Technical Manual

Human Glucagon PharmaGenie ELISA Kit

- Catalogue Code: SBRS0028
- Competitive ELISA Principle
- Research Use Only

Contents

Key features and Sample Types

Aliases:

Glucagon

Gene ID:

10068

Uniprot:

2641

Detection method:

Competitive (Quantitative)

Range:

1-1,000 pg/ml

Sensitivity:

4.77 pg/ml

Sample Types:

Cell Culture Supernatants, Serum

Reactivity:

Human

Storage & Expiry

The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C. For prepared reagent storage, see table below.

Introduction

Glucagon is a 29-amino acid peptide hormone secreted by the pancreas. Its function is to raise blood glucose levels to opposing the effect of insulin, which lowers blood glucose levels.

Glucagon is synthesized and secreted from alpha cells of the endocrine portion of the pancreas. In rodents, the alpha cells are located in the outer rim of the islet. However, alpha cells in human pancreas are distributed throughout the islet.

Glucagon and insulin are part of a feedback system that keeps blood glucose levels at a stable level. The pancreas releases glucagon when glucose levels fall too low. Glucagon causes the liver to convert stored glycogen into glucose, which is released into the bloodstream. Glucagon raises blood glucose levels. High blood glucose levels stimulate the release of insulin. Insulin allows glucose to be taken up and used by insulin-dependent tissues.

Glucagon has important clinical applications. Abnormally-elevated levels of glucagon may be caused by pancreatic tumors, such as glucagonoma, with symptoms including necrolytic migratory erythema, reduced amino acids, and hyperglycemia. It may occur alone or in the context of multiple endocrine neoplasia type 1 (MENI).

How do our ELISA kits work?

The Assay Genie Human Glucagon ELISA kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human Glucagon in serum and cell culture supernatants. This assay employs an antibody specific for human Glucagon coated on a 96- well plate. Standards and samples are pipetted into the wells and Glucagon present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human Glucagon antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of Glucagon bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Kit Contents

Each kit contains reagents for 96 assays including:

No.	Component	96-Well Kit	Storage
1	Microplate coated with anti-Human Glucagon	8 x 12	1 month at 4°C*
2	Wash Buffer Concentrated (20X)	25ml	1 month at 4°C
3	Standard Glucagon Peptide	2 vials	Do not store and reuse
4	Anti-Glycogen Polyclonal Antibody	2 vials	Do not store and reuse
5	Assay Diluent B (5X)	15ml	1 month at 4°C
6	Biotinylated Glucagon Peptide	2 vials	Do not store and reuse
7	HRP- Streptavidin Concentrate (200X)	600µl	1 month at 4°C
8	Positive Control	1 vial	Do not store and reuse
9	TMB One-Step Substrate Reagent	12ml	N/A
10	Stop Solution	8ml	N/A

*Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

Additional materials required:

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Precision pipettes to deliver 2 µl to 1 ml volumes.
3. Adjustable 1-25 ml pipettes for reagent preparation.
4. 100 ml and 1 liter graduated cylinders.
5. Absorbent paper.
6. Distilled or deionized water.
7. Log-log graph paper or computer and software for ELISA data analysis.
8. Tubes to prepare the positive control or sample dilutions.

Reagent Preparation

A. Preparation of Plate and Anti-Glucagon Antibody

1. Equilibrate plate to room temperature before opening the sealed pouch.

2. Label removable 8-well strips as appropriate for your experiment.

3. 5X Assay Diluent B should be diluted 5-fold with deionized or distilled water.

4. Briefly centrifuge the anti-Glucagon antibody vial and reconstitute with 55 µl of 1X Assay Diluent B to prepare the antibody concentrate. Pipette up and down to mix gently.

5. The antibody concentrate should then be diluted 100-fold with 1X Assay Diluent B. This is your anti-Glucagon antibody working solution, which will be used in step 2 of Assay Procedure (Section VIII).

Note: The following steps may be done during the antibody incubation procedure (step 2 of Assay Procedure)

B. Preparation of Biotinylated Glucagon

6. Briefly centrifuge the vial of biotinylated Glucagon and reconstitute with 20 µl of ddH2O before use.

7. Transfer the entire contents of the biotinylated Glucagon vial into a tube containing 10 ml of 1X Assay Diluent B. This is your Working Stock of biotinylated Glucagon. Pipette up and down to mix gently. The final concentration of biotinylated Glucagon will be 80 pg/ml.

a. Second Dilution of biotinylated Glucagon for Standards: Add 2 ml of Working Stock Biotinylated Glucagon to 2 ml of 1X Assay Diluent B. The final concentration of biotinylated Glucagon will be 40 pg/ml.

b. Second Dilution of biotinylated Glucagon for Positive Control: Add 100 µl of Working Stock biotinylated Glucagon peptide to 100 µl of the prepared Positive Control. (See section D for Positive Control preparation) The final concentration of biotinylated Glucagon will be 40 pg/ml.

c. c. Second Dilution of biotinylated Glucagon for samples: Add 125 µl of Working Stock Biotinylated Glucagon to 125 µl of prepared sample (see section E for sample preparation). This is a 2-fold dilution of your sample. The final concentration of biotinylated Glucagon will be 40 pg/ml.

C. Preparation of Standards

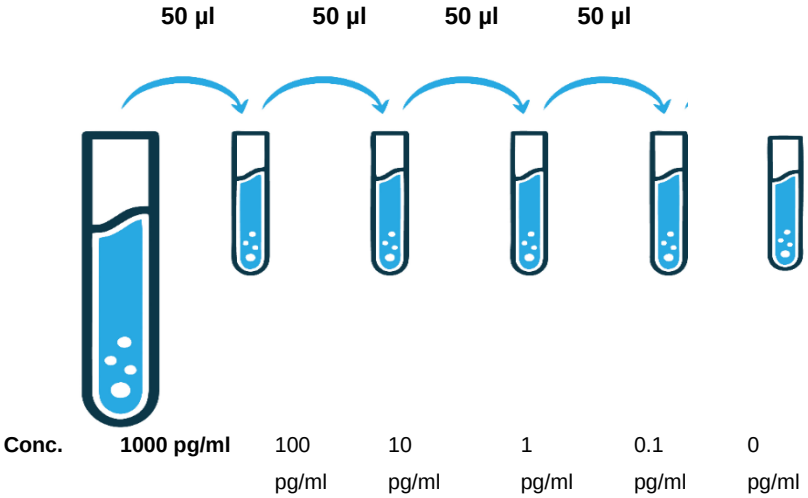
8. Label 6 microtubes with the following concentrations: 1,000 pg/ml, 100 pg/ml, 10 pg/ml, 1 pg/ml, 0.1 pg/ml and 0 pg/ml. Pipette 450 µl of biotinylated Glucagon working solution (prepared in step 7a) into each tube, except the 1,000 pg/ml (leave this one empty). It is very important to make sure the concentration of biotinylated Glucagon is 40 pg/ml in all standards.

9. Briefly centrifuge the vial of Glucagon Standard. Reconstitute with 10 µl of ddH2O and briefly vortex if desired. Pipette 8 µl of Glucagon Standard and 792 µl of 40 pg/ml biotinylated Glucagon working solution (prepared in step 7a) into the tube labeled 1000 pg/ml. Mix thoroughly. This solution serves as the first standard (1000 pg/ml Glucagon standard, 40 pg/ml biotinylated Glucagon).

10. To make the 100 pg/ml standard, pipette 50 µl of the 1000 pg/ml Glucagon standard into the tube labeled 100 pg/ml. Mix thoroughly.

11. Repeat this step with each successive concentration, preparing a dilution series as shown in the illustration below. Each time, use 450 µl of biotinylated Glucagon and 50 µl of the prior concentration until the 0.1 pg/ml is reached. Mix each tube thoroughly before the next transfer.

DILUTION SERIES



D. Positive Control Preparation

12. Briefly centrifuge the Positive Control vial and reconstitute with 100 µl of ddH2O.

13. Refer to step 7b. This is a 2-fold dilution of the Positive Control. The final concentration of biotinylated Glucagon should still be 40 pg/ml. The Positive Control is a cell culture media sample that serves as a system control to verify that the kit components are working. The resulting OD will not be used in any calculations, if no positive competition is observed please contact Assay Genie Technical Support. The Positive Control may be diluted further if desired, but be sure the final concentration of biotinylated Glucagon is 40 pg/ml.

E. Sample Preparation

14. If you wish to perform a 2-fold dilution of your sample, proceed to step 7c. If you wish to perform a higher dilution of your sample, dilute your sample with 1X Assay Diluent B before performing step 7c. EXAMPLE (to make a 4-fold dilution of sample):

a. Dilute sample 2-fold (62.5 µl of sample + 62.5 µl of 1X Assay Diluent B.).

b. Perform step 7c (125 µl of working solution biotinylated Glucagon + 125 µl of sample prepared above).

The total volume is 250 µl, enough for duplicate wells on the microplate. It is very important to make sure the final concentration of the biotinylated Glucagon is 40 pg/ml.

Note: Optimal sample dilution factors should be determined empirically, however you may reference below for recommended dilution factors for serum: Human=2X Mouse=2X Rat=2X.

F. Preparation of Wash Buffer and HRP

15. If 20X Wash Buffer Concentrate contains visible crystals, warm to room temperature and mix gently until dissolved.

16. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1X Wash Buffer.

17. Briefly centrifuge the HRP-Streptavidin vial before use.

18. Dilute the HRP-Streptavidin concentrate 200-fold with 1X Assay Diluent B.

Assay Procedure

1. Keep kit reagents on ice during reagent preparation steps. It is recommended that all standards and samples be run at least in duplicate.

2. Add 100 µl of Anti-Glucagon Antibody (See Reagent Preparation step 5) to each well. Incubate for 1.5 hours at room temperature with gentle shaking (1-2 cycle/sec). You may also incubate overnight at 4ºC.

3. Discard the solution and wash wells 4 times with 1X Wash Solution Buffer (200- 300 µl each). Washing may be done with a multichannel pipette or an automated plate washer. Complete removal of liquid at each step is essential to good assay performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

4. Add 100 µl of each standard (see Reagent Preparation Section C), Positive Control (see Reagent Preparation Section D) and sample (see Reagent Preparation Section E) to appropriate wells. Be sure to include a blank well (Assay Diluent only). Cover wells and incubate for 2.5 hours at room temperature with gentle shaking (1-2 cycles/sec) overnight or at 4ºC.

5. Discard the solution and wash 4 times as directed in Step 3. 10

6. Add 100 µl of prepared HRP-Streptavidin solution (see Reagent Preparation step 18) to each well. Incubate for 45 minutes at room temperature with gentle shaking. It

is recommended that incubation time should not be shorter or longer than 45 minutes.

7. Discard the solution and wash 4 times as directed in Step 3.

8. Add 100 µl of TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking (1-2 cycles/sec).

9. Add 50 µl of Stop Solution to each well. Read at 450 nm immediately.

Assay Procedure Summary

1. Prepare all reagents, samples and standards as instructed.

2. Add 100 µl anti-Glucagon to each well. Incubate 1 hour at room temperature.

3. Add 100 µl standard or sample to each well. Incubate 2.5 hours at room temperature

4. Add 100 µl prepared Streptavidin solution. Incubate 45 minutes at room temperature.

5. Add 100 µl TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.

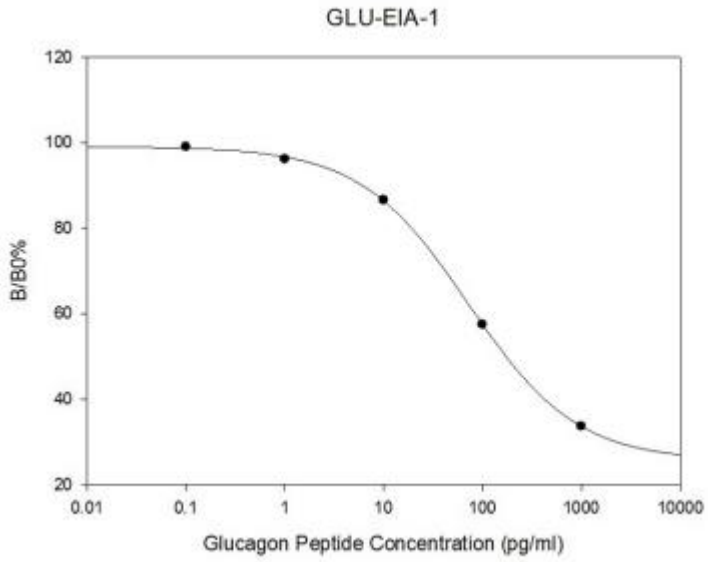
6. Add 50 µl Stop Solution to each well. Read at 450 nm immediately.

Calculation of Results

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

Typical Data

These standard curves are for demonstration only. A standard curve must be run with each assay.



B. Sensitivity

The minimum detectable concentration of Glucagon is 4.77 pg/ml.

C. Spiking and Recovery

1-1,000 pg/ml

D. Reproducibility

Intra-Assay CV%: <10%

Inter-Assay CV%: <12%

D. Assay Diagram

Recommended Plate Layout

Blank	Blank	SA1	SA1	SA9	SA9	SA17	SA17	SA25	SA25	SA33	SA33
Total Binding	Total Binding	SA2	SA2	SA10	SA10	SA18	SA18	SA26	SA26	SA34	SA34
Standard1	Standard1	SA3	SA3	SA11	SA11	SA19	SA19	SA27	SA27	SA35	SA35
Standard2	Standard2	SA4	SA4	SA12	SA12	SA20	SA20	SA28	SA28	SA36	SA36
Standard3	Standard3	SA5	SA5	SA13	SA13	SA21	SA21	SA29	SA29	SA37	SA37
Standard4	Standard4	SA6	SA6	SA14	SA14	SA22	SA22	SA30	SA30	SA38	SA38
Standard5	Standard5	SA7	SA7	SA15	SA15	SA23	SA23	SA31	SA31	SA39	SA39
Pos Control	Pos Control	SA8	SA8	SA16	SA16	SA24	SA24	SA32	SA32	SA40	SA40

Key:

Blank = Buffer Only

Total Binding = Biotin-Glucagon only

Standard 1 = 1000 pg/ml

Standard 2 = 100 pg/ml

Standard 3 = 10 pg/ml

Standard 4 = 1 pg/ml

Standard 5 = 0.1 pg/ml

Pos Control = Biotin with positive control

Specificity

Cross Reactivity: This EIA kit shows no cross-reactivity with any of the cytokines tested: Ghrelin, Nesfatin, Angiotensin II, NPY and APC.

Troubleshooting

Problem	Causes	Solutions
Low signal in samples	• Improper preparation of detection antibody • Too brief incubation times • Inadequate reagent volumes or improper dilution	• Briefly spin down vials before opening. Dissolve the powder thoroughly. • Ensure sufficient incubation time; assay procedure step 2 may be done overnight • Check pipettes and ensure correct preparation
Poor standard curve	• Inaccurate pipetting • Improper standard dilution	• Check pipettes • Briefly centrifuge Standard Protein and dissolve the powder thoroughly by gently mixing
Large CV	• Inaccurate pipetting • Air bubbles in wells	• Check pipettes • Remove bubbles in wells
High background	• Plate is insufficiently washed • Contaminated wash buffer	• Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed. • Make fresh wash buffer
Low sensitivity	• Improper storage of the ELISA kit • Stop solution	• Follow storage recommendations in the storage section of the technical manual. Keep substrate solution protected from light. • Add stop solution to each well before reading plate • Ensure correct dilution

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

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