YK142 Mouse GLP-2 EIA

FOR LABORATORY USE ONLY

Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, BELGIUM
Tel 0032 16 58 90 45 | Fax 0032 16 50 90 45
www.gentaur-worldwide.com
info@gentaur.com

Contents

I.	**Introduction**	**2**
II.	**Characteristics**	**2**
III.	**Composition**	**3**
IV.	**Method**	**4 - 5**
V.	**Notes**	**6**
VI.	**Performance Characteristics**	**7 - 8**
VII.	**Stability and Storage**	**8**
VIII.	**Reference**	**8**

- 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

- ▼ The assay kit 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 kit was consisted by 8-wells strips. The kit can be used separately.
- ▼ Precision and reproducibility
  Intra-assay CV（%） serum 4.9 - 8.8
  Inter-assay CV（%） serum11.0-14.7
  Intra-assay CV（%） plasma 3.5 - 6.0
  Inter-assay CV（%） plasma 4.9 -16.0
- ▼ Stability and Storage
  Store all of the components at 2-8℃.
  12 months from the date of manufacturing.
  The expiry date is described on the label of kit.

Contents

1) Antibody coated plate
2) Mouse GLP-2 standard
3) Labeled antigen
4) GLP-2 antibody
5) SA-HRP solution
6) Substrate buffer
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 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 streptoavidin (SA-HRP) are added to form HRP labeled streptoavidin-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 streptoavidin
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_2SO_4
9. Buffer solution	liquid	1 bottle (25 mL)	Tris-HCl buffer
10. Washing solution (concentrated)	Liquid	1 bottle (50 mL)	Concentrated saline
11. Adhesive foil		3 sheets	

MTP[*1] ….. Microtiter plate

IV. Method

< Euipment 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 75μL of labeled antigen solution into the wells first, then introduce 25μL of each of standard solutions (0, 0.412, 1.235, 3.704, 11.11, 33,33, 100 ng/mL) or samples and finally add 50μL of GLP-2 antibody into the wells.

3. Cover the plate with adhesive foil and incubate it at 4ºC 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 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 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 100μL of substrate solution into the wells, cover the plate with adhesive foil and incubate it for 30 minutes at room temperature.

10. Add 100μL of stopping solution into the wells to stop reaction.

11. Read the optical absorbance of the wells at 492nm. 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 standard curve to read mouse GLP-2 concentrations in samples from the corresponding absorbance values.

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℃. 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℃, precipitates may be observed, however they will be dissolved when diluted. Diluted washing solution is stable for 6 months at 2-8℃.

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℃.

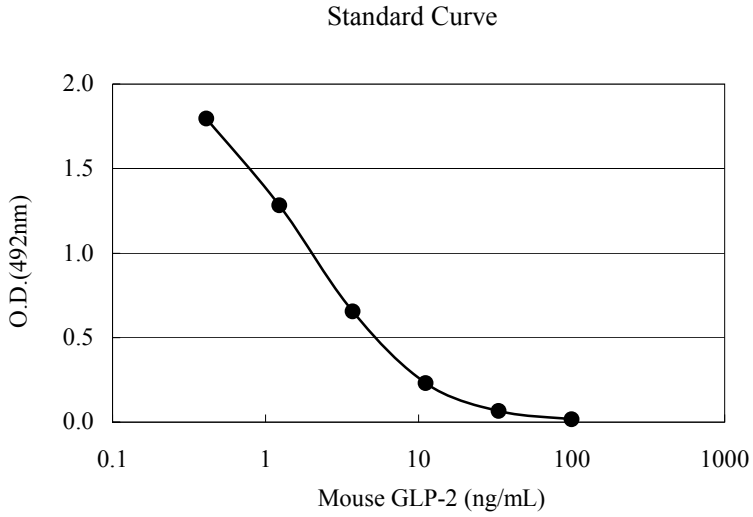
9. Read plate optical absorbance of reaction solution in wells as soon as possible after stopping color reaction.

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



Analytical recovery

< Mouse serum >

Sample No.	Muse 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℃.

< Shelf life > 12 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
4. Brubaker PL, et al: Circulating and tissue forms of intestinal growth factor, glucagon-like peptide 2. *Endocrinology* ***15***: 673-677,1997
5. Drucker D. J.: Perspective in diabetes Glucagon-like peptides. *Diabetes* ***47***: 159-169, 1998
6. Xiao Q, et al: Secretion of the intestinotropic hormone glucagon-like peptide 2 is differentially regulated by nutrients in humans. *Gastroenterology* ***117***: 99-105,1999
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)

GENTAUR