

YK160 GLP- 1 EIA Kit

For Measurement of Rat, Mouse & Human GLP-1

FOR LABORATORY USE ONLY

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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 translational processing. The mammalian proglucagon¹⁾ 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 or glycine-extended form²⁾³⁾.

GLP1 (7-36) amide and its receptor are present in several brain regions and may play a role in the physiological control of feeding⁴⁾. 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 secretagogues⁵⁾.

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 metabolism⁶⁾. Intravenous GLP-1 (7-37) and (7-36) amide could normalize fasting hyperglycaemia in type 2 diabetic patients⁷⁾. 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 action⁸⁾. 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 loss⁹⁾.

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 diabetes¹⁰⁾.

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 alternative¹¹⁾.

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 necessities.

| YK160 GLP-1 EIA Kit | Contents |
|--|---|
| <p>This assay kit can measure Rat, Mouse&Human GLP-1 within the range of 0.206~50 ng/mL. The assay is completed within 16~18 hr.+1.5 hr. With one assay kit, 41 samples can be measured in duplicate.</p> | <ol style="list-style-type: none"> 1) Antibody coated plate 2) GLP-1 standard 3) Labeled antigen 4) GLP-1 antibody 5) SA-HRP |
| <p>Test sample: plasma (rat/mouse/human) Sample volume: 30 μL The 96-well plate in kit is consisted by 8-wells strips. The kit can be used separately.</p> | <ol style="list-style-type: none"> 6) Diluent for SA-HRP 7) Substrate buffer 8) OPD tablet 9) Stopping solution |
| <p>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</p> | <ol style="list-style-type: none"> 10) Buffer solution 11) Washing solution (concentrated) 12) Adhesive foil |
| <p>Stability and Storage Store all of the components at 2~8°C. This kit is stable under the condition for 12 months from the date of manufacturing. The expiry date is stated on the package.</p> | |

II. Characteristics

This EIA kit is used for quantitative determination of rat/mouse/human GLP-1 in plasma samples. The kit is characterized by sensitive quantification and high specificity. In addition, it is not influenced by the other constituents in samples. GLP-1 standard of this kit is a highly purified synthetic product.

< Specificity >

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

< Assay 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 and biotin-avidin affinity system. To the well of the plate coated with goat anti rabbit IgG, GLP-1 standard or samples, labeled antigen and GLP-1 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-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 | Microtiter plate | 1 plate (96 wells) | Goat anti rabbit IgG antibody |
| 2. GLP-1 standard | Lyophilized | 1 vial (25 ng) | Synthetic GLP-1 (7-36) amide |
| 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 antibody |
| 5. SA-HRP | Liquid | 1 tube (0.2 mL) | HRP labeled SA |
| 6. Diluent for SA-HRP | Liquid | 1 bottle (12 mL) | Phosphate buffer |
| 7. Substrate buffer | Liquid | 1 bottle (26 mL) | Citrate buffer containing 0.015% hydrogen peroxide |
| 8. OPD tablet | Tablet | 2 tablets | o-Phenylenediamine dihydrochloride |
| 9. Stopping solution | Liquid | 1 bottle (12 mL) | 1M H ₂ SO 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 pieces | |

. 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. 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 the GLP-1 standard with 0.5 mL of buffer solution, which affords 50 ng/mL standard solution. The reconstituted standard solution (0.1 ml) is diluted with 0.2 mL of buffer solution, which yields 16.67 ng/mL standard solution. Repeat the dilution procedure to make each of 5.556, 1.852, 0.617 and 0.206 ng/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 distilled or deionized water.

3. Preparation of SA-HRP solution

The kit can be used twice separately by user's option. There are two methods to dilute SA-HRP. It should be prepared immediately before use.

. Using kit twice:

Prepare the necessary volume of SA-HRP solution for completing the assay. SA-HRP solution should be prepared 100-fold dilution by diluent for SA-HRP. For example, if assay needs 5.6 mL of SA-HRP solution, SA-HRP 0.056 mL should be diluted with 5.6 mL of diluent for SA-HRP in a clean vessel. The rest of SA-HRP and diluent for SA-HRP should be stored at 2-8°C separately until next use. SA-HRP solution should be prepared immediately before use.

. Using kit only once:

Add 120 μ L of SA-HRP into the bottle of diluent for SA-HRP and mix well. Diluted SA-HRP solution should be used only once.

4. Preparation of substrate solution:

Resolve one 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 >

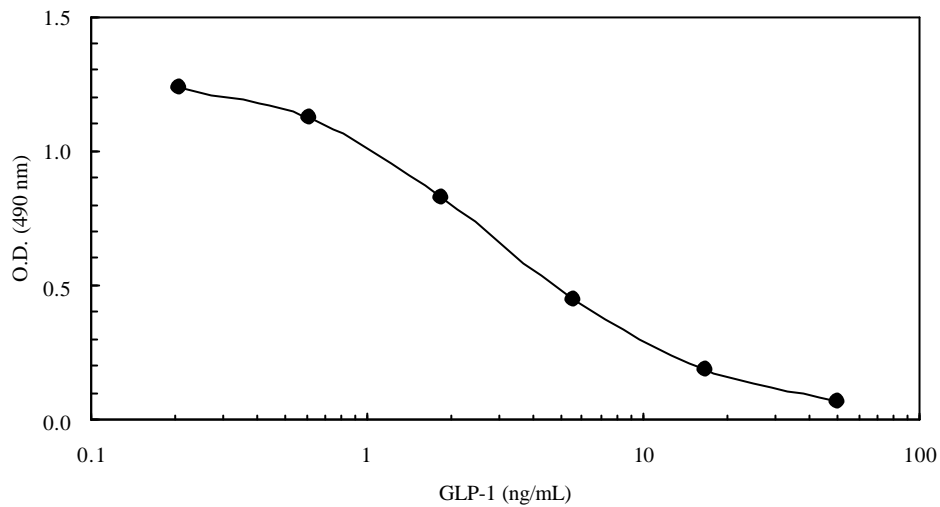
1. Bring all the reagents and samples to room temperature (20~30°C) at least 1 hour before starting assay.
2. Add 0.35 mL/well of washing solution into the wells and aspirate the washing solution in the wells. Repeat this washing procedure further twice (total 3 times). Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.
3. Fill 40 μ L of labeled antigen solution into the wells first, then introduce 30 μ L of each of standard solutions (0, 0.206, 0.617, 1.852, 5.556, 16,67, 50 ng/mL) or samples and finally add 40 μ L of GLP-1 antibody into the wells.
4. Cover the plate with adhesive foil and incubate it at 4°C 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. 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. Prepare the necessary volume of SA-HRP solution for completing the assay.
7. Pipette 100 μ L of SA-HRP solution into the wells.
8. 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.
9. Resolve one 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. Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.
11. Add 100 μ L of substrate solution containing OPD into the wells cover the plate with adhesive foil and keep it for 30 minutes at room temperature for color reaction.
12. Add 100 μ L of stopping solution into the wells to stop color reaction.
13. 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 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. GLP-1 standard, labeled antigen, SA-HRP solution and substrate solution should be prepared immediately before use.
3. During storage of washing solution (concentrated) at $2\sim 8^{\circ}\text{C}$, 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 into each well of the plate precisely. Use clean test tubes or vessels in assay, and new tip must be used for each standard and sample solution to avoid cross contamination.
5. This kit can be used dividedly in strips of the plate. In such case, the rest of reconstituted reagents (standard and labeled antigen solutions) should be stored at $2\sim 8^{\circ}\text{C}$, and used within 2 weeks, or stored at or below -30°C , and used within 1 month. The rest of SA-HRP and diluent for SA-HRP should be stored at $2\sim 8^{\circ}\text{C}$ separately until next use. SA-HRP solution should be prepared immediately before use.
6. When concentration of GLP-1 in samples is expected to exceed 50 ng/mL , the sample needs to be diluted with buffer solution to proper concentration.
7. During incubation except the case at 4°C incubation and color reaction, the plate should be shaken gently with a microtiter plate shaker to promote immunoreaction.
8. Read optical absorbance of reaction solution in the wells immediately after stopping color reaction.
9. Perform all the determination in duplicate.
10. For accurate quantification, plot a standard curve for each assay.
11. Protect reagents from strong light (e.g. direct sunlight) during storage and assay.
12. Satisfactory performance of the assay is guaranteed only when reagents in combination pack with identical lot number are used.

. Performance Characteristics

A typical standard curve



Analytical recovery

< Rat plasma >

| Added 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 >

| Added 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 | Human plasma |
|--------------------|--------------|--------------|
| Intra-assay CV (%) | 5.36 ~ 6.60 | 4.69 ~ 10.67 |
| Inter-assay CV (%) | 5.51 ~ 18.87 | 9.63 ~ 17.57 |

Crossreactivity

| Related peptides | Crossreactivity (%) |
|--------------------|---------------------|
| GLP-1 (7-36) amide | 100% |
| GLP-1 (9-36) amide | 100% |
| GLP-1 (1-36) amide | 0.3% |
| GLP-1 (1-37) | < 0.1% |
| GLP-1 (7-37) | < 0.1% |

. Stability and Storage

- < Storage > Store all of the components at 2~8°C.
- < Shelf life > The kit is stable under the condition for 12 months from the date of manufacturing.
The expiry date is stated on the package.
- < Package > For 96 tests per one kit.

. References

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Update at May 28, 2009