Human Glucagon ELISA
(Multispecies specificity)

Cat. No.: RSCYK090R

1. 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 (9 – 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 glucagon (19 – 29), synthetic glucagon as standard antigen and biotinylated glucagon as labeled antigen for the measurment of rat, mouse or human glucagon in plasma.

2. Characteristics

This EIA kit is used for quantitative determination of rat, mouse or human pancreatic glucagon in plasma sample. It has a lot of advantage to perform the assay, such as good quantification, high specificity and no influence with other body fluid factors or physiogical active substances.

Glucagon standard is highly purified synthetic product (purity: higher than 98%) and biotinylated peptide 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 and glucagon standard or samples, and biotinylated glucagon are added to the wells for competitive immunoreaction. After rinsing out excess rat, mouse or human glucagon, HRP labeled streptoavidins are added to bind to the antigen-antibody complex so that HRP labeled streptoavidin - biotinylated glucagon – antibody complexs are formed on the surface of the wells. Finally, excess HRP labeled streptoavidins are rinsed out and HRP enzyme activity is determined and the concentration of rat, mouse or human pancreatic glucagon is calculated.

3. Composition

	Component	Form	Quantity	Main Ingredient
①	Antibody coated plate	MTP*1	1 plate (96 wells)	Rabbit anti glucagon
②	Glucagon standard	lyophilized	1 vial	Synthetic glucagons (10 ng/vial)
③	Labeled antigen	lyophilized	1 vial (1.5 ng)	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 hydrochloride
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……..Microtittration plate

4. 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 microtiter plate and dispenser for approximately 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 (1,000 mL)
7)Distilled water or deionized water

Preparatory work

1. Preparation of standard solution:
   Reconstitute the standard(lyophilized Rat/human glucagon 10ng/vial) with 1mL of Buffer solution (A), which affords 10,000 pg/mL standard solution. 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 of 1,111, 370, 123, 41 pg/mL.. Buffer solution (A) is used as 0 ng/mL.

2. Preparation of labeled antigen:
   Reconstitute labeled antigen with 6mL 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

1. Warm up the reagents and samples to room temperature 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 into the wells .
3. Cover the plate with adhesive foil and incubate it at 4°C overnight (20 - 24 hours).

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 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 shake with a plate rotator.

7. 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.

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

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

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

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

12. Use the standard curve to read glucagon concentrations in samples from the corresponding absorbance values.

Procedure for 50 μL sample volume

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

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

3. Cover the plate with adhesive foil and incubate it at 4°C two overnight (44-48 hours).

4. – 12. Same as 4 – 12. of the above mentioned procedure.

5. Notes

1. Plasma 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 test tubes in small amount and frozen at or below – 30°C. Avoid repeated freezing and thawing of plasma samples.

2. Glucagon standard, Labeled antigen, and OPD 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°C.

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

4. 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 to avoid cross contamination.

5. When sample value exceeds 10 ng/mL, it needs to be diluted with buffered solution within the assay range.

6. During incubation with SA-HRP solution at room temperature, the test plate should be rotated gently by plate rotator to promote immunoreaction.

7. 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.

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

9. Perform all the determination in duplicate.

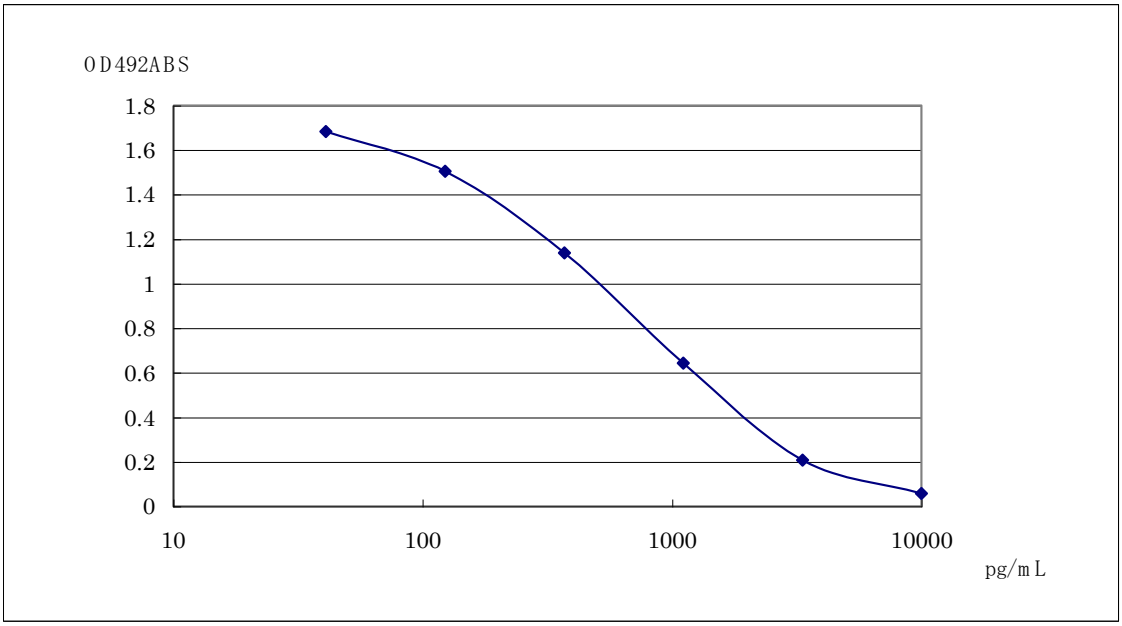
10. To quantitate accurately, always run a standard curve when testing samples.

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

6. 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	316	-
2	200	536	516	110
3	500	856	816	108
4	1000	1316	1316	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

7. 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 1 kit including standards

8. References

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Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium
http://www.gentaur-worldwide.com