GLP-2 ELISA

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

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 48-GP2HU-E01.1
Size: 96 wells
Version: February 14, 2020 - ALPCO 2.0

Intended Use

The GLP-2 ELISA kit is used for quantitative determination of human GLP-2 [both GLP-2 (1-33) and GLP-2 (3-33)] in plasma and serum samples. 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 posttranslational processing of proglucagon by prohormone convertase produces different proglucagon derived peptides (PGDPs) in both the pancreas and intestine. The most notable pancreatic PGDP is glucagon, whereas intestinal L cells produce several structurally related peptides, including glucagon-like peptide 1 (GLP-1) and 2 (GLP-2), as well as glicentin and oxyntomodulin which contain the glucagon sequence in their molecules. Among PGDPs, GLP-2 has been found to result in intestinal epithelial proliferation.

GLP-2 ELISA Performance Summary

- The assay kit can measure human GLP-2 within the range of 0.412 - 100 ng/mL
- The assay can be completed within 16-18 hr + 1.5 hr
- With one assay kit, 41 samples can be measured in duplicate
- Test sample: human plasma and serum
- Sample volume: 25 µL
- The 96-well plate of this kit consists of twelve 8-wells strips; the strips can be used separately.
- Precision and reproducibility:
    - (Human plasma)
        - Intra-assay CV (%) 3.7 - 4.8
        - Inter-assay CV (%) 13.0 - 16.4
    - (Human serum)
        - Intra-assay CV (%) 3.0 - 5.5
        - Inter-assay CV (%) 14.3 - 17.5
- Stability and storage:
    - Store all the components at 2-8℃.
    - The kit is stable stored under this condition for 19 months from the date of manufacture.
    - The expiry date is stated on the package.

Contents Summary

1. Antibody coated plate
2. Human GLP-2 standard
3. Labeled antigen
4. GLP-2 antibody
5. SA-HRP solution
6. Substrate buffer
7. OPD tablet
8. Stop solution
9. Buffer solution
10. Wash solution (concentrated)
11. Adhesive foil

Assay Characteristics

The GLP-2 ELISA kit is characterized by its sensitive quantification and high analytical specificity. In addition, it is not influenced by other constituents in samples. The kit standard, human GLP-2, is a highly purified synthetic product (purity: higher than 98%).

Analytical Specificity

This ELISA kit is highly specific to human GLP-2 and shows cross-reactivity to neither glucagon (rat/mouse/human) nor GLP-1 even at a concentration of 300 pmol/mL.

Principle of the Assay

The GLP-2 ELISA is based on a competitive enzyme immunoassay using a combination of highly specific antibody to human GLP-2 and the biotin-avidin affinity system. Standards, samples, biotinylated human GLP-2, and rabbit anti-GLP-2 antibody are added to the microwell plate wells coated in goat anti-rabbit IgG for the competitive immunoreaction. After incubation and plate washing, horse radish peroxidase (HRP)-labeled streptavidin (SA) is added to form an 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.

Materials

Component	Form	Quantity	Main Ingredient
Antibody coated plate	Microtiter plate	1 plate x 96 wells	Goat anti-rabbit IgG antibody
Human GLP-2 standard	Lyophilized	1 vial x 50ng	Synthetic human GLP-2
Labeled antigen	Lyophilized	1 vial	Biotinylated human GLP-2
GLP-2 antibody	Liquid	1 bottle x 6 mL	Rabbit anti-human GLP-2 antibody
SA-HRP solution	Liquid	1 bottle x 12 mL	HRP-labeled SA
Substrate buffer	Liquid	1 bottle x 26 mL	Citrate buffer containing 0.015% hydrogen peroxide
OPD tablet	Tablet	2 tablets	o-Phenylenediamine dihydrochloride
Stop solution	Liquid	1 bottle x 12 mL	1M H_2SO_4
Buffer solution	Liquid	1 bottle x 25 mL	Phosphate buffer
Wash solution (Concentrated)	Liquid	1 bottle x 50 mL	Concentrated saline
Adhesive foil		3 pieces	

Equipment Required, but Not Provided

1. Microtiter plate reader which can read extinction 2.5 at 490nm
2. Microtiter plate shaker capable of 100 rpm
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. Glass test tubes for preparation of standard solution

6. Graduated cylinder (1000 mL)
7. Distilled or deionized water
8. Timer

Sample Handling

An EDTA-2Na additive blood collection tube is recommended for plasma sample collection. It is strongly recommended that plasma and serum samples should be tested as soon as possible after collection. If the samples are tested later, they should be divided into aliquots and frozen at or below -30°C. Avoid repeated freezing and thawing of samples.

Reagent Preparation

1. *Preparation of standard solution*
   Reconstitute human GLP-2 standard (lyophilized, 50 ng/vial) with 0.5mL of buffer solution, which affords 100 ng/mL standard solution. Dilute 0.1 mL of the standard solution with 0.2 mL of buffer solution, which yields 33.33 ng/mL standard solution. Repeat the dilution procedure to make each of 11.11, 3.704, 1.235 and 0.412ng/mL standard solutions. Buffer solution itself is used as the 0 ng/mL.

2. *Preparation of labeled antigen solution*
   Reconstitute labeled antigen with 6 mL of buffer solution.

3. *Preparation of substrate solution*
   Resolve one OPD tablet with 12 mL of substrate buffer. It should be prepared immediately before use.

4. *Preparation of wash solution*
   Dilute 50 mL of wash solution (concentrated) to 1000 mL with distilled or deionized water.

5. Other reagents are ready to use.

Assay Procedure

1. Bring all the reagents and samples to room temperature (20-30°C) at least 1 hour before starting the assay.

2. Add 0.35mL per well of wash solution into the wells of the plate. Aspirate the solution. Repeat this wash procedure twice (total 3 times). Finally, invert the plate and tap it onto an absorbent surface, such as paper towels, to ensure blotting of most residual wash solution.

3. Add 40µL of labeled antigen solution into the wells first. Then add 25µL of each standard (0, 0.412, 1.235, 3.704, 11.11, 33.33 and 100 ng/mL) or sample into the designated wells. Finally add 50µL of GLP-2 antibody into the wells.

4. Cover the plate with adhesive foil and incubate it at 4ºC for 16 - 18 hours without shaking.

5. After incubation, remove the adhesive foil, aspirate the solution in the wells and wash the wells 3 times with approximately 0.35 mL per well of wash solution. Finally, invert the plate

and tap it onto an absorbent surface, such as paper towels, to ensure blotting of most residual wash solution.

6. Pipette 100µL of SA-HRP solution into each of the wells.

7. Cover the plate with adhesive foil and incubate it at room temperature (20 - 30ºC) for 1 hour. During the incubation, the plate should be shaken with a microtiter plate shaker set to 100 rpm.

8. Dissolve one OPD tablet with 12 mL of substrate buffer. It should be prepared immediately before use.

9. Remove the adhesive foil, aspirate the solution in the wells and wash the wells 5 times with approximately 0.35 mL per well of wash solution. Finally, invert the plate and tap it onto an absorbent surface, such as paper towels, to ensure blotting of most residual wash solution.

10. Add 100µL of substrate solution into each the wells, cover the plate with adhesive foil and incubate it for 30 minutes at room temperature.

11. Add 100µL of stop solution into each of the wells to stop the color reaction.

12. Read optical absorbance of the solution in the wells at 490 nm.

Calculation of Results

The dose-response curve of this assay fits best to a 4- (or 5-) parameter logistic equation. The results of unknown samples can be calculated with any computer program having a 4- (or 5-) parameter logistic function. Otherwise calculate mean absorbance values of wells containing standards and plot a standard curve on semi-logarithmic graph paper (abscissa: concentration of standard; ordinate: absorbance values). Use the average absorbance of each sample to determine the corresponding value by simple interpolation from this standard curve.

Notes

1. Human GLP-2 standard, labeled antigen and substrate solution should be prepared immediately before use. If a full plate is not required to be used for testing, unused strips and the rest of the reconstituted reagents (human GLP-2 standard and labeled antigen) should be stored below -30°C.

2. During storage of wash solution (concentrated) at 2 - 8°C, precipitates may be observed. However, they will be dissolved when diluted. Diluted wash solution is stable for 6 months at 2 - 8°C.

3. As pipetting may affect precision of the assay, pipette the standard solutions and samples into each well of the plate precisely. Use clean test tubes or vessels in the assay, and new tips must be used for each standard solution or sample to avoid cross-contamination.

4. When the concentration of GLP-2 in a sample is expected to exceed 100 ng/mL, the sample needs to be diluted with buffer solution to an appropriate concentration.

5. During incubation (except at 4°C and color reaction), the plate should be shaken gently with a microtiter plate shaker to promote immunoreaction.

6. Perform all the determinations in duplicate.

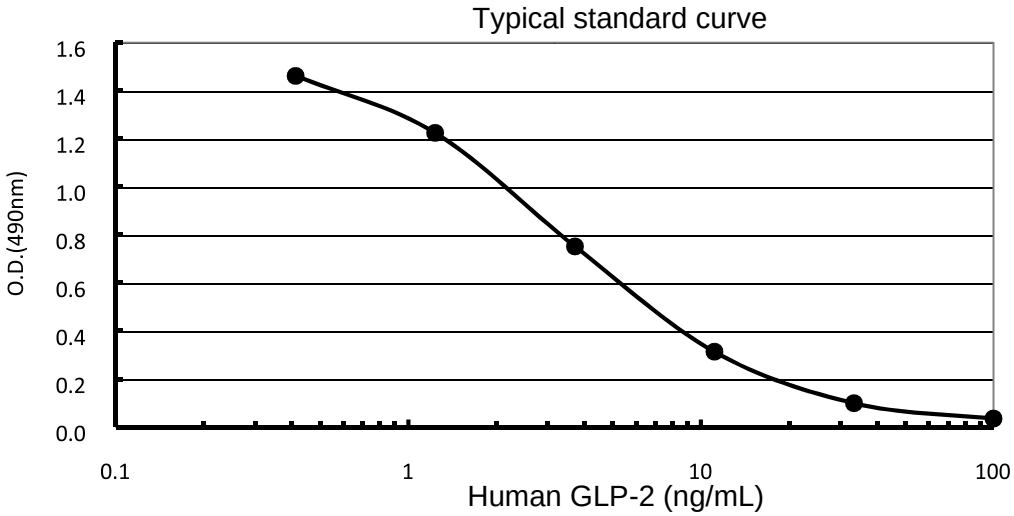
7. Read the optical absorbance of each well immediately after stopping the color reaction.

8. For accurate quantification, plot a standard curve for each assay.

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

10. Satisfactory performance of the assay is guaranteed only when reagents with the identical lot number are used.

Performance Characteristics



Analytical Recovery

Human plasma 1

Added human GLP-2 (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
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

Added human GLP-2 (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
	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

Added human GLP-2 (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0	3.16		
2	4.90	5.16	95
5	6.89	8.16	84.4
10	14.58	13.16	110.8

Human serum 2

Added human GLP-2 (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0	4.31		
2	5.21	6.31	82.6
5	7.14	9.31	76.7
10	14.07	14.31	98.3

Precision and Reproducibility

	Human plasma	Human serum
Intra-assay CV (%)	3.7 - 4.8	3.0 - 5.5
Inter-assay CV (%)	13.0 - 16.4	14.3 - 17.5

Stability and Storage

Storage
Store all the components at 2 - 8°C.

Shelf life
The kit is stable under this condition for 19 months from the date of manufacture. The expiry date is stated on the package.

References

1. Philippe J: Structure and pancreatic expression of the insulin and glucagon gene. Endocr Rev 12: 252 - 271, 1991
2. Mojsov S et al : Preproglucagon gene expression in pancreas and intestine diversifies the level of post-transcriptional processing. J Biol Chem 261: 11880 – 11889, 1986
3. Drucker D J et al : Induction of intestinal epithelial proliferation by glucagon-like peptide 2. Proc Natl Acad Sci USA 93: 7911 – 7916, 1996