KAMIYA BIOMEDICAL COMPANY

Human GLP-2 EIA

For the quantitative determination of GLP-2 in human plasma and serum.

Cat. No. KT-385

For Research Use Only.

PRODUCT INFORMATION

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INTENDED USE
The Human GLP-2 EIA is for the quantitative determination of GLP-2 in human plasma and serum. For research use only. Not for use in diagnostic procedures.

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 sample constituents. The Human GLP-2 Calibrator is a highly purified synthetic product (purity: > 98%).

PRINCIPLE
This EIA kit is based on a competitive enzyme immunoassay using a highly specific antibody to human GLP-2 combined with a biotin-avidin affinity system. The 96-well plate is coated with goat anti-rabbit IgG and GLP-2 Calibrators or samples, biotinylated human GLP-2 and rabbit anti-GLP-2 antibody are added to the wells for a competitive immuno-reaction. After incubation and washing, HRP-labeled streptoavidin (SA-HRP) is added to form HRP-labeled SA-biotinylated GLP-2-antibody complex on the surface of the wells. Finally, HRP enzyme activity is determined by o-Phenylenediamine dihydrochloride (OPD) and the concentration of human GLP-2 is calculated.

COMPONENTS

Component	Form	Quantity	Main Ingredient
1. Antibody-Coated Plate	Microtiter Plate	1 plate (96-well)	Goat anti-rabbit IgG antibody
2. GLP-2 Calibrator	Lyophilized	1 vial (50 ng/vial)	Synthetic human GLP-2
3. Labeled Antigen	Lyophilized	1 vial	Biotinylated human GLP-2
4. GLP-2 Antibody	Liquid	1 bottle (6 mL)	Rabbit anti-human GLP-2 antibody
5. SA-HRP Solution	Liquid	1 bottle (12 mL)	HRP-labeled streptoavidin (SA)
6. Substrate Buffer	Liquid	1 bottle (26 mL)	Citrate buffer with 0.015% hydrogen peroxide
7. OPD Tablet	Tablet	2 tablets	o-Phenylenediamine dihydrochloride
8. Stop Solution	Liquid	1 bottle (12 mL)	1 M H_2SO_4
9. Buffer Solution	Liquid	1 bottle (25 mL)	Phosphate buffer
10. Wash Solution Concentrate	Liquid	1 bottle (50 mL)	Concentrated saline
11. Plate Seal		3 sheets	

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
- Glass 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 human GLP-2 Calibrator (lyophilized 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, and 0.412 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 Buffer Solution.

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

3. 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.

4. 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.

5. 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 (Human GLP-2 Calibrator and labeled antigen) should be stored below -30°C.

SPECIMEN COLLECTION AND HANDLING

EDTA-2Na additive blood collection tubes are recommended for plasma sample collection. It is strongly recommended that plasma and serum samples 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°C 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 0.35 mL/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 onto an absorbent surface, such as paper toweling, to ensure removal of most of the residual Wash Solution.
3. Add 40 µL of Labeled Antigen Solution into wells. Then add 25 µL of the prepared Calibrator Solutions (0, 0.412, 1.235, 3.704, 11.11, 33.33, and 100 ng/mL) or samples. Next, add 50 µL of GLP-2 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 3 times with approximately 0.35 mL/well of diluted Wash Solution. Finally, invert the plate and tap onto an absorbent surface, such as paper toweling, to ensure removal of most of the residual 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 5 times with approximately 0.35 mL/well of diluted Wash Solution. Finally, invert the plate and tap onto an absorbent surface, such as paper toweling, to ensure removal of most of the residual Wash 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.

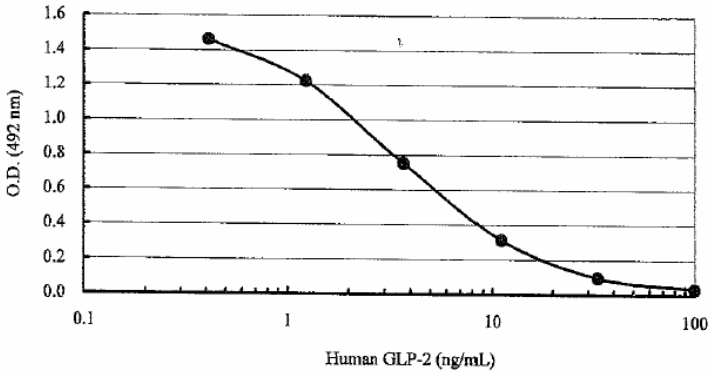
RESULTS

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-2 concentrations in samples from the corresponding absorbance values.

When a sample value is expected to exceed 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	GLP-2 Added (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
Human Plasma 1	0	4.82		
	2	6.10	6.82	89.4
	5	7.60	9.82	77.4
	10	14.77	14.82	99.7
Human Plasma 2	0	4.03		
	2	5.19	6.03	86.1
	5	6.96	9.03	77.1
	10	13.85	14.03	98.7
Human Serum 1	0	3.16		
	2	4.90	5.16	95.0
	5	6.89	8.16	84.4
	10	14.58	13.16	110.8
Human	0	4.31		

Serum 2	2	5.21	6.31	82.6
	5	7.14	9.31	76.7
	10	14.07	14.31	98.3

Precision and reproducibility

- Intra-assay CV (%) Human Plasma 3.7 – 4.8
  Human Serum 3.0 – 5.5

- Inter-assay CV (%) Human Plasma 13.0 – 16.4
  Human Serum 14.3 – 17.5

Assay Range

0.412 – 100 ng/mL

Cross-Reactivity

No cross-reactivity with neither glucagon (rat, mouse, or human) nor GLP-1, even at a concentration of 300 pmol/mL.

FOR RESEARCH USE ONLY

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