COSMO BIO CO., LTD.
Inspiration for Life Science

# Glucagon EIA

## For determination of Rat, Mouse or Human Glucagon

## Cat. No. YII-YK090-EX

**FOR LABORATORY USE ONLY**

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## Contents



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

**YII-YK090-EX 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**

- ▼ The assay kit can measure Glucagon in the range of 50-10000 pg/mL
- ▼ Test 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, urine (rat, mouse, human)
  Sample volume: 100µL or 50 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 (%) 3.3-5.1
  Inter-assay CV (%) 7.3-18.9
- ▼ Stability and Storage
  Store all of the components at 2-8°C.
  12 months from the date of manufacturing.
  The expiry date is described on the label of kit.

**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 sample. The kit is characterized for sensitive quantification, high specificity and no influence with other components in plasma and needlessness of sample pre-treatment.

Glucagon standard used in the 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 sample is based on a competitive enzyme immunoassay using combination with highly specific antibody to glucagon and biotin-avidin affinity system. The 96-wells 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) are 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 | MTP[*1] | 1 plate (96 wells) | Rabbit anti glucagon antibody |
| 2. | Glucagon standard | Lyophilized | 1 vial | Synthetic pancreatic glucagon ( 10ng/vial) |
| 3. | Labeled antigen | Lyophilized | 1 vial | Biotinylated pancreatic glucagon |
| 4. | SA-HRP solution | Liquid | 1 bottle (12 mL) | HRP labeled streptoavidin |
| 5. | Substrate buffer | Liquid | 1 bottle (26 mL) | 0.015% Hydrogen peroxide |
| 6. | OPD tablet | Tablet | 2 tablets | o-Phenylenediamine dihydrochloride |
| 7. | Stopping solution | Liquid | 1 bottle (12 mL) | 2N $H_2SO_4$ |
| 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 | |

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

2) Preparation of labeled antigen solution：
Reconstitute labeled antigen with 6 mL of Buffer solution (B).

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＞

< Procedure for 100µL sample volume >

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

2. Fill 100µL of each of standard solutions ( 0, 41, 123, 370, 1111, 3333, 10000 pg/mL) or samples, then introduce 50µL 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, 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 (20-30°C) for 1 hour. During the incubation, the plate should be shake with a microtiter plate shaker.

7. Take off the adhesive foil, aspirate and wash the wells three times with approximately 0.35 mL/well of washing solution.

8. Add 100µL of substrate solution into the wells, cover the plate with adhesive foil and incubate it for 20 minutes at room temperature.

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

10. Read the optical absorbance of the wells at 490nm. 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 glucagon concentrations in samples from the corresponding absorbance values.

< Procedure for 50µL sample volume >

1. Bring all the reagents and samples return to room temperature before beginning the test.

2. Fill 50µL of each of standard solutions (0, 41, 123, 370, 1111, 3333, 10000 pg/mL) or samples, and then introduce 50µL 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, Shaker not need)

4-10. Same as 4.-10. of the above mentioned procedure for 100µL sample volume.

## Ⅴ. Notes

1. EDTA-2Na additive blood collection tube is recommended for the plasma collection and adds aprotinin 500KIU for every 1 mL blood immediately. Sample 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 -20°C and thawing before test. 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 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. Using clean test tubes or vessels in assay and use new tip for each sample to avoid cross contamination.

5. When sample value exceeds 10000 pg/mL, the sample needs to be diluted with Buffer solution (A) to proper concentration.

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

7. Perform all the determination in duplicate.

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

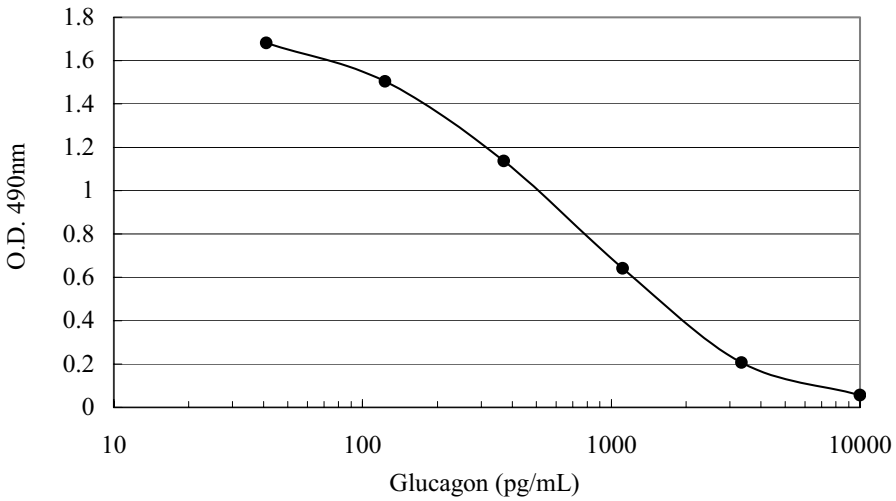
9. For accurate quantification, plot a standard curve each assay.

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

11. Satisfactory performance of the assay 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

50-10,000 pg/mL

## Ⅶ. Stability and Storage

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

＜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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2. Unger, R.H., Eisentraut, A.M., McCall, M.S. and Madison, L.L. (1961): Glucagon antibodies and an immunoassay for glucagon. *J. Clin. Invest.,* ***40*** : 1280-1289

3. Nishino, T., Kodaira, T., Shin, S., Imagawa, K., Shima, K., Kumahara, Y., Yanaihara, C. and Yanaihara, N.(1981): Glucagon radioimmunoassay with use of antiserum to glucagon C-terminal fragment. *Clin. Chem.,* ***27*** : 1690-1697

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**Manufactured by Yanaihara Institute Inc.**


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