Glucagon ELISA Kit

Enzyme Immunoassay for the quantification of Glucagon in Human, Mouse, Rat, Pig serum, plasma, cell culture supernatants

Catalog number: ARG81293

distributed in the US/Canada by:
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INTRODUCTION

The protein encoded by this gene is actually a preproprotein that is cleaved into four distinct mature peptides. One of these, glucagon, is a pancreatic hormone that counteracts the glucose-lowering action of insulin by stimulating glycogenolysis and gluconeogenesis. Glucagon is a ligand for a specific G-protein linked receptor whose signalling pathway controls cell proliferation. Two of the other peptides are secreted from gut endocrine cells and promote nutrient absorption through distinct mechanisms. Finally, the fourth peptide is similar to glicentin, an active enteroglucagon. [provided by RefSeq, Jul 2008]

Glucagon plays a key role in glucose metabolism and homeostasis. Regulates blood glucose by increasing gluconeogenesis and decreasing glycolysis. A counterregulatory hormone of insulin, raises plasma glucose levels in response to insulin-induced hypoglycemia. Plays an important role in initiating and maintaining hyperglycemic conditions in diabetes.

GLP-1 is a potent stimulator of glucose-dependent insulin release. Play important roles on gastric motility and the suppression of plasma glucagon levels. May be involved in the suppression of satiety and stimulation of glucose disposal in peripheral tissues, independent of the actions of insulin. Have growth-promoting activities on intestinal epithelium. May also regulate the hypothalamic pituitary axis (HPA) via effects on LH, TSH, CRH, oxytocin, and vasopressin secretion. Increases islet mass through stimulation of islet neogenesis and pancreatic beta cell proliferation. Inhibits beta cell apoptosis.

GLP-2 stimulates intestinal growth and up-regulates villus height in the small intestine, concomitant with increased crypt cell proliferation and decreased enterocyte apoptosis. The gastrointestinal tract, from the stomach to the colon

is the principal target for GLP-2 action. Plays a key role in nutrient homeostasis, enhancing nutrient assimilation through enhanced gastrointestinal function, as well as increasing nutrient disposal. Stimulates intestinal glucose transport and decreases mucosal permeability.

Oxyntomodulin significantly reduces food intake. Inhibits gastric emptying in humans. Suppression of gastric emptying may lead to increased gastric distension, which may contribute to satiety by causing a sensation of fullness.

Glicentin may modulate gastric acid secretion and the gastro-pyloro-duodenal activity. May play an important role in intestinal mucosal growth in the early period of life. [UniProt]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for Glucagon has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Glucagon present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for Glucagon is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Glucagon bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm.The concentration of Glucagon in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Standard (Lyophilized)	2 X 1 ng/vial	4°C
Standard/Sample diluent	1 X 16 ml	4°C
Antibody conjugate concentrate	2 vials (60 µl)	4°C
Antibody diluent buffer	16 ml	4°C
HRP-Streptavidin concentrate	2 vials (60 µl)	4°C (Protect from light)
HRP-Streptavidin diluent buffer	16 ml	4°C
20X Wash buffer	25 ml	4°C
TMB substrate	12 ml	4°C (Protect from light)
STOP solution	12 ml	4°C
Plate sealer	4 strips	Room temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: read at 610-650 nm as the reference wave length)
- Microplate shaker (shaking amplitude 3 mm; approx. 600 rpm-700) or orbital shaker
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Prior to beginning the assay procedure, bring all reagents to room temperature (20-25°C).
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernatants - Remove particulates by centrifugation for 10 min at 1000 x g and aliquot & store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

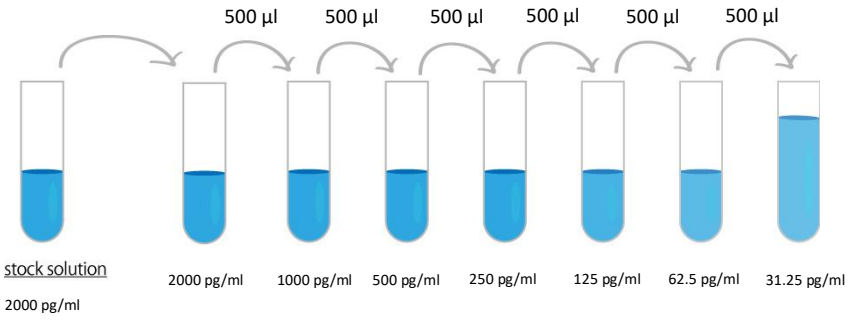
Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma on ice using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer.
- 1X Antibody conjugate: 5-10 minutes before use (freshly prepared is recommended), dilute 100X antibody conjugate concentrate into Antibody diluent buffer with Antibody diluent buffer to yield 1X detection antibody solution.
- 1X HRP-Streptavidin Solution: 5-10 minutes before use (freshly prepared is recommended), dilute 100X HRP-Streptavidin concentrate solution into HRP-Streptavidin diluent buffer to yield 1X HRP-Streptavidin Solution buffer.

- **Sample**: If the initial assay found samples contain Glucagon higher than the highest standard, the samples can be diluted with Standard/Sample diluent and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.
  (It is recommended to do pre-test to determine the suitable dilution factor).
- **Standards**: Reconstitute the standard with 0.5 ml Standard/Sample diluent to yield a stock concentration of 2000 pg/ml. Allow the stock standard to sit for at least 15 minutes with gentle agitation to make sure the standard is dissolved completely before making serial dilutions. The Standard/ Sample diluent serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted with Standard/ Sample diluent as according to the suggested concentration below: 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.25 pg/ml.



ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl of standards, samples and zero controls (Standard/Sample diluent) into wells. Incubate on a microplate shaker at 700 rpm for 120 minutes at RT.
3. Aspirate each well and wash, repeating the process three times for a total four washes. Wash by filling each well with 1× Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add 100 µl 1X Antibody conjugate into each well. Cover wells and incubate on a microplate shaker at 700 rpm for 120 minutes at RT.
5. Aspirate each well and wash as step 3.
6. Add 100 µl of 1X HRP-Streptavidin solution to each well. Cover wells and incubate on a microplate shaker at 700 rpm for 20 minutes at RT.
7. Aspirate each well and wash as step 3.
8. Add 100 µl of TMB Reagent to each well. Incubate for 20-25 minutes at RT in dark.
9. Add 50 µl of Stop Solution to each well. The color of the solution should change from blue to yellow. Gently tap the plate to ensure thorough mixing

10. Read the OD with a microplate reader at 450nm immediately. (Optional: read at 610-650 nm as the reference wave length). It is recommended read the absorbance within 5 minutes after adding the stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

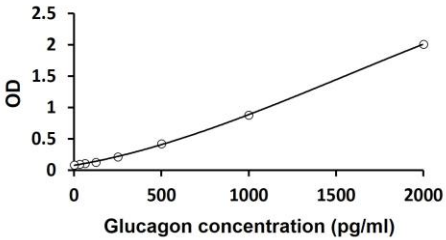
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Glucagon ranged from 31.25- 2000 pg/ml. The mean MDD was 15 pg/ml.

Specificity

This assay recognizes natural and recombinant Glucagon. No significant cross-reactivity or interference with the factors below was observed:

Glucentin-Related Polypeptide (aa 21-50), Glucagon-Like Peptide 1 (aa 92-128) and Glucagon-Like Peptide 2 (aa 146-178)

Intra-assay and Inter-assay precision

The CV values of both intra and inter precision fall below 10%.

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For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.

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