Mouse GLP-2 ELISA

For quantitative determination of mouse GLP-2 in serum or plasma samples.

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

Catalog Number: 48-GP2MS-E01
Size: 96 Wells
Version: March 5, 2020 – ALPCO 2.0

Intended Use

The Mouse GLP-2 ELISA kit is used for the quantitative determination of mouse GLP-2 [both GLP-2 (1-33) and GLP-2 (3-33) in serum or plasma samples. For research use only. Not for use in diagnostic procedures.

Introduction

The proglucagon gene is expressed in both pancreatic A cell and intestinal L cell. Tissue-specific post-translational processing of proglucagon by the prohormone convertase produce the different proglucagon derived peptides (PGDPs) in both the pancreas and intestine. The most notable pancreatic PGDP is glucagon, whereas the intestinal L cell produces 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 the PGDPs, GLP-2 has recently been found to show intestinal epithelial proliferation.

Mouse GLP-2 ELISA Performance Summary

- The assay can measure GLP-2 in the range of 0.412 – 100 ng/mL
- The assay completes within 16-18 hr. + 1.5 hr.
- With one assay kit, 41 samples can be measured in duplicate
- Test sample: mouse serum or plasma
- Sample Volume 25 µL
- The 96-well plate in the kit consists of 8-wells strips that can be used separately.
- Precision and reproducibility:
    - Serum:
        - Intra-assay CV (%) 5 – 9
        - Inter-assay CV (%) 11 – 15
    - Plasma
        - Intra-assay CV (%) 4 – 6
        - Inter-assay CV (%) 5 – 16
- Stability and Storage:
- Store all components at 2-8 °C
- This kit is stable 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) Mouse GLP-2 standard
3) Labeled
4) SA-HRP solution
5) OPD tablet
6) Stop solution
7) Buffer solution
8) Wash solution (concentrated)
9) Adhesive foil

Assay Characteristics

The kit is characterized for sensitive quantification, high specificity, and no influence with other components in serum or plasma. No sample pre-treatment is necessary. The mouse GLP-2 standard is a highly purified synthetic product.

Analytical Specificity

This ELISA kit has high specificity for mouse GLP-2 and shows no cross reactivity with mouse glucagon or mouse GLP-1 even at the concentration of 300 pmol/mL.

Principle of the Assay

This ELISA kit, for determination of mouse GLP-2 in serum or plasma samples, is based on a competitive enzyme immunoassay using a combination of a highly specific antibody to rat GLP-2 (strong cross reactivity to mouse GLP-2) and biotin-avidin affinity system. The 96-wells plate is coated with goat anti-rabbit IgG antibody. Mouse GLP-2 standard or samples, labeled antigen, and anti-rat GLP-2 polyclonal antibody are added to the wells for competitive immunoreaction. After incubation and plate washing, HRP labeled streptavidin (SA-HRP) is added to form HRP labeled streptavidin-biotinylated rat GLP-2-antibody complex on the surface of the wells. Finally, HRP enzyme activity is determined by the absorbance given off in the reaction with o-Phenylenediaminedihydrochloride (OPD) and the concentration of mouse GLP-2 is calculated.

Materials

Component	Form	Quantity	Main Ingredient
1. Antibody coated plate	Microtiter Plate	1plate (96wells)	Goat anti-rabbit IgG
2. Mouse GLP-2 standard	lyophilized	1 vial	Synthetic mouse GLP-2 (50ng/vial)
3.Labeled antigen	lyophilized	1 vial	Biotinylated rat GLP-2
4. GLP-2 Antibody	liquid	1bottle (6 mL)	Rabbit anti-rat GLP-2
5. SA-HRP solution	liquid	1bottle (12 mL)	HRP labeled streptavidin
6. Substrate buffer	liquid	1 bottle (26 mL)	0.015% Hydrogen Peroxide
7. OPD tablet	tablet	2 tablets	o-Phenylenediamine dihydrochloride
8. Stop Solution	liquid	1 bottle (12 mL)	2N H_2SO_4
9. Buffer solution	liquid	1 bottle (25 mL)	Phosphate buffer
10. Wash solution	liquid	1 bottle (50 mL)	Concentrated saline
11. Adhesive foil		3 sheets	

Equipment Required, but Not Provided

1. Timer Distilled water or deionized water
2. Microtiter plate reader which can read extinction up to 2.5 at 490 nm
3. Microtiter plate shaker capable of 100 rpm
4. Washing device for microtiter plate and dispenser with aspiration system
5. Micropipettes, multi-channel pipettes for 8 wells or 12 wells and their tips
6. Test tubes for preparation of standard solution
7. Graduated cylinder (1000 mL)
8. Timer

Sample Handling

Plasma or serum 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 amounts and frozen at or below -30℃. Avoid repeated freezing and thawing of plasma or serum samples.

Reagent Preparation

1) Preparation of standard solution:
Reconstitute the standard (lyophilized mouse GLP-2 50ng/vial) with 0.5 mL of Buffer solution, to make 100 ng/mL of standard solution. Take 0.1 ml of the reconstituted standard solution and dilute with 0.2 mL of Buffer solution, to make 33.33ng/mL standard solution. Repeat the same dilution process, serially, to make standards of 11.11, 3.704, 1.235, 0.412 ng/mL. Buffer solution is used as 0 ng/mL.

2) Preparation of labeled antigen:
Reconstitute labeled antigen with 9 mL of Buffer solution.

3) Preparation of substrate solution:
Dissolve OPD tablet with 12 mL of substrate buffer. It should be prepared immediately before use.

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

5) Other reagents are ready for use.

Procedure

1. Bring all the reagents and samples to room temperature before beginning the test.
2. Pipette 75µL of labeled antigen solution into the wells first, then pipette 25µL of each of the standards and/or samples. Then pipette 50µL of GLP-2 antibody into the wells.
3. Cover the plate with adhesive foil and incubate at 4ºC for 16 ~ 18 hours. (Still, shaker not needed)
4. Take off the adhesive foil, aspirate the contents of the wells, and wash the wells three times with approximately 0.35 mL/well of wash solution.
5. Pipette 100µL of SA-HRP solution into the wells.
6. 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 plate shaker.
7. Dissolve OPD tablet with 12 mL of substrate buffer. **It should be prepared immediately before use.**
8. Take off the adhesive foil, aspirate and wash the wells five times with approximately 0.3 mL/well of wash solution.
9. Add 100µL of substrate solution into the wells, cover the plate with adhesive foil and incubate for 30 minutes at room temperature.
10. Add 100µL of stop solution into the wells to stop reaction.
11. Read the absorbance of the wells at 490 nm with a microplate reader.

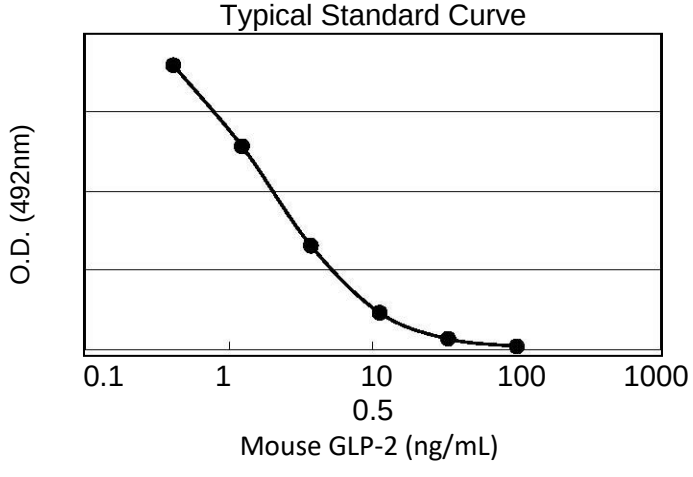
Calculation of Results

The dose-response curve of this assay fits best to a 4- or 5- parameter logistic equation. The results can be calculated with any computer program capable of analyzing a 4- or 5- parameter logistic equation. Otherwise, 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 average absorbance of each sample to determine the corresponding concentration by simple interpolation off of the standard curve.

Notes

1. Mouse GLP-2 standard, labeled antigen, and substrate solution should be prepared immediately before use.
2. During storage of the wash solution (concentrated) at 2-8°C, precipitates may be observed, however they will dissolve when diluted. Diluted wash solution is stable for 6 months at 2-8°C.
3. Pipetting operations may affect the precision of the assay, pipette standard solutions or samples into each well of plate precisely. Use clean test tubes or vessels in assay and use new tip for each sample to avoid cross contamination.
4. When sample value exceeds 100 ng/mL, dilution will be necessary to bring the value onto the standard curve.
5. During incubation with SA-HRP solution at room temperature, the test plate should be shaken gently by plate shaker to promote immunoreaction.
6. Perform all the determinations in duplicate.
7. Reconstituted standard and labeled antigen should be stored at or below -30°C.
8. Read plate optical absorbance of reaction solution in wells as soon as possible after stopping color reaction.
9. To quantify accurately, always run a standard curve when testing samples.
10. Protect reagents from strong light (e.g. direct sunlight) during storage and assay.
11. Satisfactory performance of the test is guaranteed only when reagents are used from combination pack with identical lot number.

Performance Characteristics



Analytical recovery

Mouse Serum

Sample No.	Mouse GLP-2 added (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
1	0	0.98		
2	2	2.80	2.98	94.0
3	5	5.59	5.98	93.5
4	20	20.24	20.98	96.5

Mouse Plasma

Sample No.	Mouse GLP-2 added (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
1	0	1.13		
2	2	2.89	3.13	92.3
3	5	5.73	6.13	93.5
4	20	22.36	21.13	105.8

Precision and reproducibility

- Intra-assay/Mouse serum CV（%） 5-9
- Inter-assay/Mouse serum CV（%） 11-15
- Intra-assay/Mouse plasma CV（%） 4-6
- Inter-assay/Mouse plasma CV（%） 5-16

Assay range
0.412-100 ng/mL

Stability and Storage

Storage: Store all components at 2-8℃.
Shelf life: 19 months from date of manufacture. The expiry date is described on the label of kit.
Package: For 96 tests per one kit including standards.

References

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7. Kato I, et al: Synthesis of Rat Glucagon-like peptide(GLP)-2 and its biological and immunochemical studies. *Peptide Science **1999***: N.Fujii(Ed). The Japanese Peptide Society (2000)