KAMIYA BIOMEDICAL COMPANY

Rat, Mouse and Human GLP-1 EIA

For the quantitative determination of GLP-1 in rat, mouse and human plasma.

Cat. No. KT-384

For Research Use Only. Not for Use in Diagnostic Procedures.

PRODUCT INFORMATION

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INTENDED USE
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INTRODUCTION
GLP-1 is a peptide hormone from the intestinal mucosa, which is produced from its precursor, proglucagon, by post-translational 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 acid peptide and is produced in the human small intestine and pancreas, in either C-terminal-amidated or glycine-extended form.

GLP-1 (7-36) amide and its receptor are present in several brain regions and may play a role in the physiological control of feeding. 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.

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 draining rather than by altering extrapancreatic glucose metabolism. Intravenous GLP-1 (7-37) and (7-36) amide could normalize fasting hyperglycemia in type 2 diabetic patients. Hyperglycemia 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. 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.

A G-protein-coupled receptor, GPR120, which is abundantly expressed in the 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 KT-384) 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.

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. Advantages of this assay include high sensitivity, high specificity and no interference from other components in plasma samples. The GLP-1 calibrator of this kit is a highly purified synthetic product.

PRINCIPLE
This EIA kit is based on a competitive enzyme immunoassay using a highly specific antibody combined with a biotin-avidin affinity system. The 96 well plate is coated with goat anti-rabbit IgG and GLP-1 Calibrators or samples, biotinylated human GLP-1 and GLP-1 antibody are added to the wells for a competitive immuno-reaction. After incubation and rinsing excess GLP-1, HRP-labeled streptoavidins (SA-HRP) are added to bind to the antigen-antibody complex so that HRP-labeled streptoavidin-biotinylated GLP-1-antibody complexes are formed 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.

COMPONENTS

Component	Form	Quantity	Main Ingredient
1. Antibody-Coated Plate	MTP[*1]	1 plate (96-well)	Goat Anti-Rabbit IgG
2. GLP-1 Calibrator	Lyophilized	1 vial (25 ng/vial)	Synthetic GLP-1 (7-36) amide
3. Labeled Antigen	Lyophilized	1 vial	Biotinylated GLP-1 (7-36) amide
4. GLP-1 Antibody	Liquid	1 vial (6 mL)	Rabbit anti-GLP-1 (7-36) amide
5. SA-HRP	Liquid	1 tube (0.2 mL)	HRP-Labeled Streptoavidin
6. SA-HRP Diluent	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. Stop Solution	Liquid	1 bottle (12 mL)	1M H_2SO_4
10. Buffer Solution	Liquid	1 bottle (10 mL)	Phosphate buffer
11. Wash Solution Concentrate	Liquid	1 bottle (50 mL)	Concentrated saline
12. Plate Seal		3 sheets	

MTP[*1] ..... Microtiter plate

MATERIALS REQUIRED BUT NOT PROVIDED

- Photometer for microtiter plate (plate reader), which can read absorbance up to 2.5 at 490 nm
- Rotator for microtiter plate
- Washing device for microtiter plate and dispenser with an aspiration system
- Micropipettes, multi-channel pipettes for 8 wells or 12 wells and their tips
- Test tubes for preparation of Calibrator Solution
- Graduated cylinder (1,000 mL)
- Distilled water or de-ionized water

PRECAUTIONS

Protect reagents from strong light (e.g. direct sunlight) during storage and assay.

Satisfactory performance of the test is guaranteed only when reagents are used from a kit with identical lot number.

As pipetting operations may affect the precision of the assay, precisely pipette the prepared Calibrator Solutions or samples into corresponding wells. Use a new tip for each sample and calibrator to avoid cross-contamination. Use clean test tubes or vessels.

Always run a calibration curve when testing samples.

REAGENT PREPARATION

1. Preparation of Calibrator Solutions: Reconstitute the GLP-1 Calibrator (lyophilized GLP-1, 25 ng/vial) with 0.5 mL of Buffer Solution, giving a 50 ng/mL Calibrator Solution after reconstitution. 0.1 mL of the reconstituted Calibrator Solution is diluted with 0.2 mL of Buffer Solution to yield a 16.67 ng/mL Calibrator Solution. Repeat the serial dilution to make Calibrator Solutions at 5.556, 1.852, 0.617, 0.206 ng/mL. Buffer Solution is used as the zero calibrator (0 ng/mL).

   Note: Calibrator Solution must be prepared immediately before assay. Use clean test tubes or vessels.

2. Preparation of Labeled Antigen: Reconstitute the Labeled Antigen with 6 mL of distilled water.

   Note: Labeled Antigen must be prepared immediately before assay. Use clean test tubes or vessels.

3. Preparation of SA-HRP Solution: Add 120 µL of SA-HRP into the bottle of SA-HRP Diluent and mix well.

   Note: Diluted SA-HRP Solution must be prepared immediately before assay. Use clean test tubes or vessels.

4. Preparation of Substrate Solution: Dissolve one OPD Tablet in 12 mL of Substrate Buffer.

Note: Substrate Solution must be prepared immediately before assay. Use clean test tubes or vessels.

5. Preparation of Wash Solution: Dilute 50 mL of Wash Solution Concentrate to 1,000 mL with distilled or de-ionized water. Diluted Wash Solution is stable for 6 months at 4°C.

   Note: During storage of the Wash Solution Concentrate at 4°C, precipitates may be observed, however, they will dissolve when diluted.

6. Other reagents are ready for use.

STORAGE

Store kit at 4°C. This kit can be used dividedly in strips of the plate. In such case, the rest of reconstituted reagents (calibrator and labeled antigen solutions) should be stored at 4°C and used within 2 weeks, or stored at or below -30°C and used within 1 month.

SPECIMEN COLLECTION AND HANDLING

EDTA-2Na additive blood collection tube is recommended for plasma sample collection. 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 amounts and frozen at -30℃ or below. Avoid repeated freeze/thaw cycles.

ASSAY PROTOCOL

1. Warm the reagents and samples to room temperature (20-30°C) at least 1 hour before beginning the test.
2. Add 350 µL/well of diluted Wash Solution into the wells and aspirate the Washing Solution. Repeat this washing procedure twice, for a total of 3 washing steps. 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. Add 40 µL of Labeled Antigen Solution into wells. Then add 30 µL of the prepared Calibrator Solutions (0, 0.206, 0.617, 1.852, 5.556, 16.67, 50 ng/mL) or samples. Next, add 40 µL of GLP-1 Antibody into the wells.
4. Cover the plate with a Plate Seal and incubate at 4°C overnight (16-18 hours). Plate rotator is not needed for this incubation.
5. Remove the Plate Seal and aspirate the solution in the wells. Wash the wells 4 times with approximately 0.35 mL/well of diluted Wash 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 100 µL of diluted SA-HRP Solution into each of the wells.
7. Cover the plate with a Plate Seal and incubate at room temperature for 1 hour. During the incubation, the plate should be rotated on a plate rotator.
8. Remove the Plate Seal, aspirate and wash the wells 5 times with approximately 0.35 mL/well of diluted Wash 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.
9. Add 100 µL of Substrate Solution (dissolved OPD tablet) into the wells, cover the plate with a Plate Seal and incubate for 30 minutes at room temperature.
10. Add 100 µL of Stop Solution into the wells to stop the reaction.
11. Read the optical absorbance of the wells at 490 nm. The optical absorbance of reaction solution in wells should be read as soon as possible after stopping the color reaction.

Note: Perform all determinations in duplicate.

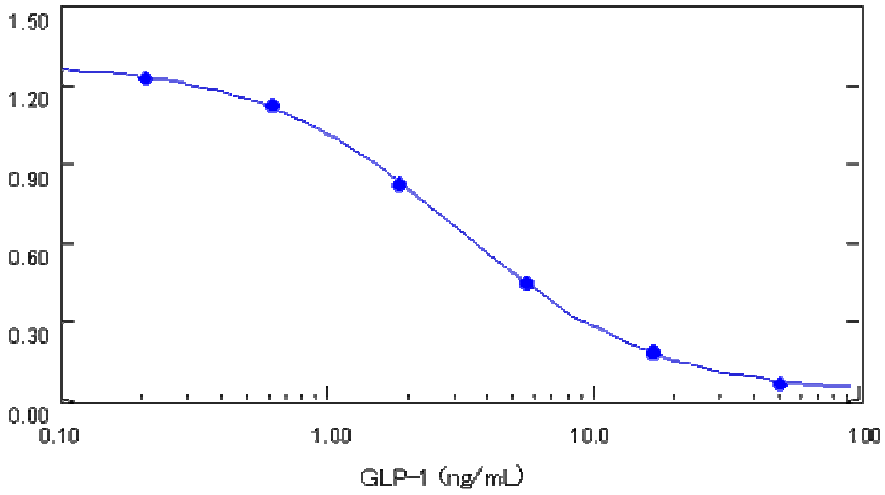
RESULTS

Calculate mean absorbance values of wells containing the Calibrators and plot a calibration curve on semilogarithmic graph paper (abscissa: concentration of Calibrators; ordinate: absorbance values of Calibrators). Use the calibration curve to read GLP-1 concentrations in samples from the corresponding absorbance values.

When a sample value exceeds 50 ng/mL, it must be diluted with Buffer Solution and re-assayed until the sample value is within the assay range.

PERFORMANCE

Typical Calibration Curve (example only, a new calibration curve for each run must be established by the end-user)



Analytical Recovery

Sample	GLP-1 Added (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
Rat Plasma	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	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

Cross-Reactivity

Related Peptides	Cross-reactivity (%)
GLP-1 (1-37)	<0.1%
GLP-1 (1-36) amide	0.3%
GLP-1 (7-37)	<0.1%
GLP-1 (7-36) amide	100%
GLP-1 (9-36) amide	100%

Precision and reproducibility

- Intra-assay CV (%) Rat Plasma 5.36 – 6.60
  Human Plasma 4.69 – 10.67
- Inter-assay CV (%) Rat Plasma 5.51 – 18.87
  Human Plama 9.63 – 17.57

Assay Range
0.206 – 50 ng/mL

Cross-Reactivity
No cross-reactivity with rat, human or mouse glucagons, human glicentin and rat, mouse, human GLP-2.

FOR RESEARCH USE ONLY

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