



GLP-1 EIA Kit For Measurement of Rat, Mouse & Human GLP-1

Cat. No. YII-YK160

Price: 682 Euro/96 well
2YK160 GLP-1 EIA Kit.

Introduction

GLP-1 is a peptide hormone from the intestinal mucosa, which is produced from its precursor, proglucagon by posttranslational processing. The mammalian proglucagon 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 form^{1,2,3}. GLP-1 (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 improve 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 0.206 ng/mL) for rat/mouse/human GLP-1 (YK160) as a useful tool for these necessities.

YK160 GLP-1 EIA Kit Contents

The assay kit can measure Rat, Mouse & Human¹ 1) Antibody coated plate GLP-1 in the range of 0.206 - 50 ng/mL. 2) GLP-1 standard The assay completes within 16-18 hr. 1.5 hr. 3) Labeled antigen With one assay kit, 41 samples can be measured 4) GLP-1 antibody in duplicate. 5) SA-HRP 6) Diluent for SA-HRP Test sample: plasma (rat/mouse/human) Sample volume: 30 µL 7) Substrate buffer The 96-well plate in kit was consisted by 8) OPD tablet 9) Stopping solution 8-wells strips. The kit can be used separately. 10) Buffer solution 11) Washing solution (concentrated) Rat plasma measurement Intra-assay CV 5.36 - 6.60 Inter-assay CV 5.51 - 18.87 12) Adhesive foil 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 °C 12 months from the date of manufacturing. The expiry date is described on the label of kit. 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 streptavidin (SA-HRP) are added to form HRP labeled streptavidin-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.

4III. Composition

Component Form Quantity Main Ingredient

1. Antibody coated plate MTP*11 plate (96 wells) Goat anti-rabbit IgG2. 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 (6mL) Rabbit anti-GLP-1(7-36)amide 5. SA-HRP liquid 1 tube (0.2mL) HRP labeled streptavidin 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 2 tablets-Phenylenediamine dihydrochloride 9. Stopping solution liquid 1 bottle (12 mL) 2N H₂SO₄ 10. Buffer solution liquid 1 bottle (10mL) Phosphate buffer 11. Washing solution liquid 1 bottle (50 mL) Concentrated saline (Concentrated) 12. Adhesive foil 3 sheets MTP*1..... Microtiter plate

5IV. 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 120 L 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.

6 < 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 40 L of labeled antigen solution into the wells first, then introduce 30 L of each of standard solutions (0, 0.206, 0.617, 1.852, 5.556, 16.67, 50ng/mL) or samples and finally add 40 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. 6. Add 120 L of SA-HRP into the bottle of diluent for SA-HRP and mix well. 7. Pipette 100 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 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 0.35mL/well of washing solution. 11. Add 100 L of substrate solution into the wells, cover the plate with adhesive foil and incubate it for 30 minutes at room temperature. 12. Add 100 L 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.

7V. 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°C. 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°C. 3. During storage of washing solution (concentrated) at 2-8°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 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 4incubation 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.

8VI.

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
 CV5.366.60 Inter-assay CV5.5118.87 Human plasma Intra-assay CV4.6910.67 Inter-assay CV9.6317.57 Assay
 range0.206 50 ng/mL

9VII.

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. References1. Heinrich, G. (1984) Glucagon gene sequence. Four of six exons encode separate functional domains of rat pre-proglucagon. J. Biol. Chem.,259, 14082-14087 2. Orskov, C. (1994) Tissue and plasma concentrations of amidated and glycine-extended glucagon-like peptide I in humans. Diabetes.,43, 535-539 3. Yanaihara N. (1990) Recent advances in gastrointestinal hormones (article in Japanese).Hum. Cell.,3, 1-8 4. Asarian, L. (1998) Intracerebroventricular glucagons-like peptide-1 (7-36)amide inhibits sham feeding in rats without eliciting satiety, Physiol. Behav.,64, 367-372 5. Mojsov, S. (1990) Both amidated and nonamidated forms of glucagon-like peptide I are synthesized in the rat intestine and the pancreas. J. Biol. Chem.,265,8001-80086.Vella, A. (2000) Effect of glucagon-like peptide1 (7-36)amide on glucose effectiveness and insulin action in people with type 2 diabetes. Diabetes.,49,611-6177. Nauck, MA. (1998) Normalization of fasting glycaemia by intravenous GLP-1 ([7-36 amide] or [7-37]) in type 2 diabetic patients. Diabet. Med.,15, 937-945 8. Nauck, MA. (2004) Blood glucose control in healthy subject and patients receiving intravenous glucose infusion or total parenteral nutrition using glucagon-like peptide 1. Regul. Pept., 118, 89-97 9. Naslund, E. (2004) Prandial subcutaneous injections of glucagon-like peptide-1 cause weight loss in obese human subjects. Br. J. Nutr.,91, 439-44610.Akira H. (2005) Free fatty acids regulate gut incretin glucagon-ike peptide-1 secretion through GRP120. Nature. Med.,11, 90-9411.Holst, JJ. (2002) Therapy of type 2 diabetes mellitus based on the actions ofglucagon-like peptide-1 Diabetes Metab. Res. Rev.,18, 430-441