



## **Glucagon (Human, Mouse, Rat) ELISA**

For the quantitative determination of glucagon in human, mouse,  
or rat plasma

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

Catalog Number: 48-GLUHU-E01  
Size: 96 wells  
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### **ALPCO Diagnostics**

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## I. Introduction

According to many studies on glucagon immunoassays, it is 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 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, Yanaihara, et al. succeeded in producing pancreatic glucagon specific antibodies using synthetic peptides with the C-terminal fragment (19-29) of glucagon as an immunogen in 1981.

This ELISA kit has been developed by using polyclonal antibodies 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 (Human, Mouse, Rat) ELISA

- This assay kit can measure glucagon within the range of 50-10,000 pg/mL.
- The assay duration changes according to the sample volume:  
100  $\mu$ L, 20-24 hr + 1.5 hr  
50  $\mu$ L, 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: 100  $\mu$ L or 50  $\mu$ L
- The 96-well plate of this kit consists of 12 8-well strips. The strips can be divided and 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 these conditions for 12 months from the date of manufacturing. The expiry date is stated on the package label.

### Contents

- 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

## II. Characteristics

This ELSIA kit is used for the quantitative determination of rat, mouse, or human pancreatic glucagon in plasma samples. This kit is characterized by sensitive quantification and high specificity. There is no need for sample pre-treatment and other components of plasma do not interfere with the assay.

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

### *Specificity*

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

### *Assay Principle*

This ELISA kit for the determination of rat, mouse, or human pancreatic glucagon in plasma samples is based on a competitive enzyme immunosorbent assay using a combination of a highly specific antibody to glucagon and a biotin-avidin affinity system. The 96-well plate is coated with rabbit anti-glucagon antibody. Glucagon standards, samples, and the labeled antigen are added to the wells for a competitive immunoreaction. After incubation and plate washing, HRP-labeled streptavidin (SA-HRP) is added to form HRP labeled streptavidin-biotinylated pancreatic glucagon-antibody complexes 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 streptavidin
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. Stop solution	Liquid	1 bottle (12 ml)	1 M H <sub>2</sub> SO <sub>4</sub>
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. Wash 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) Deionized or distilled water

### *Preparatory work*

- 1) Preparation of standard solution:  
Reconstitute the standard (lyophilized rat/mouse/human glucagon 10 ng/vial) with 1 ml of buffer solution A, which yields a 10,000 pg/ml standard solution. Dilute 0.5 ml of the reconstituted standard solution with 1.0 ml of buffer solution A, which yields a 3,333 pg/ml standard solution. Repeat the same dilution to make each of the standard solutions: 1,111; 370; 123; and 41 pg/ml. Buffer solution A is used as the 0 pg/ml standard.
- 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 1,000 ml with deionized or distilled water.
- 5) Other reagents are ready for use.

### *Procedure*

#### *Procedure for 100 $\mu$ l sample volume*

1. Bring all the reagents and samples to room temperature (20-30°C) before starting the assay.
2. Pipette 100  $\mu$ l of each of the standard solutions (0; 41; 123; 370; 1,111; 3,333; 10,000 pg/ml) and samples, then add 50  $\mu$ l of labeled antigen solution to the wells.
3. Cover the plate with adhesive foil and incubate it at 4°C for 20-24 hours. (Plate shaker is not required for this step.)

4. After the incubation, take off the adhesive foil, aspirate the solution in the wells, and wash the wells three times with approximately 0.35 ml/well of wash solution. Finally, invert the plate and tap it onto an absorbent surface, such as paper towels, to ensure that most of the residual wash solution is gone.
5. Pipette 100  $\mu$ l 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. Dissolve one OPD tablet with 12 ml of substrate buffer. It should be prepared immediately before use.
8. Take off the adhesive foil, aspirate the solution in the wells, and wash the wells three times with approximately 0.35 ml/well of wash solution. Finally, invert the plate and tap it onto an absorbent surface, such as paper towels, to ensure that most of the residual wash solution is gone.
9. Add 100  $\mu$ l of substrate solution (containing OPD) to the wells, cover the plate with adhesive foil, and incubate the plate for 20 minutes at room temperature for the color reaction.
10. Add 100  $\mu$ l of stop solution to the wells to stop the color reaction.
11. Read the optical absorbance of the wells at 490 nm. Calculate mean absorbance values of standard solutions and plot a standard curve on semilogarithmic 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.

*Procedure for 50  $\mu$ l sample volume*

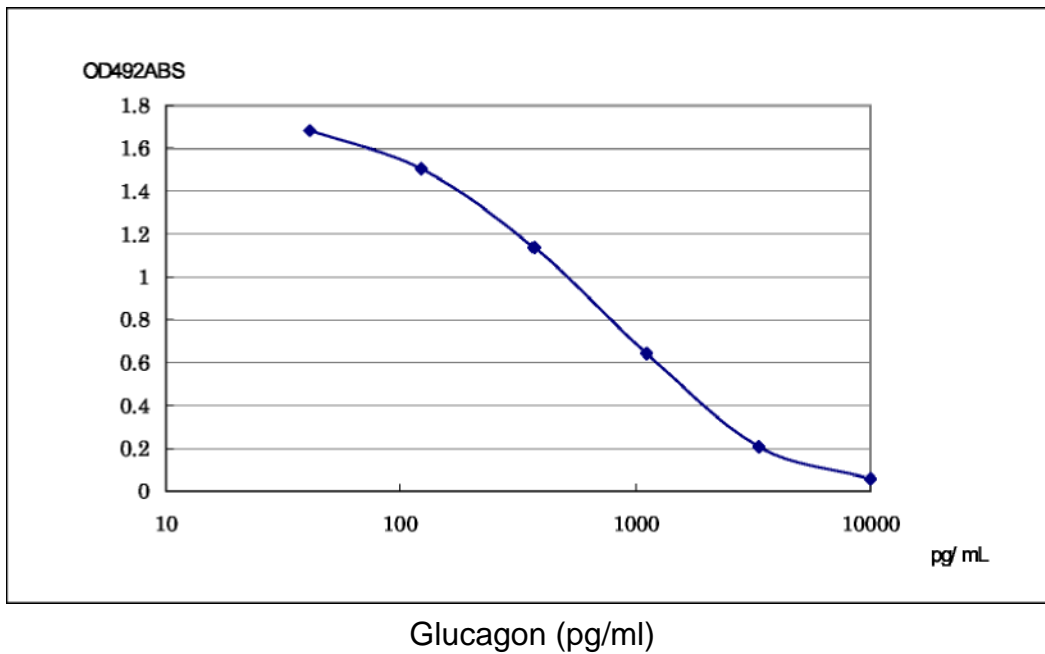
1. Bring all the reagents and samples to room temperature (20-30°C) before starting the assay.
2. Pipette 50  $\mu$ l of each of the standard solutions ( 0; 41; 123; 370; 1,111; 3,333; 10,000 pg/ml) and samples, then add 50  $\mu$ l of labeled antigen solution to the wells.
3. Cover the plate with adhesive foil and incubate it at 4°C for 44-48 hours. (Plate shaker is not required for this step.)
- 4.-11. Same as steps 4-11 of the procedure above for the 100  $\mu$ l sample volume.

## V. Notes

1. It is recommended that EDTA-2Na additive blood collection tubes are used for the plasma sample collection and that aprotinin 500KIU is immediately added for every 1 ml of blood. It is also strongly recommended that plasma samples should be used as soon as possible after collection. If the samples are to be tested later, they should be divided into aliquots and frozen at or below  $-30^{\circ}\text{C}$ , and then thawed before assaying. Avoid repeated freezing and thawing of samples.
2. The glucagon standard solution, labeled antigen solution, and substrate solution should be prepared immediately before use. The strips of the plate can be separated and used at different times. In such cases, the reconstituted reagents (standard and labeled antigen) should be stored at or below  $-30^{\circ}\text{C}$  between assay runs.
3. Precipitates may be observed during storage of the wash solution (concentrated) at  $2-8^{\circ}\text{C}$ ; however, they will dissolve during the dilution of the wash solution.
4. Pipetting operations may affect the precision of the assay. Pipette standard solutions and samples into the wells of the plate precisely. Use clean test tubes or vessels in the assay, and a new tip must be used for each standard and sample solution to avoid cross contamination.
5. When the concentration of glucagon in a sample is expected to exceed 10,000 pg/ml, the sample needs to be diluted with buffer solution A to bring the concentration within the assay's range.
6. The step 6 incubation (after the addition of the SA-HRP solution) requires that the plate be shaken gently with a microtiter plate shaker to promote the immunoreaction. The  $4^{\circ}\text{C}$  incubation and the color reaction incubation do not require the use of a plate shaker.
7. Perform all the determinations in duplicate.
8. Read the optical absorbance of the reaction solution in the wells immediately after stopping the 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 with identical lot numbers 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* The kit is stable under the conditions of storage specified for 12 months from the date of manufacturing. The expiry date is stated on the package label.

*Package* For 96 tests per one kit.

## VIII. References

- 1) Unger, R.H., Eisentraut, A.M., McCall, M.S., Keller, S., Lanz, H.C. and Madison, L. L. (1959): Glucagon antibodies and their use for immunoassay for glucagon. *Proc. Soc. Exp. Biol. Med.*, **102**: 621-623.
- 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.
- 4) Jaspan, J.B., and Rubenstein, A.H. (1977): Circulating glucagon: Plasma profiles and metabolism in health and disease. *Diabetes.*, **26**: 887-902.
- 5) Glucagon related peptides (1993): (Okuno, G., Ohneta, A. and Shima, K. ed.) pp. 52-65. Ishiyaku, Tokyo.