

YK142 Mouse GLP-2 EIA

FOR LABORATORY USE ONLY

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- Please read all the package insert carefully before beginning the assay -

YK142 Mouse GLP-2 EIA kit

I. Introduction

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

YK142 Mouse GLP-2 EIA Kit	Contents
▼ The assay kit can measure GLP-2 in the range of 0.412 - 100 ng/mL	1) Antibody coated plate
▼ The assay completes within 16-18 hr. + 1.5 hr.	2) Mouse GLP-2 standard
▼ With one assay kit, 41 samples can be measured in duplicate	3) Labeled antigen
▼ Test sample: mouse serum or plasma Sample volume: 25 μ L	4) GLP-2 antibody
▼ The 96-well plate in kit was consisted by 8-wells strips. The kit can be used separately.	5) SA-HRP solution
▼ Precision and reproducibility Intra-assay CV (%) serum 4.9 - 8.8 Inter-assay CV (%) serum 11.0-14.7 Intra-assay CV (%) plasma 3.5 - 6.0 Inter-assay CV (%) plasma 4.9 -16.0	6) Substrate buffer
▼ Stability and Storage Store all of the components at 2-8°C. 19 months from the date of manufacturing. The expiry date is described on the label of kit.	7) OPD tablet
	8) Stopping solution
	9) Buffer solution
	10) Washing solution (concentrated)
	11) Adhesive foil

II. Characteristics

This EIA kit is used for quantitative determination of mouse GLP-2 [both GLP-2 (1-33) and GLP-2 (3-33)] in serum or plasma samples. The kit is characterized for sensitive quantification, high specificity and no influence with other components in serum or plasma and needlessness of sample pre-treatment. Mouse GLP-2 standard is highly purified synthetic product.

< Specificity >

The EIA kit has high specificity to mouse GLP-2 and shows no cross reactivity with mouse glucagon and mouse GLP-1 even in the concentration of 300 pmol/mL.

< Test Principle >

This EIA kit for determination of mouse GLP-2 in serum or plasma samples is based on a competitive enzyme immunoassay using combination of 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) are 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 o-Phenylenediamine dihydrochloride (OPD) and the concentration of mouse GLP-2 is calculated.

III. Composition

Component	Form	Quantity	Main Ingredient
1. Antibody coated plate	MTP ^{*1}	1 plate(96 wells)	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	1 bottle (6 mL)	Rabbit anti rat GLP-2
5. SA-HRP solution	liquid	1 bottle (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. Stopping solution	liquid	1 bottle (12 mL)	2N H ₂ SO ₄
9. Buffer solution	liquid	1 bottle (25 mL)	Phosphate buffer
10. Washing solution (concentrated)	Liquid	1 bottle (50 mL)	Concentrated saline
11. Adhesive foil		3 sheets	

MTP^{*1} Microtiter plate

IV. Method

< Equipment required >

- 1) Photometer for microtiter plate (Plate reader) which can read extinction 2.5 at 490 nm
- 2) Microtiter plate shaker
- 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) Test tubes for preparation of standard solution
- 6) Graduated cylinder (1,000 mL)
- 7) Distilled water or deionized water

< Preparatory work >

- 1) Preparation of standard solution:

Reconstitute the standard (lyophilized mouse GLP-2 50ng/vial) with 0.5mL of Buffer solution, which affords 100 ng/mL standard solution. The 0.1ml of the reconstituted standard solution is diluted with 0.2 mL of Buffer solution that yields 33.33ng/mL standard solution. Repeat the same dilution to make each standard of 11.11, 3.704, 1.235, 0.412ng/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:

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

- 4) Preparation of washing solution:

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

- 5) Other reagents are ready for use.

< Procedure >

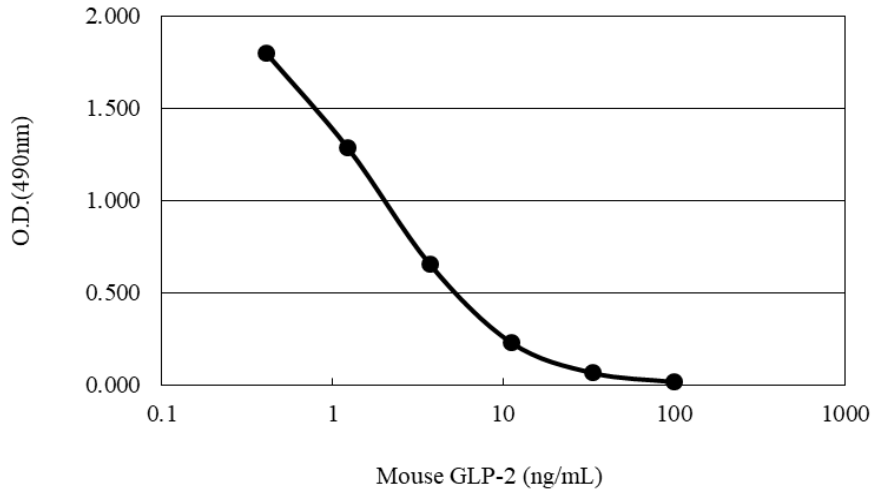
1. Bring all the reagents and samples to room temperature before beginning the test.
2. Fill 75 μ L of labeled antigen solution into the wells first, then introduce 25 μ L of each of standard solutions (0, 0.412, 1.235, 3.704, 11.11, 33,33, 100 ng/mL) or samples and finally add 50 μ L of GLP-2 antibody into the wells.
3. Cover the plate with adhesive foil and incubate it at 4°C for 16 ~ 18 hours.(Still, shaker not need)
4. Take off the adhesive foil, aspirate the solution in the wells and wash the wells three times with approximately 0.35 mL/well of washing solution.
5. Pipette 100 μ L of SA-HRP solution into the wells.
6. Cover the plate with adhesive foil and incubate it at room temperature (20-30°C) for 1 hour. During the incubation, the plate should be shake with a plate shaker.
7. Resolve 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 washing solution.
9. Add 100 μ L of substrate solution into the wells, cover the plate with adhesive foil and incubate it for 30 minutes at room temperature.
10. Add 100 μ L of stopping solution into the wells to stop reaction.
11. Read the optical absorbance of reaction mixture in each well at 490 nm with a microtiter plate reader. 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 semilogarithmic 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.

V. Notes

1. 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 amount and frozen at or below -30°C . Avoid repeated freezing and thawing of plasma or serum samples.
2. Mouse GLP-2 standard, labeled antigen, and substrate solution should be prepared immediately before use.
3. During storage of washing solution (concentrated) at $2-8^{\circ}\text{C}$, precipitates may be observed, however they will be dissolved when diluted. Diluted washing solution is stable for 6 months at $2-8^{\circ}\text{C}$.
4. Pipetting operations may affect the precision of the assay, pipette standard solutions or samples into each well of plate precisely. Using clean test tubes or vessels in assay and use new tip for each sample to avoid cross contamination.
5. When sample value exceeds 100 ng/mL, it needs to be diluted with buffered solution to proper concentration.
6. During incubation with SA-HRP solution at room temperature, the test plate should be shake gently by plate shaker to promote immunoreaction.
7. Perform all the determination in duplicate.
8. The kit can be used separately. In this case, the standard and label antigen should be store at or below -30°C .
9. Read plate optical absorbance of reaction solution in wells as soon as possible after stopping color reaction.
10. To quantitate accurately, always run a standard curve when testing samples.
11. Protect reagents from strong light (e.g. direct sunlight) during storage and assay.
12. Satisfactory performance of the test is guaranteed only when reagents are used from combination pack with identical lot number.

VI. Performance Characteristics

Standard Curve



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 (%) 4.9-8.8
- Inter-assay/Mouse serum CV (%) 11.0-14.7
- Intra-assay/Mouse plasma CV (%) 4.2-6.0
- Inter-assay/Mouse plasma CV (%) 4.9-16.0

Assay range

0.412-100 ng/mL

VII. Stability and Storage

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

VIII. References

1. Philippe J.: Structure and pancreatic expression of the insulin and glucagon genes. *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
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5. Drucker D. J.: Perspective in diabetes Glucagon-like peptides. *Diabetes* **47**: 159-169, 1998
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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)

<Manufacturer>

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