COSMO BIO CO., LTD.
Inspiration for Life Science

Rat Glicentin ELISA Kit

Cat. No. YII-YK111-EX

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Contents

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

YII-YK111-EX **Rat Glicentin EIA kit**

Ⅰ. Introduction

Glicentin is a 69-amino-acid peptide containing glucagon and oxyntomodulin sequences in the molecule. It is suggested that glicentin and oxyntomodulin are produced in the intestinal L-cells and glucagon in A-cells in the pancreas, these peptides are derived from a common precursor by two different tissue-specific processing pathways. In 1983, the amino acid sequence of human glicentin was deduced by Bell et al. from the genomic sequence of human preproglucagon. Glicentin is a major form of gut glucagon-like immunoreactants (Gut GLIs).
In mammalian small intestine, proglucagon is processed into glicentin, oxyntomodulin, and glucagon –like peptide (GLP)-1 and –2. GLP-1(7-37) and GLP-1(7-36) amide have been isolated from the intestine and pancreas. It has been known that the GLP-1 sequence is well conserved between species in all mammals studied. Using synthetic peptides, several investigators have demonstrated that in contrast to GLP-1(1-37), truncated GLP-1(7-36) amide and GLP-1(7-37) have several physiological effects. However, the physiological role of glicentin, a major gut glucagon, is still unclear. It has been known that the circulating level of plasma glicentin-like peptides increases significantly nutrient ingestion.
Yanaihara institute Inc. has succeeded in developing a specific and convenient ELISA kit for determination of Rat glicentin in blood and tissues.

Ⅱ. Characteristics

This EIA kit is used for quantitative determination of rat glicentin in plasma or serum sample. It has a lot of advantage to perform the assay, such as good quantification, no influence with other body fluid factors or physiological active substances and needlessness of sample pre-treatment. Glicentin standard used in the kit is a highly purified synthetic product and the quantity is measured by protein determination.

＜Specificity＞
The EIA kit does not exhibit cross-reactions with human glucagon, human GLP-1, human GLP-2, oxyntomodulin and rat glicentin.

＜Test Principle＞
This ELISA kit for determination of human glicentin in plasma or serum sample is based on a sandwich enzyme immunoassay. During first reaction, the human glicentin in standards or samples binds to the anti human glicentin monoclonal antibodies which are coated on the surface of the microtitration plate. After rinsing out excess glicentin, rabbit anti human glicentin antibodies are added to bind to the antigen-antibody complex. Then, excess antibodies are rinsed out and HRP labeled antibodies (Donkey anti rabbit IgG antibodies) are added to bind to the antibody-antigen-antibody complex. Finally, HRP enzyme activity is determined and the concentration of human glicentin is calculated.

Ⅲ. Composition

Component	Form	Quantity	Main ingredient
1. Antibody coated plate	MTP*1	1 plate (96 wells)	Anti human glicentin monoclonal antibody
2. Glicentin standard	lyophilized	1 vial (4 ng)	Human glicentin (recombinant)
3. First antibody	liquid	1 bottle (12 mL)	Rabbit Anti human glicentin
4. Second antibody	liquid	1 bottle (12 mL)	HRP−labeled anti rabbit IgG (from donkey)
5. Buffered solution (A)	liquid	1 bottle (10 mL)	Phosphate buffer
6. Buffered solution (B)	liquid	1 bottle (10 mL)	Phosphate buffer
7. OPD tablet	tablet	2 tablets	o-Phenylenediamine hydrochloride
8. Substrate buffer	liquid	1 bottle (24 mL)	0.015% H_2O_2 containing citrate buffer
9. Stopping solution	liquid	1 bottle (12 mL)	2N-H_2SO_4
10. Washing solution (concentrated)	liquid	1 bottle (50 mL)	10 fold−concentrated saline
11. Adhesive foil		3 sheets	

MTP*1. . . . . Microtitration plate

Ⅳ. Method

＜Equipment required＞

1）Photometer for microtitration plate（plate reader）、which can read extinction 2.5 at 490 nm

2）Rotator for microtitration plate

3）Washing device for microtitration plate and dispenser for approximate 0.3 mL 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（1000 mL）

7）Distilled water or deionized water

＜Preparatory work＞

1）Preparation of standard solution：
Reconstitute the standard (lyophilized human glicentin 4 ng / vial) with 1mL of buffered solution (A), which affords 4000 pg/mL standard solution. The reconstituted standard solution is diluted with the same volume of buffered solution (A) (e.g. 0.2 mL standard solution + 0.2 mL buffered solution), that yields 2000 pg/mL standard solution. Repeat the dilution to make 1000, 500, 250, 125 and 62.5 pg/mL standard solutions, respectively. Buffered solution (A) is used as 0 pg/mL solution.

2) Preparation of substrate solution：
Resolve OPD tablet with 11mL of substrate buffer. It should be prepared immediately before use.

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

4) Other reagents are ready for use.

＜Procedure＞

1. Warm up all the reagents and samples to room temperature before beginning the test.

2. Remove solution from wells of antibody coated plate and add 300 μL/well of washing solution into the wells. Aspirate the washing solution in the wells.

3. Fill 75 μL of buffered solution (B) into wells first, then introduce 25 μL each of standard solutions (0, 62.5, 125, 250, 500, 1000, 2000, 4000 pg/mL) or samples into the wells.

4. Cover the plate with adhesive foil and incubate it at room temperature overnight (18 ～ 24 hours).
   During incubation, the plate should be rotated with a plate rotator.

5. Take off the adhesive foil, aspirate the solution in the wells and wash the wells five times with approximately 0.3 mL/well of washing solution.

6. Pipette 100 μL of first antibody into the wells.

7. Cover the plate with adhesive foil and incubate it at room temperature (20～30℃) for 2 hours.
   During incubation, the plate should be rotated with a plate rotator.

8. Take off the adhesive foil, aspirate the solution in the wells and wash the wells five times with approximately 0.3 mL/well of washing solution.

9. Pipette 100 μL of second antibody into the wells.

10. Cover the plate with adhesive foil and incubate it at room temperature (20～30℃) for 2 hours.
    During incubation, the plate should be rotated with a plate rotator.

11. Take off the adhesive foil, aspirate the solution in the wells and wash the wells five times with approximately 0.3 mL/well of washing solution.

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

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

14. Read the optical absorbance of the wells at 490nm..

15. Calculate mean absorbance values of wells containing standards and plot a standard curve on logarithmic graph paper (abscissa: concentration of standard; ordinate: absorbance values.)

16. Use the standard curve to read glicentin concentrations in samples from the corresponding absorbence values.

Ⅴ. Notes

1. Plasma or serum samples must be used as soon as possible after collection. If the samples are to be tested at a later time, they should be divided into tubes in small amount and frozen at or below − 30℃. Avoid repeated freezing and thawing of plasma or serum samples.

2. Glicentin standard solution and substrate solution should be prepared immediately before use in assay using clean test tubes or vessels. Diluted washing solution is stable for 6 months at 2 to 8℃.

3. As antibody coated plate are divisible, make sure the number of wells required, and strips of the plate which are not used for the test should be removed from the holder and stored for later use. The strips not used can be used within two weeks if stored at 2 to 8℃.

4. During storage of washing solution (concentrated) at 2 to 8℃, precipitates may be observed, however they will be dissolved when diluted.

5. As pipetting operations may affect with the precision of the assay, pipette precisely standard solutions or samples into each well of plate. And use new tip for each sample and for dilution of the standard solution to avoid cross contamination.

6. When sample value exceeds 4.0 ng/mL, it needs to be diluted with the buffered solution within the assay range.

7. During incubation except over night reaction and color reaction, the test plate should be rotated gently by plate rotator to promote immunoreaction.

8. During continuous rotation of test plate, the plate rotator may be heated up. It is recommended to place styrene form or plywood between the plate and the rotator.

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

10. Perform all the determination in duplicate.

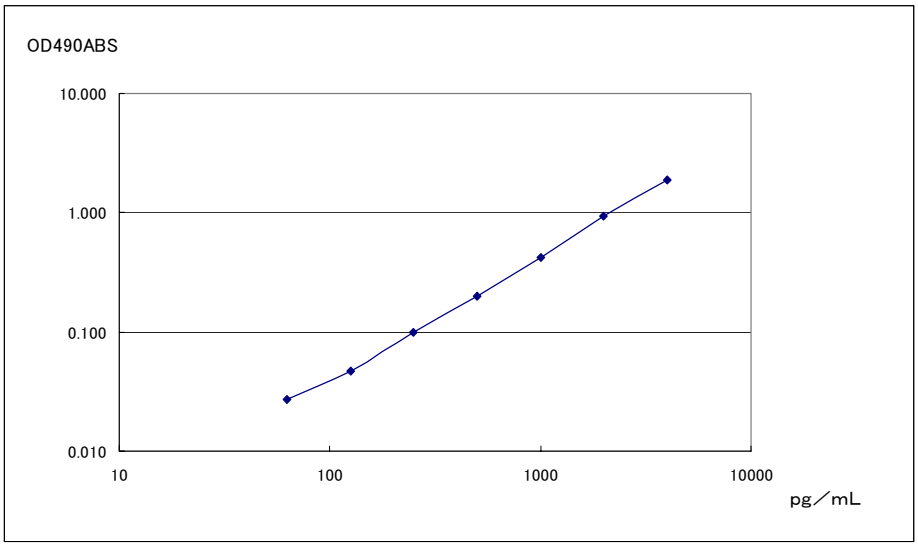
11. To quantitate accurately, always run a standard curve in each plate when testing samples..

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

13. Satisfactory performance of the test is guaranteed only when reagents are used from combination pack with identical lot number.

Ⅵ. Performance Characteristics

Typical standard curve



Analytical recovery

＜Human serum 1＞

Glicentin added pg/mL	Observed pg/mL	Expected pg/mL	Recovery %
0.00	282.96		
97.56	327.00	380.52	85.94
190.50	382.27	473.46	80.74
447.80	616.94	730.76	84.42
1330.00	1290.49	1612.96	80.01

＜Human serum 2＞

Glicentin added pg/mL	Observed pg/mL	Expected pg/mL	Recovery %
0.00	7.54		
97.56	117.55	105.10	111.85
190.50	187.59	198.04	94.72
447.80	464.08	455.34	101.92
1330.00	1418.30	1337.54	106.04

＜Human serum 3＞

Glicentin added pg/mL	Observed pg/mL	Expected pg/mL	Recovery %
0.00	140.32		
97.57	186.17	237.88	78.26
190.50	312.35	330.82	94.42
447.80	711.58	588.12	120.99
1330.00	1490.59	1470.32	101.38

Dilution Test

Dilution	Undiluted	1 / 2	1 / 4	1 / 8
Serum 1	883.5	934.7	836.6	948.8
Serum 2	326.1	344.3	385.8	383.6
Serum 3	412.0	471.8	445.0	433.2

Precision and reproducibility

- Intra-assay CV(%) 2.8 ～ 5.5
- Inter-assay CV(%) 3.9 ～ 5.1

Assay range

62.5 － 4000 pg / mL

Ⅶ. Stability and Storage

＜Storage＞ Store all of the components at 2 to 8℃.

＜Shelf life＞ 3 month from the date of manufacturing
The expiry date is described on the label of kit.

＜Package＞ For 96 tests per 1 kit including standards

Ⅷ. References

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