Version 20-04/24

User's Manual

Human Glucagon ELISA Kit

REF **DEIA2827**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

Glucagon ELISA kit is an assay intended to measure the pancreatic hormone glucagon in plasma and serum. Glucagon measurements are used in the diagnosis and treatment of patients with various disorders of carbohydrate metabolism, including diabetes mellitus, hypoglycemia, and hyperglycemia.

General Description

Glucagon is a 29 amino acid polypeptide processed from proglucagon (residues 33-61) in pancreatic alpha cells. In intestinal L-cells proglucagon is cleaved into glicentin, corresponding to proglucagon residues no. 1-69. Glicentin can further be processed into oxyntomodulin, corresponding to proglucagon residues no. 33-69. Moreover, a fragment of glucagon corresponding to its C-terminal part (residues no. 19-29), also designated mini-glucagon, is reported to be present in the pancreas in low amounts compared to the total glucagon content.

In general, glucagon has an effect opposite that of insulin, i.e. it raises blood glucose levels. It causes the liver to convert glycogen into glucose, which is then released into the blood stream. With longer stimulation, glucagon action in the liver results in a glucose-sparing activation of free fatty acid oxidation and production of ketones. During hypoglycaemia, glucagon secretion offers a protective feedback mechanism, defending the organism against damaging effects of glucose deficiency in the brain and nerves.

Principles of Testing

Glucagon ELISA Kit is a solid phase two-site enzyme immunoassay. It is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the glucagon molecule. During the first incubation glucagon in the sample reacts with anti-glucagon antibodies bound to microplate wells. After washing, peroxidase conjugated anti-glucagon antibodies are added. After a second incubation and a simple washing step, the bound conjugate is detected by reaction with 3,3',5,5'-tetramethylbenzidine (TMB). The reaction is stopped by adding acid to give a colorimetric endpoint that is read spectrophotometrically.

Reagents And Materials Provided

Each Glucagon ELISA kit contains a plate sealer and reagents for 96 wells, sufficient for 42 samples and one calibrator curve in duplicate. The expiry date for the complete kit is stated on the outer label. The recommended storage temperature is 2-8°C.

1.Coated Plate, 1 plate, 96 wells (8-well strips), Ready for Use

Mouse monoclonal anti-glucagon coated plate.

For unused microplate strips, reseal the bag using adhesive tape, store at 2-8°C and use within 2 months.

2.Calibrators 1, 2, 3, 4, 5, 5 vials, 1000 µL, Lyophilized

Synthetic glucagon, Color coded yellow, Concentration stated on vial label.

Add 1000 µL redistilled water per vial.

Reconstituted Calibrators are stable for 1 month at 2-8°C.

If reconstituted Calibrators are to be used for longer than 1 month, aliquote and store at -20°C. Aliquoted Calibrators are stable for at least 2 months at -20°C.

Avoid repeated freeze/thaw cycles.

3.Calibrator 0, 1 vial, 5 mL, Ready for Use

Color coded yellow

4.Assay Buffer, 1 bottle, 22 mL, Ready for use

Color coded red

5.Enzyme Conjugate 11X, 1 bottle, 2.2 mL, Preparation see below.

Mouse monoclonal anti-glucagon

6.Enzyme Conjugate Buffer, 1 bottle, 22 mL, Ready for use

Color coded blue.

7.Wash Buffer 21X, 1 bottle, 50 mL

Dilute with 1000 mL redistilled water to make wash buffer 1X solution.

Storage after dilution: 2-8°C for 2 months.

8.Substrate TMB, 1 bottle, 22 mL, Ready for Use

Colorless solution

Note! Light sensitive!

9.Stop Solution, 1 vial, 7 mL, Ready for Use

0.5 M H_2SO_4

Materials Required But Not Supplied

1. Pipettes with appropriate volumes (repeating pipettes preferred for addition of enzyme conjugate 1X solution, Assay Buffer, Substrate TMB and Stop Solution)
2. Tubes, beakers and cylinders for reagent preparation
3. Redistilled water
4. Magnetic stirrer
5. Vortex mixer
6. Microplate reader with 450 nm filter
7. Microplate shaker (700-900 cycles per minute, orbital movement)
8. Refrigerator (2-8°C) with room for microplate shaker
9. Microplate washing device with overflow function (recommended but not required)

Storage

Store the kit at 2°C - 8 °C.

Specimen Collection And Preparation

Serum, plasma or cellculture medium can be used. However, glucagon in serum, EDTA plasma and cell culture medium samples will be sensitive to storage conditions and freeze-thaw cycles. It is recommended to keep samples on ice when thawing them and preparing the assay. Return to freezer as soon as possible. Addition of aprotinin to EDTA plasma samples will not improve stability.

Serum

Collect blood by venipuncture, allow to clot and separate the serum by centrifugation. Store samples at -80°C and avoid freeze-thaw cycles. Avoid storing samples at room temperature or 2-8°C.

Plasma

EDTA plasma

Collect blood by venipuncture into tubes containing EDTA as anticoagulant, and separate the plasma fraction by centrifugation. Store samples at -80°C and avoid freeze-thaw cycles. Avoid storing samples at room temperature or 2-8°C.

Stabilized EDTA plasma

For studies in which low levels of glucagon need to be detected, it may be beneficial to use sample collection tubes specifically optimized for stabilization, since this will prevent the degradation of glucagon. Store samples at -80°C and avoid freeze-thaw cycles. Avoid storing samples at room temperature or 2-8°C.

Cell culture medium

Note that different chemicals used in cell culture media can interfere with the assay (such as sodium azide (NaN_3) and beta-mercaptoethanol). Avoid freeze/ thaw, do not store samples in room temperature, samples should be kept on ice during use. Samples should be diluted at least 2X with Calibrator 0.

Preparation of samples

No dilution is normally required for serum and plasma, but samples above Calibrator 5 should be diluted with Calibrator 0. Dilution in Calibrator 0 is recommended for all cell culture medium samples.

Reagent Preparation

Preparation of enzyme conjugate 1X solution

Prepare the needed volume of enzyme conjugate 1X solution by dilution of Enzyme Conjugate 11X (1+10) in Enzyme Conjugate Buffer according to the table below. When preparing enzyme conjugate 1X solution for the whole plate, pour all of the Enzyme Conjugate Buffer into the Enzyme Conjugate 11X bottle. Mix gently. Use within 1 week.

Number of strips	Enzyme Conjugate 11X	Enzyme Conjugate Buffer
12 strips	1 vial	1 vial
8 strips	1300 µL	13 mL
4 strips	700 µL	7 mL

Assay Procedure

Prepare a calibrator curve for each assay run. All reagents and samples must be brought to room temperature before use.

1. Reconstitute Calibrators with 1000 µL redistilled water.
2. Pipette 25 µL each of Calibrators, controls and samples in duplicate into appropriate wells.
3. Add 200 µL Assay Buffer to each well and attach the plate sealer.
4. Incubate on plate shaker (700-900 rpm) over night (18-22 h) at 2-8°C.
5. Prepare wash buffer 1X solution by diluting Wash Buffer 21X with 1000 mL redistilled water. Prepare the needed volume of enzyme conjugate 1X solution by dilution of Enzyme Conjugate 11X (1+10) in Enzyme Conjugate Buffer.
6. Wash 6 x 700 µL using an **automatic** plate washer with overflow wash function. The chosen program should fill all the wells with wash buffer in each cycle and ensure that the wells are never left without wash buffer (e.g. Plate Mode). Do not use additional soak! Invert and tap the plate firmly against absorbent paper after the final wash. Or **manually**, Discard the reaction volume by inverting the microplate over a sink. Hold the plate vertically over a sink and fill the wells by spraying wash buffer into the wells with a wash bottle. Discard the wash buffer 1X solution, tap firmly several times against absorbent paper to remove excess liquid. Repeat 5 times. Avoid prolonged soaking during washing procedure.
7. Add 200 µL enzyme conjugate 1X solution to each well.
8. Incubate on plate shaker (700-900 rpm) 1h at room temperature (18-25°C).
9. Wash as described in step 6.
10. Add 200 µL Substrate TMB per well.
11. Incubate on the bench for 30 min at room temperature (18-25°C).
12. Add 50 µL Stop Solution to each well. Place plate on a shaker for approximately 5 seconds to ensure mixing.
13. Read optical density at 450 nm. Read within 30 minutes.
14. Apply curve fitting directly on raw data (A450 nm). Preferably use 5-parametric logistic regression with automatic weighing on relative weights ($1/y^2$).

Note! Be extra careful not to contaminate the Substrate TMB with enzyme conjugate solution.

Quality Control

Commercial controls and/or internal plasma pools with low, intermediate and high glucagon concentrations should routinely be assayed as samples and results charted from day to day. It is good laboratory practice to record the following data for each assay: kit lot number, dilution and/or reconstitution dates of kit components, OD values for the blank, Calibrators and controls.

Laboratories should follow government regulations or accreditation requirements for quality control frequency.

Calculation

The concentration of glucagon is obtained by plotting the absorbance of the Calibrators, except for Cal 0, versus their concentration. It is important to use an appropriate curve fitting model that represent the true dose-response relationship to get accurate results. It is every laboratory's responsibility to try out the functionality of the chosen curve fitting model and used software. **Note** that weighting of the curve fit is

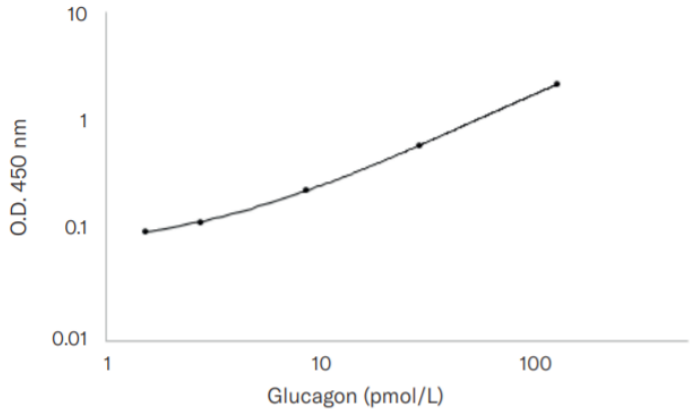
important to get a proper fit at the low range of the standard curve, especially when the measuring range is wide.

The Glucagon ELISA is validated with Five parameter logistic with weighting $1/y^2$, using Magellan (Tecan) software.

Typical Standard Curve

Wells	Identity	$A_{450\ nm}$	Mean conc. pmol/L
1A–B	Calibrator 0	0.066/0.067	
1C–D	Calibrator 1*	0.099/0.100	
1E–F	Calibrator 2*	0.121/0.117	
1G–H	Calibrator 3*	0.238/0.239	
2A–B	Calibrator 4*	0.597/0.624	
2C–D	Calibrator 5*	2.235/2.179	
2E–F	Sample 1	0.141/0.138	3.68
2G–H	Sample 2	0.283/0.276	11.1
3A–B	Sample 3	1.929/1.780	99.3

*Concentration stated on vial label.



Conversion factor

1 pmol/L = 3.5 pg/mL

Reference Values

Good practice dictates that each laboratory establishes its own expected range of values. The following results may serve as a guide until the laboratory has gathered sufficient data of its own. Fasting levels for 121 tested, apparently healthy individuals, yielded a median of 6.5 pmol/L and central 95% reference range of ≤1.5-18 pmol/L analyzed in plasma.

Precision

QC samples (P800) were analyzed in 4 replicates over 6 different occasions on one kitlot and one instrument system by three laboratory technicians.

Sample	Mean value pmol/L	Accuracy %	Coefficient of variation Repeatability %*	Coefficient of variation Within laboratory precision %**
QC_{LLOQ}	1.5	96	5.6	8.3
QC_{Low}	4.3	90	6.3	13
QC_{Medium}	11	105	14	16
QC_{High}	101	101	2.1	10
QC_{ULOQ}	130	96	5.1	7.0

*Within-run variation
**Between-run variation

Detection Range

1.5-130 pmol/L (5-453 pg/mL)

Detection Limit

The detection limit is 0.75 pmol/L (2.61 pg/mL) as determined by the methodology described in ISO11843-Part 4.

Lower Limit of Quantification, LLOQ, is 1.5 pmol/L (5.95pg/mL)as determined according to FDA/EMA guidelines.

The Upper Limit of Quantification, ULOQ, is 130 pmol/L (453pg/mL) as determined according to FDA/EMA guidelines.

Specificity

No cross-reactivity to glicentin, proglucagon 1-16, and glucagon 3-29.

Substance	Cross-reaction %	Concentrations tested pmol/L
Glicentin	n.d.	300
Oxyntomodulin	4.0	300
Proglucagon 1-61	n.d.	25
Glucagon 3-29	n.d.	250

n.d. = not detected

Linearity

Dilutional linearity

Cell culture medium were spiked above the highest calibrator concentration and subsequently diluted for analysis in the assay. Nominal values were used for calculation. Mean recovery for dilutional linearity is 105 % (102-110%) with a precision of the final concentration across all dilutions of 4 %.

High Dose Hook

Effect Samples with a concentration up to 50 nmol/L can be measured without giving falsely low results.

Interferences

Interference data at low (1.5 pmol/L) and high (102 pmol/L) concentrations of glucagon are presented below. The substance is concluded to interfere if the recovery value is not within 100 ± 25 % of the nominal concentration.

	Concentration (pmol/L)	Recovery %	
		Low conc.	High conc.
Glicentin	3	77	107
	12	<Cal 1	104
	30	89	114
	120	<Cal 1	107
	300	117	114
Oxyntomodulin	3	106	96
	12	118	97
	30	171	114
	120	455	112
	300	1293	136
Proglucagon 1-61	5	132	110
	23	126	111
Glucagon 3-29	5	102	110
	25	104	109
	125	113	102

Selectivity

Lipemic samples do not interfere in the assay.

Certain levels of hemoglobin (>50 mg/dL) can interfere in the assay.

Precautions

1. For in vitro diagnostic use in EU/EEA, UK, US and Canada.
2. Regulatory Status in the rest of the world: For Research Use Only. Not for use in diagnostic procedures.
3. All samples should be handled as capable of transmitting infections.
4. Each well can only be used once.
5. The Stop Solution contains <5% Sulphuric acid.

The Stop Solution is labeled:**Danger**

H318 - Causes serious eye damage.

H315 - Causes skin irritation.

P280 - Wear protective gloves. Wear eye or face protection.

P264 - Wash hands thoroughly after handling.

P302 + P352 + P362 + P364 - IF ON SKIN: Wash with plenty of soap and water. Take off contaminated clothing and wash it before reuse.

P332 + P313 - If skin irritation occurs: Get medical attention.

P305 + P351 + P338 + P310 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact

lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or physician.

6. The Enzyme Conjugate Buffer, Cal 0, 1, 2, 3, 4, 5, Wash Buffer and Assay Buffer contain <0.06% 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H-isothiazol-3-one (3:1).

The Enzyme Conjugate Buffer, the Calibrators, Wash Buffer and Assay Buffer are labeled: **Warning**

H317 - May cause an allergic skin reaction.

P280 - Wear protective gloves.

P261 - Avoid breathing vapour.

P272 - Contaminated work clothing should not be allowed out of the workplace.

P302 + P352 - IF ON SKIN: Wash with plenty of soap and water.

P333 + P313 - If skin irritation or rash occurs: Get medical attention.

P501 - Dispose of contents and container in accordance with all local, regional, national and international regulations.

Limitations

Grossly lipemic samples do not interfere in the assay. Certain levels of hemoglobin (>50 mg/dL) can interfere in the assay. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical findings have been evaluated.