Quantikine® ELISA

Glucagon Immunoassay

Catalog Number DGCG0

For the quantitative determination of Glucagon concentrations in cell culture supernates, serum, and plasma

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Glucagon is a 29 amino acid (aa) peptide produced by the pancreas that plays a critical role in glucose metabolism and homeostasis (1-4). The Glucagon precursor mRNA is expressed by alpha cells (α-cells) of the pancreas, L cells of the intestine, and in the brain (1, 2). Only the pancreatic α-cells express the prohormone convertase PC2, also called PCSK2, which is required to produce Glucagon (2). Intestinal L cells instead express the prohormone convertase PC1, which processes the precursor to the Glucagon-overlapping peptides glicentin and oxyntomodulin. L cells also produce two Glucagon-like peptides, GLP-1 and GLP-2 that are derived from the same Glucagon precursor and influence glucose metabolism, but do not share any common sequence with Glucagon (1, 2). The aa sequence of the mature Glucagon peptide is identical in human, mouse, rat, pig, dog, horse, cow, sheep, and Xenopus.

In normal metabolism, Glucagon is secreted in response to low blood glucose (hypoglycemia) and downregulated in response to high blood glucose (hyperglycemia). Although Glucagon binding sites are found in liver, brain, pancreas, kidney, intestine, and adipose tissue, the main activity of Glucagon receptors occurs in the liver, where Glucagon stimulates gluconeogenesis and glycogenolysis, thereby increasing blood glucose (1-4). It is particularly important that the brain receive sufficient glucose, since it is unable to store more than a minute quantity. Therefore the release of Glucagon from α-cells is under control by both hormones and neurotransmitters, and is very responsive to circulating glucose concentration. Insulin, and/or the zinc that islet β cells secrete with it, downregulates Glucagon secretion in intact islets (5, 6). Glucagon secretion is also downregulated by the neurotransmitter γ-aminobutyric acid (GABA), somatostatin produced by islet δ-cells, and GLP-1, but is enhanced by the neurotransmitter L-glutamate, amino acids (especially arginine), and Glucagon itself (2-4, 7). Through receptors on the α-cells, these substances affect potassium, sodium, and calcium channel activity and alter intracellular calcium concentration (2-4). Glucose suppression of Glucagon secretion is probably indirect, acting through paracrine signals from other islet cells (8).

Like insulin, Glucagon is dysregulated in type 2 diabetes (T2D) and contributes to its pathology (2-4). Glucagon secretion is less responsive to insulin-mediated suppression in times of high circulating glucose, causing glucagonemia, and increasing the risk of hyperglycemia. Glucagon is also regulated by some of the same messengers that regulate insulin (10-12). Leptin inhibits α-cell glucagon secretion and stimulates β-cell insulin secretion, but glucagon blunts the leptin-mediated insulin secretion (10). Islet α-cells express ghrelin receptors and respond to ghrelin by increasing Glucagon secretion (11). Glucocorticoids, activated by 11β-HSD1, depress Glucagon secretion in hypoglycemia and insulin secretion in hyperglycemia (12). Although genetic polymorphisms of the Glucagon receptor are associated with T2D, downregulation of Glucagon secretion or deletion of the Glucagon receptor in mice that are susceptible to T2D actually improves glycemic control (13, 14).

The Quantikine Glucagon Immunoassay is a 4.5 hour solid-phase ELISA designed to measure Glucagon in cell culture supernates, serum, and plasma. It contains synthetic Glucagon as the standard. The antibodies were raised against a human Glucagon synthetic peptide. This immunoassay has been shown to accurately quantitate human, mouse, rat, and porcine Glucagon.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for Glucagon has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Glucagon present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for Glucagon is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution 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 and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- Buffers containing Tween® detergents should be avoided with this assay. Only use the Wash Buffer included in this kit.
- If samples generate values higher than the highest standard, dilute the samples with calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Glucagon Microplate	894070	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against Glucagon.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.*
Glucagon Standard	894072	2 vials of synthetic Glucagon in a buffer with preservatives; lyophilized. *Refer to vial label for reconstitution volume.*	Discard after use. Use a new standard for each assay.
Glucagon Conjugate	894071	21 mL of a monoclonal antibody against Glucagon conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-110	895967	2 vials (11 mL/vial) of a buffered protein base with preservatives.	
Calibrator Diluent RD5-59	895968	21 mL of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895222	100 mL of a 10-fold concentrated solution of buffered surfactant with preservatives.	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm
- Pipettes and pipette tips
- Deionized or distilled water
- Squirt bottle, manifold dispenser, or automated microplate washer
- 1000 mL graduated cylinder
- Test tubes for dilution of standards
- Glucagon Controls (optional; R&D Systems®, Catalog # QC100).

PRECAUTION

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

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

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and 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 at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

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

Note: *Citrate plasma has not been validated for use in this assay.*
Samples should be used within 8 hours of preparation or thawing (See reference 15).
Samples with abnormally high levels of Albumin interfere in this assay.

REAGENT PREPARATION

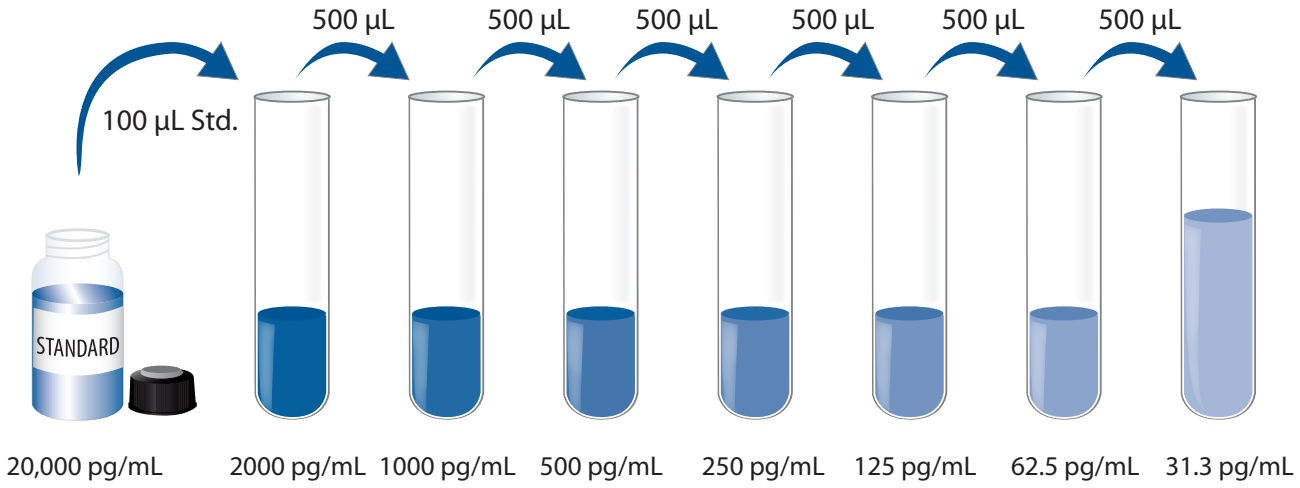
The conjugate must be kept cold during use. Bring all other reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 100 mL of Wash Buffer Concentrate to 900 mL of deionized or distilled water to prepare 1000 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Glucagon Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Glucagon Standard with deionized or distilled water. This reconstitution produces a stock solution of 20,000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 30 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5-59 into the 2000 pg/mL tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 pg/mL standard serves as the high standard. Calibrator Diluent RD5-59 serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

The conjugate must be kept cold during use. Bring all other reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Wash and aspirate the plate a total of two times with Wash Buffer prior to assay. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good 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 150 µL of Assay Diluent RD1-110 to each well.
5. Add 50 µL of standard, control, or sample per well. Cover with the adhesive strip provided. Incubate for 3 hours at room temperature.
6. Aspirate each well and wash as directed in step 3, repeating the process three times for a total of four washes.
7. Add 200 µL of **cold** Glucagon Conjugate to each well. Cover with a new adhesive strip. Incubate for 1 hour at **2-8 °C**. **If running more than one kit, do not stack the plates.**
8. Repeat the aspiration/wash as in step 6.
9. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
10. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

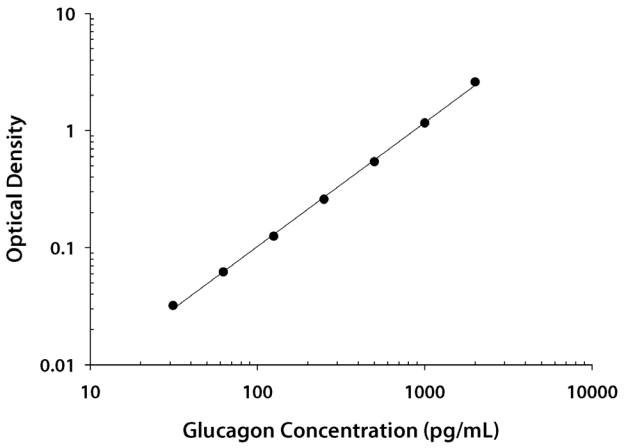
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the Glucagon concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

If the samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.019 0.020	0.020	—
31.3	0.051 0.052	0.052	0.032
62.5	0.082 0.082	0.082	0.062
125	0.141 0.149	0.145	0.125
250	0.279 0.279	0.279	0.259
500	0.556 0.570	0.563	0.543
1000	1.169 1.193	1.181	1.161
2000	2.588 2.642	2.615	2.595

PRECISION

Intra-Assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	315	618	1024	354	653	1080
Standard deviation	10.3	22.0	27.6	30.7	37.8	63.6
CV (%)	3.3	3.6	2.7	8.7	5.8	5.9

RECOVERY

The recovery of Glucagon spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	98	91-108%
Human serum (n=4)	102	95-111%
Human EDTA plasma (n=4)	98	86-107%
Human heparin plasma (n=4)	96	88-100%
Mouse serum (n=2)	99	92-106%
Rat serum (n=1)	93	86-101%
Porcine serum (n=2)	97	91-107%

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of Glucagon were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Human serum (n=4)	Human EDTA plasma (n=4)	Human heparin plasma (n=4)	Mouse serum (n=2)	Rat serum (n=1)	Porcine serum (n=2)
1:2	Average % of Expected	98	102	100	102	99	105	103
	Range (%)	92-101	94-113	94-113	95-109	90-108	———	103-103
1:4	Average % of Expected	95	100	99	98	98	92	111
	Range (%)	88-99	89-107	90-108	94-103	95-100	———	108-115
1:8	Average % of Expected	94	97	95	96	92	103	102
	Range (%)	90-98	88-107	89-106	92-103	91-94	———	101-104
1:16	Average % of Expected	95	96	101	97	105	116	100
	Range (%)	87-101	85-104	93-106	93-104	103-107	———	99-10

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of Glucagon ranged from 2.12-14.7 pg/mL. The mean MDD was 6.37 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified natural porcine pancreas-derived Glucagon.

The NIBSC/WHO First International Standard for Glucagon 69/194 was evaluated in this kit. The dose response curve of the WHO Standard material parallels the Quantikine® standard curve. To convert sample values obtained with the Quantikine® kit to approximate NIBSC (69/194) units, use the equation below.

NIBSC (69/194) approximate value (IU/mL) = 0.886 x 10^{-9} x Quantikine® Glucagon value (pg/mL).

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy subjects were evaluated for the presence of Glucagon in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	% Detectable (pg/mL)	Range (pg/mL)
Human serum (n=37)	105	92	ND-224
Human EDTA plasma (n=37)	109	95	ND-226
Human heparin plasma (n=37)	100	95	ND-221
Mouse serum (n=10)	72.2	50	ND-96.3
Rat serum (n=17)	188	94	ND-932
Porcine serum (n=10)	213	100	60.3-497

ND=Non-detectable

Cell Culture Supernates - Human peripheral blood leukocytes were cultured in DMEM supplemented with 5% fetal bovine serum, 50 mM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were then cultured unstimulated or stimulated with 10 µg/mL PHA for 1 or 5 days. Aliquots of the cell culture supernates were removed and assayed for levels of natural Glucagon. No detectable levels were observed.

SPECIFICITY

This assay recognizes natural Glucagon. The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range Glucagon control were assayed for interference. No significant cross-reactivity or interference was observed.

Natural proteins:

Gastric Inhibitory Polypeptide
Glucagon-like Peptide 1
Glucagon-like Peptide 2
Glicentin-related Polypeptide

Oxyntomodulin does not interfere but does cross-react < 12% in this assay.

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