KAMIYA BIOMEDICAL COMPANY

Rat GLP-2 EIA

For the quantitative determination of GLP-2 in rat serum or plasma.

Cat. No. KT-373

For Research Use Only.

PRODUCT INFORMATION

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INTENDED USE
The Rat GLP-2 (Glucagon-Like Peptide-2) EIA is for the quantitative determination of GLP-2 in rat serum or plasma. For research use only.

INTRODUCTION
The proglucagon gene is expressed in both pancreatic A cells and intestinal L cells. Tissue-specific post-translational processing of proglucagon by the prohormone convertase produces the different proglucagon derived peptides (PGDPs) in both pancreas and intestine. The most notable pancreatic PGDP is glucagon, whereas the L cells produce 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. Advantages of this assay include sensitive quantification, high specificity and no interference from other body fluid factors or physiologically active substances. The Rat GLP-2 Calibrator is highly purified synthetic product.

PRINCIPLE
This EIA kit is based on a competitive enzyme immunoassay using a highly specific antibody to rat GLP-2 and a biotin/avidin-affinity system. The 96-well plate is coated with goat anti-rabbit IgG antibody. Rat GLP-2 Calibrator or samples, biotinylated rat GLP-2 and anti-rat GLP-2 polyclonal antibody antibody are added to the wells for a competitive immuno-reaction. After plate rinsing, HRP-labeled streptoavidins are added to bind to the antigen-antibody complex so that HRP-labeled streptoavidin-biotinylated rat GLP-2-antibody complexes are formed on the surface of the wells. Finally, excess HRP-labeled streptoavidins are rinsed, HRP-enzyme activity is determined, and the concentration of rat GLP-2 is calculated.

COMPONENTS

Component	Form	Quantity	Main Ingredient
1. Antibody-Coated Plate	MTP[*1]	1 plate (96-well)	Goat Anti-rabbit IgG
2. GLP-2 Calibrator	Lyophilized	1 vial (50 ng/vial)	Synthetic rat GLP-2
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 Concentrate	Liquid	1 bottle (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 hydrochloride
9. Stop Solution	Liquid	1 bottle (12 mL)	2 N H_2SO_4
10. Buffer Solution	Liquid	1 bottle (35 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 492 nm
- Rotator for microtiter plate
- Washing device for microtiter plate and dispenser for approximately 0.3 mL with 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 to avoid cross-contamination.

Always run a calibration curve when testing samples.

REAGENT PREPARATION

1. Preparation of Calibrator Solutions: Reconstitute the GLP-2 Calibrator (lyophilized rat GLP-2, 50 ng/vial) with 0.5 mL of Buffer Solution, giving a 100 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 33.33 ng/mL Calibrator Solution. Repeat the serial dilution to make Calibrator Solutions at 11.11, 3.704, 1.235, 0.412 and 0.137 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 Labeled Antigen with 9 mL of Buffer Solution.

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

3. Preparation of SA-HRP diluted solution: Add 0.12 mL of SA-HRP Concentrate to the bottle of SA-HRP Diluent (12 mL) and mix thoroughly.

   Note: 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.

SPECIMEN COLLECTION AND HANDLING

Plasma or serum 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 or below –30°C. Avoid repeated freeze/thaw cycles.

ASSAY PROTOCOL

1. Warm the reagents and samples to room temperature (20-30°C) before beginning the test.
2. Add 300 µL of Wash Solution into wells. Aspirate the Wash Solution in the wells. Repeat this washing procedure twice.
3. Add 75 µL of Labeled Antigen solution into the wells. Then add 25 µL of the prepared Calibrator Solutions (0, 0.137, 0.412, 1.235, 3.704, 11.11, 33.33, 100 ng/mL) or samples. Next, add 50 µL of rat GLP-2 Antibody to the wells.
4. Cover the plate with the Plate Seal and incubate at 4°C overnight (16 -18 hours).
5. Remove the Plate Seal and aspirate the solution in the wells. Wash the wells three times with approximately 0.3 mL/well of Wash Solution.
6. Pipette 100 µL of 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 five times with approximately 0.3 mL/well of Wash Solution.
9. Add 100 µL of Substrate Solution 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.

During continuous rotation of test plate, the plate rotator may be heated. It is recommended to place polystyrene foam or plywood between the plate and the rotator.

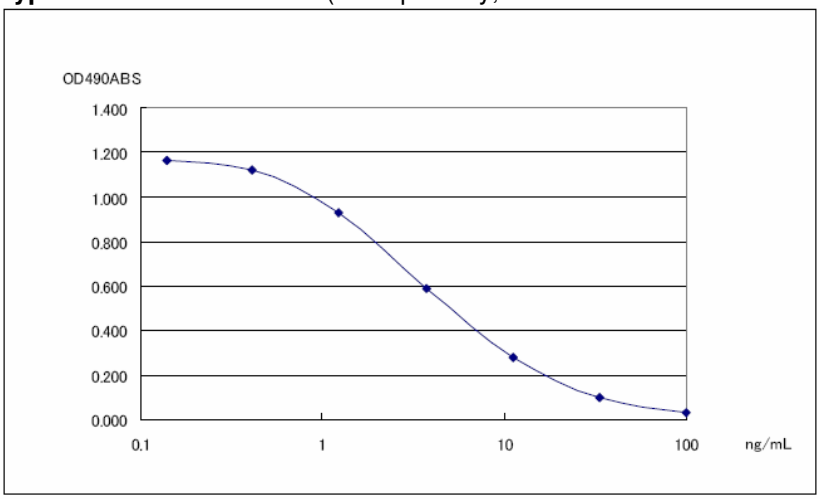
RESULTS

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-2 concentrations in samples from the corresponding absorbance values.

When a sample value exceeds 100 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	Rat GLP-2 Added (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
Rat Serum	0	1.96		
	1	2.62	2.96	88.51
	3	4.33	4.96	87.30
	6	7.08	7.96	88.88
Rat Plasma	0	1.56		
	1	2.17	2.56	84.74
	3	3.63	4.56	79.58
	6	6.00	7.56	79.42

Precision and reproducibility

- Intra-assay CV (%) Rat Serum 6.03 – 16.13
  Rat Plasma 0.48 – 8.48

Assay Range

0.137 – 100 ng/mL

Cross-Reactivity

Does not cross-react with rat Glucagon and rat GLP-1 within the range of 300 pmol/mL.

FOR RESEARCH USE ONLY

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