  Intra-assay CV（%） 4.69 -10.67
  Inter-assay CV（%） 9.63 - 17.57
- ▼ Stability and Storage
  Store all of the components at 2-8℃.
  12 months from the date of manufacturing.
  The expiry date is described on the label of kit.

Contents

1) Antibody coated plate
2) GLP-1 standard
3) Labeled antigen
4) GLP-1 antibody
5) SA-HRP
6) Diluent for SA-HRP
7) Substrate buffer
8) OPD tablet
9) Stopping solution
10) Buffer solution
11) Washing solution (concentrated)
12) Adhesive foil

II. Characteristics

This EIA kit is used for quantitative determination of rat/mouse/human GLP-1 in plasma samples. The kit is characterized for sensitive quantification, high specificity and no influence with other components in plasma samples. GLP-1 standard is highly purified synthetic product.

< Specificity >

The EIA kit has high specificity to rat/mouse/human GLP-1 and shows cross reactivity neither rat/human/mouse glucagons, human glicentin nor rat/mouse/human GLP-2.

< Test Principle >

This EIA kit for determination of rat/mouse/human GLP-1 in plasma samples is based on a competitive enzyme immunoassay using combination of highly specific antibody to GLP-1 (7-36) amide with biotin-avidin affinity system. The 96 wells plate is coated with goat anti rabbit IgG antibody. GLP-1 standard or samples, labeled antigen and GLP-1 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 GLP-1-antibody complex on the surface of the wells. Finally, HRP enzyme activity is determined by o-Phenylenediamine dihydrochloride (OPD) and the concentration of GLP-1 is calculated.

III. Composition

Component	Form	Quantity	Main Ingredient
1. Antibody coated plate	MTP[*1]	1 plate (96 wells)	Goat anti rabbit IgG
2. GLP-1 standard	lyophilized	1 vial	Synthetic GLP-1 (7-36) amide (25ng/vial)
3. Labeled antigen	lyophilized	1 vial	Biotinylated GLP-1 (7-36) amide
4. GLP-1 antibody	liquid	1 bottle (6 mL)	Rabbit anti-GLP-1 (7-36) amide
5. SA-HRP	liquid	1 tube (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 dihydrochloride
9. Stopping solution	liquid	1 bottle (12 mL)	2N H_2SO_4
10. Buffer solution	liquid	1 bottle (10 mL)	Phosphate buffer
11. Washing solution (Concentrated)	Liquid	1 bottle (50 mL)	Concentrated saline
12. 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 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) 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 GLP-1 standard with 0.5mL of buffer solution, which affords 50ng/mL standard solution. The 0.1ml of the reconstituted standard solution is diluted with 0.2 mL of buffer solution that yields 16.67ng/mL standard solution. Repeat the same dilution to make each standard of 5.556, 1.852, 0.617, 0.206ng/mL. Buffer solution is used as 0ng/mL.

2) Preparation of labeled antigen:
Reconstitute labeled antigen with 6 mL of distilled water.

3) Preparation of SA-HRP solution
Add 120μL of SA-HRP into the bottle of diluent for SA-HRP 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. Before beginning the test bring all the reagents and samples to room temperature.

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).

3. Fill 40μL of labeled antigen solution into the wells first, then introduce 30μL of each of standard solutions (0, 0.206, 0.617, 1.852, 5.556, 16,67, 50ng/mL) or samples and finally add 40μL of GLP-1 antibody into the wells.

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

5. Take off the adhesive foil, aspirate the solution in the wells and wash the wells 4 times with approximately 0.35 mL/well of washing solution.

6. Add 120μL of SA-HRP into the bottle of diluent for SA-HRP and mix well.

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

8. 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.

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

10. Take off the adhesive foil, aspirate and wash the wells 5 times with approximately 0.35 mL/well of washing solution.

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

12. Add 100μL of stopping solution into the wells to stop reaction.

13. Read the optical absorbance of the wells at 492nm. 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 GLP-1 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 tested later, 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. GLP-1 standard, labeled antigen, SA-HRP solution and substrate solution should be prepared immediately before use. Using clean test tubes or vessels in assay. Diluted washing solution is stable for 6 months at 2-8℃.

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

4. Pipetting operations may affect the precision of the assay, pipette standard solutions or samples precisely into each well of plate. In addition, use new tip for each sample to avoid cross contamination.

5. When sample value exceeds 50 ng/mL, it needs to be diluted with buffer solution to proper concentration.

6. During incubation except 4℃ incubation and color reaction, the test plate should be shake gently by plate shaker to promote immunoreaction.

7. During continuous shaking of test plate, the plate shaker may be heated up. It is recommended to place styrene foam or plywood between the plate and the shaker.

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.

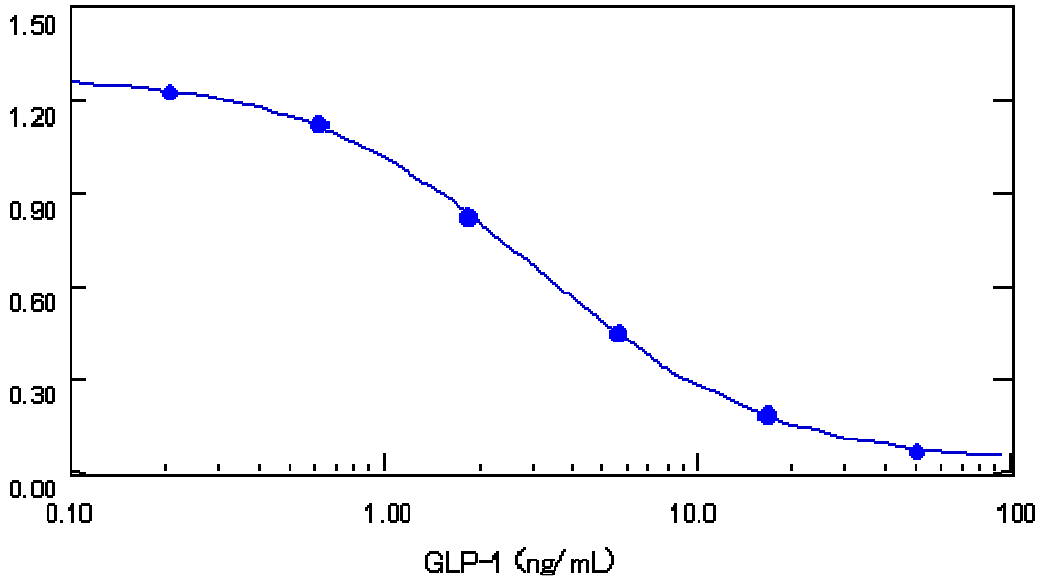
10. To quantitate accurately, always run a standard curve when testing samples.

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

< Rat plasma >

Add GLP-1 (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0	0.66		
0.5	1.28	1.16	110.4
2.0	2.73	2.66	102.6
8.0	7.72	8.66	89.20

< Human plasma >

Add GLP-1 (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0	0.66		
0.5	1.18	1.16	101.7
2.0	2.60	2.66	97.7
8.0	7.45	8.66	86.0

Precision and reproducibility

Rat plasma

- Intra-assay CV(%) 5.36 ～ 6.60
- Inter-assay CV(%) 5.51 ～ 18.87

Human plasma

- Intra-assay CV(%) 4.69 ～ 10.67
- Inter-assay CV(%) 9.63 ～ 17.57

Assay range

0.206 ~ 50 ng/mL

VII. Stability and Storage

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

< Shelf life > 12 months from the date of manufacturing
The expiry date is described on the label of kit.

< Package > For 96 tests per one kit including standards

VIII. References

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