YK090 Glucagon EIA

For determination of Rat, Mouse or Human Glucagon

FOR LABORATORY USE ONLY

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

YK090 Glucagon EIA Kit

Ⅰ. Introduction

According to many studies on glucagon immunoassay, it has been established that the antibody against the C-terminal fragment (19-29) of glucagon has specific binding with pancreatic glucagon, whereas the antibody against the N- terminal fragment (1-19) of glucagon has specific binding with both of pancreatic and intestinal glucagon (total glucagon). Once, 30K by Unger et. al had been widely used as an antibody specific for the C- terminal fragment of glucagon, but Nishino, Shima and Yanaihara et. al succeeded in producing pancreatic glucagon specific antibody using synthetic peptide with the C-terminal fragment (19-29) of glucagon as immunogen in 1981.

This EIA kit has been developed by using polyclonal antibody against glucagons (19-29), synthetic pancreatic glucagon as glucagon standard and biotinylated pancreatic glucagon as labeled antigen for the measurement of rat, mouse or human glucagon in plasma.

YK090 Glucagon EIA Kit

- ▼ This assay kit can measure Glucagon within the range of 41-10000 pg/mL
- ▼ The assay duration change according the sample volume: 100μL, 20-24 hr.＋1.5 hr.
  50μL, 44-48 hr.＋1.5 hr
- ▼ With one assay kit, 41 samples can be measured in duplicate
- ▼ Test sample: plasma (rat, mouse, human)
  Sample volume: 100μL or 50 μL
- ▼ The 96-well plate of this kit consisted by 8-wells strips. The kit can be used separately.
- ▼ Precision and reproducibility
  Intra-assay CV（%） 3.3-5.1
  Inter-assay CV（%） 7.3-18.9
- ▼ Stability and storage
  Store all of the components at 2-8°C.
  This kit is stable under the condition for12 months from the date of manufacturing.
  The expiry date is stated on the package.

Contents

1) Antibody coated plate
2) Glucagon standard
3) Labeled antigen
4) SA-HRP solution
5) Substrate buffer
6) OPD tablet
7) Stopping solution
8) Buffer solution (A)
9) Buffer solution (B)
10) Washing solution (concentrated)
11) Adhesive foil

Ⅱ. Characteristics

This EIA kit is used for quantitative determination of rat, mouse or human pancreatic glucagon in plasma samples. The kit is characterized by sensitive quantification and high specificity. In addition, it is not influence by other components in plasma samples and sample pre-treatment is unnecessary.

Glucagon standard used in kit system is highly purified synthetic product (purity: higher than 98%) and biotinylated pancreatic glucagon is purified by HPLC.

＜Specificity＞

The EIA kit has high specificity to pancreatic glucagon and shows no cross reactivity with intestinal glucagon, GLP-1 and GLP-2.

＜Test Principle＞

This EIA kit for determination of rat, mouse or human pancreatic glucagon in plasma samples is based on a competitive enzyme immunoassay using combination of highly specific antibody to glucagon and biotin-avidin affinity system. The 96-well plate is coated with rabbit anti glucagon antibody. Glucagon standard or samples, and labeled antigen are added to the wells for competitive immunoreaction. After incubation and plate washing, HRP labeled streptoavidin (SA-HRP) is added to form HRP labeled streptoavidin-biotinylated pancreatic glucagon-antibody complex on the surface of the wells. Finally, HRP enzyme activity is determined by o-phenylenediamine dihydrochloride (OPD) and the concentration of rat, mouse or human pancreatic glucagon is calculated.

Ⅲ. Composition

	Component	Form	Quantity	Main Ingredient
1.	Antibody coated plate	Microtiter plate	1 plate (96 wells)	Rabbit anti glucagon antibody
2.	Glucagon standard	Lyophilized	1 vial (10 ng)	Synthetic glucagon
3.	Labeled antigen	Lyophilized	1 vial	Biotinylated glucagon
4.	SA-HRP solution	Liquid	1 bottle (12 mL)	HRP labeled streptoavidin
5.	Substrate buffer	Liquid	1 bottle (26 mL)	Citrate buffer containing 0.015% hydrogen peroxide
6.	OPD tablet	Tablet	2 tablets	o-Phenylenediamine dihydrochloride
7.	Stopping solution	Liquid	1 bottle (12 mL)	1M H_2SO_4
8.	Buffer solution (A)	Liquid	1 bottle (10 mL)	Phosphate buffer including serum
9.	Buffer solution (B)	Liquid	1 bottle (10 mL)	Phosphate buffer
10.	Washing solution (Concentrated)	Liquid	1 bottle (50 mL)	Concentrated saline
11.	Adhesive foil		4 sheets	

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 plasma samples:
   EDTA-2Na additive blood collection tube is recommended for the plasma sample collection and adds aprotinin 500KIU for every 1 mL blood immediately. It is strongly recommended that plasma samples should 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 (below -70°C is more stable.) and thawing before assay. Avoid repeated freezing and thawing of samples.

2. Preparation of standard solution：
   Reconstitute the standard (lyophilized Rat/mouse/human glucagon 10ng/vial) with 1mL of buffer solution (A), which affords 10,000 pg/mL standard solution. The reconstituted standard solution (0.5ml) is diluted with 1.0 mL of buffer solution (A), which yields 3,333 pg/mL standard solution. Repeat the same dilution to make each of standard solution 1,111, 370, 123, and 41 pg/mL. Buffer solution (A) is used as 0 pg/mL.

3. Preparation of labeled antigen solution：
   Reconstitute labeled antigen with 6 mL of buffer solution (B).

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

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

6. Other reagents are ready for use.

<Procedure>

< Procedure for 100 μL sample volume >

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

2. Fill 100 μL of each of standard solutions (0, 41, 123, 370, 1111, 3333, 10000 pg/mL) or samples, then introduce 50 μL of labeled antigen solution into the wells.

3. Cover the plate with adhesive foil and incubate it at 4°C for 20-24 hours.(Still, plate shaker not need)

4. After incubation, take off the adhesive foil, aspirate the solution in the wells and wash the wells 3 times with approximately 0.35 mL/well of washing solution. Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.

5. Pipette 100 μL 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 shaken with a microtiter plate shaker.

7. Resolve one 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 3 times with approximately 0.35 mL/well of washing solution. Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.

9. Add 100 μL of substrate solution containing OPD into the wells, cover the plate with adhesive foil and keep it for 20 minutes at room temperature for color reaction.

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

< Procedure for 50 μL sample volume >

1. Bring all the reagents and samples return to room temperature before starting assay.
2. Fill 50 μL of each of standard solutions (0, 41, 123, 370, 1111, 3333, 10000 pg/mL) or samples, and

then introduce 50 μL of labeled antigen solution into the wells.
3. Cover the plate with adhesive foil and incubate it at 4°C for 44-48 hours. (Still, plate shaker not need)
4. Same as 4.-11. of the above mentioned procedure for 100 μL sample volume.

Ⅴ. Notes

1. EDTA-2Na additive blood collection tube is recommended for the plasma sample collection and adds aprotinin 500KIU for every 1 mL blood immediately. It is strongly recommended that plasma samples should 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 (below -70°C is more stable.) and thawing before assay. Avoid repeated freezing and thawing of samples.

2. Glucagon standard solution, labeled antigen solution and substrate solution should be prepared immediately before use. This kit can be used dividedly in strips of the plate, In such case, the rest of reconstituted reagents (standard and labeled antigen solution) should be stored at or below –30°C.

3. During storage of washing solution (concentrated) at 2-8°C, precipitates may be observed, however they will be dissolved when diluted.

4. 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 new tip must be used for each standard and sample solution to avoid cross contamination.

5. When concentration of glucagon in samples is expected to exceed 10000 pg/mL, the sample needs to be diluted with buffer solution (A) to proper concentration.

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

7. Perform all the determination in duplicate.

8. Read optical absorbance of reaction solution in the wells immediately after stopping color reaction.

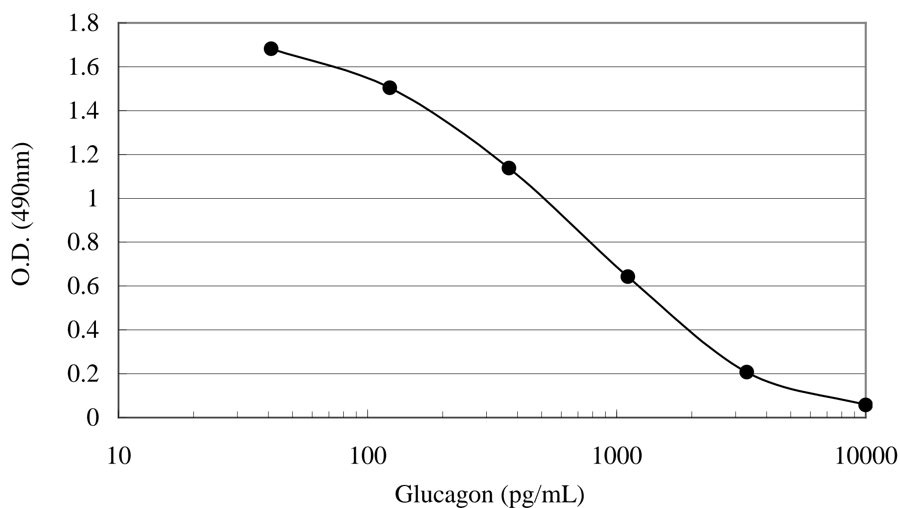
9. For accurate quantification, plot a standard curve for each assay.

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

11. Satisfactory performance of the assay is guaranteed only when reagents in combination pack with identical lot number are used.

Ⅵ. Performance Characteristics

Typical standard curve



Analytical recovery

＜Human plasma＞

Sample No.	Glucagon added (pg/mL)	Observed (pg/mL)	Expected (pg/mL)	Recovery (%)
1	0	316	-	-
2	200	536	516	110
3	500	856	816	108
4	1,000	1,316	1,316	101

Precision and reproducibility

- Intra-assay CV（%） 3.3-5.1
- Inter-assay CV（%） 7.3-18.9

Assay range

41-10,000 pg/mL

Ⅶ. Stability and Storage

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

＜Shelf life＞ The kit is stable under the condition for 12 months from the date of manufacturing. The expiry date is stated on the package.

＜Package＞ For 96 tests per one kit.

Ⅷ. References

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