Mercodia
Developing diagnostics

Mercodia Glucagon ELISA

Directions for Use

10-1271-01
REAGENTS FOR 96 DETERMINATIONS

For Research Use Only

Manufactured by

Mercodia AB, Sylveniusgatan 8A,
SE-754 50 Uppsala,
Sweden

EXPLANATION OF SYMBOLS USED ON LABELS

	Reagents for 96 determinations
	Expiry date
	Store between 2–8°C
	Lot No.

INTENDED USE

Mercodia Glucagon ELISA 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.

SUMMARY AND EXPLANATION OF THE TEST

Glucagon is a 29 amino acid polypeptide processed from proglucagon 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. These peptides are released simultaneously upon stimulation. 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.

PRINCIPLE OF THE PROCEDURE

Mercodia Glucagon ELISA 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 incubation glucagon in the sample reacts with peroxidase-conjugated anti-glucagon antibodies (clone E6A11K) and anti-glucagon antibodies (clone M5F9S) bound to microplate wells. A simple washing step removes unbound enzyme labelled antibody. 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.

WARNINGS AND PRECAUTIONS

- For research use only. Not for use in diagnostic procedures.
- Not for internal or external use in humans or animals.
- Contents of this kit and their residues must not be allowed to come into contact with ruminating animals or swine.
- The Stop Solution in this kit contains 0.5M H_2SO_4. Follow routine precautions for handling hazardous chemicals.
- All samples should be handled as capable of transmitting infections.
- Each well can only be used once.

MATERIAL REQUIRED BUT NOT PROVIDED

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

REAGENTS FOR 1 X 96 KIT

Each Mercodia Glucagon ELISA kit (10-1271-01) contains reagents for 96 wells, sufficient for 42 samples and one Calibrator curve in duplicate. For larger series of assays, use pooled reagents from packages bearing identical lot numbers. The expiry date for the complete kit is stated on the outer label. The recommended storage temperature is 2–8°C.

Coated Plate Mouse monoclonal anti-glucagon	1 plate	96 wells 8-well strips	Ready for Use
For unused microplate strips, reseal the bag using adhesive tape, store at 2–8°C and use within 2 months.			
Calibrators 1, 2, 3, 4, 5 Synthetic glucagon Color coded yellow Concentration stated on vial label. Storage after reconstitution: 2–8°C for 1 month. For storage of reconstituted Calibrators for more than 1 month, store at -20°C.	5 vials	1000 µL	Lyophilized Add 1000 µL redistilled water per vial.
Calibrator 0 Color coded yellow	1 vial	5 mL	Ready for Use
Enzyme Conjugate 11X Mouse monoclonal anti-glucagon	1 vial	2.2 mL	Preparation, see below
Enzyme Conjugate Buffer Color coded blue.	1 vial	22 mL	Ready for use
Wash Buffer 21X Storage after dilution: 2-8°C for 2 months.	1 bottle	50 mL	Dilute with 1000 mL redistilled water to make wash buffer 1X solution.
Substrate TMB Colorless solution *Note! Light sensitive!*	1 bottle	22 mL	Ready for Use
Stop Solution 0.5 M H_2SO_4	1 vial	7 mL	Ready for Use

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. Mix gently.
Use within 1 day.

Number of strips	Enzyme Conjugate 11X	Enzyme Conjugate Buffer
12 strips	1 vial	1 vial
6 strips	1000 µL	10 mL
4 strips	700 µL	7 mL

SPECIMEN COLLECTION AND HANDLING

Serum or EDTA plasma can be used. However, glucagon in serum or EDTA plasma samples will be sensitive to storage conditions and freeze-thaw cycles. 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. Avoid storing samples at room temperature or 2-8°C for longer than 2 hours. Store samples at -80°C and avoid freeze-thaw cycles.

Plasma

EDTA plasma

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

EDTA plasma in BD P800 tubes

Collecting samples in Becton Dickinson (BD) P800 tubes containing lyophilized protease inhibitors and DPP-IV inhibitors will yield (20 – 30%) higher glucagon values than EDTA plasma not collected in BD P800 tubes or serum because of improved stability. Samples from P800 tubes are stable for up to 6 hours at room temperature or 2-8°C, and up to 4 freeze-thaw cycles in cryo vials. Store samples at -80°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).

Preparation of samples

No dilution is normally required, however, samples above the obtained value of Calibrator 5 should be diluted with Calibrator 0. *Note!* Buffers containing sodium azide (NaN_3) cannot be used for sample dilution.

TEST PROCEDURE

Prepare a calibrator curve for each assay run.

1. Prepare enzyme conjugate 1X solution and wash buffer 1X solution.
2. Prepare sufficient microplate wells to accommodate Calibrators, controls and samples in duplicate.
3. Pipette 25 µL each of Calibrators, controls and samples into appropriate wells.
4. Add 200 µL enzyme conjugate 1X solution to each well and attach the plate sealer.
5. Incubate on a plate shaker (700-900 rpm) over night (18-22h) at 2–8°C.
6. Wash 6 times with 700 µL wash buffer 1X solution per well using an automatic plate washer with overflow-wash function. After final wash, invert and tap the plate firmly against absorbent paper. Do not include soak step in washing procedure.
   Or manually,
   Discard the reaction volume by inverting the microplate over a sink. Add 350 µL wash solution to each well. 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 Substrate TMB.
8. Incubate on the bench for 15 minutes at room temperature (18–25°C).
9. Add 50 µL Stop Solution to each well.
   Place plate on a shaker for approximately 5 seconds to ensure mixing.
10. Read optical density at 450 nm and calculate results.
    Read within 30 minutes.

Note! To prevent contamination between the conjugate and substrate, separate pipettes are recommended.

INTERNAL QUALITY CONTROL

Commercial controls and/or internal serum 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 OF RESULTS

Computerized calculation

The concentration of glucagon is obtained by computerized data reduction of the absorbance for the Calibrators, except for Calibrator 0, versus the concentration using four parameter logistic.

Manual Calculation

1. Plot the absorbance values obtained for the Calibrators, except for Calibrator 0, against the glucagon concentration on log-log paper and construct a calibrator curve.
2. Read the concentration of the samples from the calibrator curve.

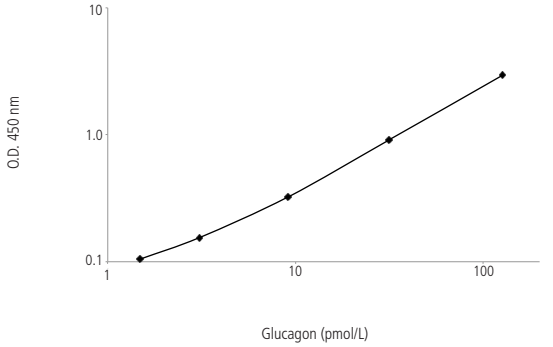
Example of results

Wells	Identity	A_{450}	Mean conc. pmol/L
1A–B	Calibrator 0	0.067/0.069	
1C–D	Calibrator 1*	0.106/0.104	
1E–F	Calibrator 2*	0.152/0.157	
1G–H	Calibrator 3*	0.331/0.316	
2A–B	Calibrator 4*	0.902/0.923	
2C–D	Calibrator 5*	2.949/2.945	
2E–F	Sample 1	0.154/0.155	3.14
2G–H	Sample 2	0.191/0.193	4.43
3A–B	Sample 3	0.569/0.562	18.1

* Concentration stated on vial label.

Calibrator curve

A typical calibrator curve is shown here. Do not use this curve to determine actual assay results.



LIMITATIONS OF THE PROCEDURE

Grossly lipemic, icteric or haemolyzed samples do not interfere in the assay.
Separate pipettes should be used when pipetting the conjugate and the substrate.

EXPECTED VALUES

Good practice dictates that each laboratory establishes its own expected range of values.

PERFORMANCE CHARACTERISTICS

Detection limit

Detection limit is defined as the Capability of Detection according to ISO11843-Part 1. Capability of Detection should be seen as part of a method validation, rather than the lowest concentration that can be measured.

The detection limit is 1 pmol/L as determined with the methodology described in ISO11843-Part 4.

Concentrations of samples with absorbances below Calibrator 1 should not be calculated, but expressed as less than or equal to (≤) the concentration indicated on the vial for Calibrator 1.

Recovery

Recovery upon addition 96-101% (Mean 98%)
Recovery upon dilution 81–96% (Mean 86%)

Hook effect

Samples with a concentration up to at least 8 µmol/L can be measured without giving falsely low results.

Precision

Each sample was analyzed in 4 replicates on 39 different occasions.

Sample	Mean value pmol/L	Coefficient of variation		
		within assay %	between assay %	total assay %
1	3.0	5.1	8.1	8.5
2	5.2	3.6	9.4	9.5
3	21.9	3.3	7.3	7.5

Specificity

Oxyntomodulin	4.4%
Glicentin	0.8%
Mini-glucagon	<0.1%
GLP-1	<0.3%
GLP-2	<0.3%
GRPP	<0.0005%

CALIBRATION

Mercodia Glucagon ELISA is calibrated against WHO 1st International reference preparation 69/194.

WARRANTY

The performance data presented here was obtained using the procedure indicated. Any change or modification in the procedure not recommended by Mercodia AB may affect the results, in which event Mercodia AB disclaims all warranties expressed, implied or statutory, including the implied warranty of merchantability and fitness for use.

Mercodia AB and its authorized distributors, in such event, shall not be liable for damages indirect or consequential.

REFERENCES

Bagger JI *et al* (2011) Glucagon antagonism as a potential therapeutic target in type 2 diabetes. *Diabetes, Obesity and Metabolism* 13:965-971.

Holst J *et al* (2004) Role of incretin hormones in the regulation of insulin secretion in diabetic and nondiabetic humans. *Am J Physiol Endocrinol Metab* 287:E199-206.

Holst J (2010) Glucagon and Glucagon-Like Peptides 1 and 2. *Results Probl Cell Differ* 50:121-135.

Young A (2005) Inhibition of Glucagon Secretion. *Advances in Pharmacology* 52:151-171.

SUMMARY PROTOCOL SHEET

Mercodia Glucagon ELISA

Add Calibrators, controls* and samples	25 µL
Add enzyme conjugate 1X solution and attach plate sealer	200 µL
Incubate	Over night (18-22 h) at 2-8°C on a plate shaker, 700-900 rpm
Wash plate with wash buffer 1X solution	700 µL, 6 times
Add Substrate TMB	200 µL
Incubate	15 minutes at 18-25°C
Add Stop Solution	50 µL Shake for 5 seconds to ensure mixing
Measure A_{450}	Evaluate results

* Not provided

For full details, see page 6.