Glucagon (Human, Mouse, Rat) ELISA

For the quantitative determination of Glucagon in human, mouse and rat plasma

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 48-GLUHU-E01
Size: 96 wells
Version: February 14, 2020 – ALPCO 2.0

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Intended Use

The Glucagon (Human, Mouse, Rat) ELISA kit is used for the quantitative determination of rat, mouse or human pancreatic glucagon in plasma samples. For research use only. Not for use in diagnostic procedures.

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). Originally, a 30K protein identified by Unger et. al had been widely used to produce antibodies specific for the C-terminal fragment of glucagon, but in 1981, Nishino, Shima, Yanaihara et. al succeeded in producing a pancreatic glucagon specific antibody using a synthetic peptide representative of the C-terminal fragment (19-29) of glucagon as the immunogen.

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

Glucagon ELISA Performance Summary

- This assay kit can measure Glucagon within the range of 41-10,000 pg/mL
- The assay duration varies 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 50µL
- The 96-well plate of this kit consists of 8-wells strips that 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 this condition for 12 months from the date of manufacture.
    - The expiry date is stated on the package.

Contents Summary

1. Antibody coated plate
2. Glucagon standard
3. Labeled antigen
4. SA-HRP solution
5. Substrate buffer
6. OPD tablet
7. Stop solution
8. Buffer solution (A)

9. Buffer solution (B)
10. Wash solution (concentrated)
11. Adhesive foil

Assay Characteristics

The kit is characterized by sensitive quantification and high analytical specificity. In addition, it is not influenced by other components in plasma samples and sample pre-treatment is unnecessary.

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

Analytical Specificity

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

Principle of the Assay

This ELISA kit for determination of rat, mouse or human pancreatic glucagon in plasma samples is based on a competitive enzyme immunoassay using a combination of a highly specific antibody to glucagon and the biotin-avidin affinity system. The 96-well plate is coated with rabbit anti-glucagon antibody. Glucagon standard or samples and biotinylated pancreatic glucagon antigen are added to the wells for competitive immunoreaction. After incubation and plate washing, HRP-labeled streptavidin (SA-HRP) is added to form HRP-labeled streptavidin-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.

Materials

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

Equipment Required, but Not Provided

1. Microtiter plate reader which can read extinction 2.5 at 490 nm
2. Microtiter plate shaker capable of 100 rpm
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 (1000 mL)
7. Distilled water or deionized water

Sample Handling

Preparation of plasma samples:
EDTA-2Na additive blood collection tube is recommended for the plasma sample collection and aprotinin (500kIU for every 1 mL blood) should be added immediately. It is strongly recommended that plasma samples should be tested as soon as possible after collection. If the samples are tested later, they should be aliquoted into test tubes and frozen at or below -30°C (below -70°C is recommended) and thawed prior to analysis. Avoid repeated freezing and thawing of samples.

Reagent Preparation

1. *Preparation of standard solution*
   Reconstitute the standard (lyophilized rat/mouse/human glucagon, 10ng/vial) with 1mL of buffer solution (A), which yields a 10,000 pg/mL standard solution. Take 0.5 mL of the reconstituted standard solution and dilute with 1.0 mL of buffer solution (A), which yields a 3333 pg/mL standard solution. Repeat the same dilution to make each of standard solutions (1111, 370, 123, and 41 pg/mL). Buffer solution (A) is used as 0 pg/mL.

2. *Preparation of labeled antigen solution*
   Reconstitute labeled antigen with 6 mL of buffer solution (B).

3. *Preparation of substrate solution*
   Dissolve one OPD tablet with 12 mL of substrate buffer. It should be prepared immediately before use.

4. *Preparation of wash solution*
   Dilute 50 mL of wash solution (concentrated) to 1000 mL with distilled or deionized water.

5. Other reagents are ready for use.

Assay Procedures

Procedure for 100 µL sample volume

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

2. Add 100 µL of each of standard solution (0, 41, 123, 370, 1111, 3333, 10000 pg/mL) or sample into the designated wells. Then add 50 µL of labeled antigen solution into each well.

3. Cover the plate with adhesive foil and incubate it at 4°C for 20-24 hours (no shaking, plate shaker not need).

4. After incubation, remove the adhesive foil, aspirate the wash solution in the wells and wash the wells 3 times with approximately 0.35 mL/well of wash solution. Finally, invert the plate and tap it onto an absorbent surface, such as a paper towel, to ensure blotting of most of the residual wash 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 set to 100 rpm.

7. Dissolve one OPD tablet with 12 mL of substrate buffer. It should be prepared immediately before use.

8. Remove adhesive foil, aspirate and wash the wells 3 times with approximately 0.35 mL/well of wash solution. Finally, invert the plate and tap it onto an absorbent surface, such as paper towel, to ensure blotting of most of the residual wash 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 to occur.

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

11. Read optical absorbance of the solution in the wells at 490 nm with a microplate reader.

Procedure for 50 µL sample volume

1. Bring all the reagents and samples return to room temperature before starting assay.

2. Add 50 µL of each of standard solution (0, 41, 123, 370, 1111, 3333, 10000 pg/mL) or sample into the designated wells. Then add 50 µL of labeled antigen solution into each well.

3. Cover the plate with adhesive foil and incubate it at 4°C for 44-48 hours (no shaking, plate shaker not need).

4. Follow the same steps 4-10 of the above mentioned procedure for 100 µL sample volume.

Calculation of Results

The dose response curve of the assay fits best with 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 semi-logarithmic graph paper (abscissa: concentration of standard antigen; ordinate: absorbance value). Use the average absorbance of each sample to determine the corresponding value by simple interpolation from the standard curve.

Notes

1. EDTA-2Na additive blood collection tube is recommended for the plasma sample collection and aprotinin (500kIU for every 1 mL of blood) should be added immediately. It is strongly recommended that plasma samples should be tested as soon as possible after collection. If the samples are tested later, they should be aliquoted into test tubes and frozen at or below -30°C (below -70°C is recommended) and thawed prior to analysis. Avoid repeated freezing and thawing of samples.

2. Glucagon standard solution, labeled antigen solution and substrate solution should be prepared immediately before use. Use only enough plate strips as required for the analysis. If all the strips are not required, the remainder of the reconstituted reagents (standard and labeled antigen solution) should be stored at or below -30°C.

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

4. Pipetting technique may affect the precision of the assay. Pipette standard solutions or samples into each well of plate precisely. Use clean test tubes or vessels during the analysis, and new pipette tips must be used for each standard and sample solution to avoid cross contamination.

5. When the concentration of glucagon in samples is expected to exceed 10,000 pg/mL, the sample needs to be diluted with buffer solution (A) prior to analysis.

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 determinations in duplicate.

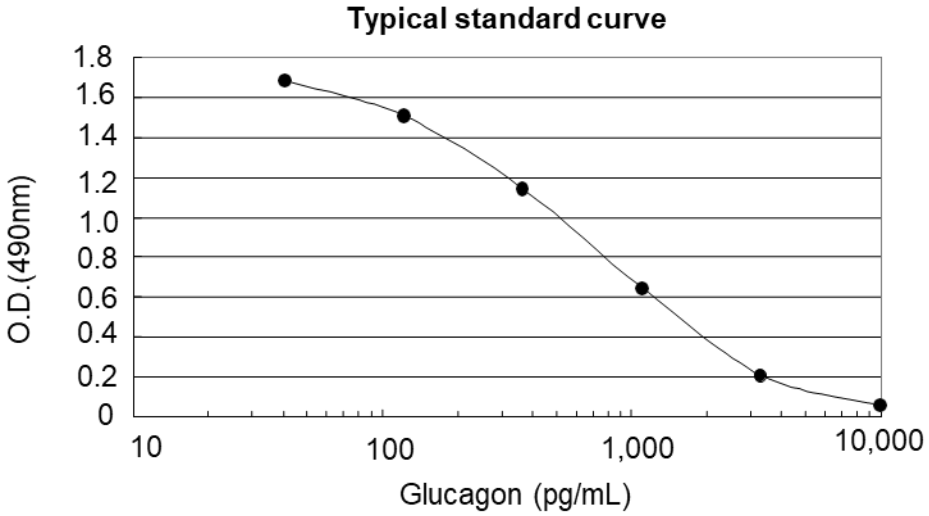
8. Read optical absorbance of reaction solution in the wells immediately after stopping the color reaction.

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

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

11. Satisfactory performance of the assay is guaranteed only when reagents from the same lot number are used.

Performance Characteristics



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	1000	1316	1316	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

Stability and Storage

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

Shelf life

The kit is stable under this condition for 12 months from the date of manufacturing. The expiry date is stated on the package.

References

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