Mercodia
Developing diagnostics

Mercodia Glicentin ELISA

Directions for Use

10-1273-01
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.

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INTENDED USE

Mercodia Glicentin ELISA provides a method for the quantitative determination of human glicentin in serum, EDTA plasma and cell culture medium.

SUMMARY AND EXPLANATION OF THE TEST

Glicentin, a polypeptide composed of 69 amino acids, is derived from the posttranslational cleavage of proglucagon in the gut. The sequence of glicentin also include the sequences of glucagon, mini-glucagon, oxyntomodulin and GRPP, all of which are derived from proglucagon,.

Observed biological actions of glicentin are stimulation of insulin secretion, inhibition of gastric acid secretion, control of gut motility, and stimulation of gut growth. These properties are also attributed to GLP-1 and GLP-2, making it unclear if the high concentrations of glicentin used in some studies resulted in minor activation of GLP-1 and GLP-2 receptors.

PRINCIPLE OF THE PROCEDURE

Mercodia Glicentin 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 glicentin molecule. During incubation glicentin in the sample reacts with anti-human glicentin antibodies bound to microtitration well (clone C7H9G). After washing, which removes non-reactive plasma components, a peroxidase conjugated anti-glicentin antibody (clone RG-OC5) recognizes the glicentin bound to the solid phase. After a second incubation and a simple washing step that removes unbound enzyme labeled 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, then 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.
- The content of this kit and their residues must not be allowed to come into contact with ruminating animals or swine.
- All samples should be handled as capable of transmitting infections.
- Each well can only be used once.
- 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 wa ter. 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.

- 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 and Wash 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.

MATERIAL REQUIRED BUT NOT PROVIDED

- Pipettes with appropriate volumes (repeating pipettes preferred for addition of Assay Buffer, 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)
- Microplate washing device with overflow function (recommended but not required)

REAGENTS FOR 1 X 96 KIT

Each Mercodia Glicentin ELISA kit (10-1273-01) 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-glicentin	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 8 weeks.			
Calibrators 1, 2, 3, 4, 5 Synthetic glicentin Color coded yellow Concentration stated on vial label. Storage after reconstitution: 2–8°C for 2 weeks. For storage of reconstituted Calibrators for more than 2 weeks, 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-glicentin	1 vial	2.2 mL	Preparation, see below
Enzyme Conjugate Buffer Color coded blue.	1 vial	22 mL	Ready for use
Assay Buffer Color coded red	1 vial	12 mL	Ready for use
Wash Buffer 21X Storage after dilution: 2-8°C for 8 weeks.	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.

Number of strips	Enzyme Conjugate 11X	Enzyme Conjugate Buffer
12 strips	1 vial	1 vial
6 strips	1000 µL	10.0 mL
4 strips	700 µL	7.0 mL

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

SPECIMEN COLLECTION AND HANDLING

Serum or EDTA plasma can be used. However, glicentin in serum or EDTA plasma samples could 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 up to 30% higher glicentin values than EDTA plasma not collected in BD P800 tubes or serum because of improved stability. Store samples at -80°C. Avoid repeated freezing and thawing.

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) can not be used for sample dilution.

TEST PROCEDURE

Prepare a calibrator curve for each assay run. The product has been optimized and validated without plate sealer.

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 100 µL Assay Buffer to each well.
5. Incubate on a plate shaker (700-900 rpm) for 1 hour at room temperature (18-25°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 enzyme conjugate 1X solution to each well.
8. Incubate on a plate shaker (700-900 rpm) for 1 hour at room temperature (18-25°C).
9. Wash as described in 6.
10. Add 200 µL Substrate TMB.
    Incubate for 15 minutes on the bench at room temperature (18–25°C).
11. Add 50 µL Stop Solution to each well.
    Place plate on a shaker for approximately 5 seconds to ensure mixing.
12. Read optical density at 450 nm and calculate results.
    Read within 30 minutes.

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

INTERNAL QUALITY CONTROL

Commercial controls and/or internal serum pools with low, intermediate and high glicentin 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 glicentin is obtained by computerized data reduction of the absorbance for the Calibrators, except for Calibrator 0, versus the concentration using cubic spline regression or four parameter logistic.

Manual Calculation

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

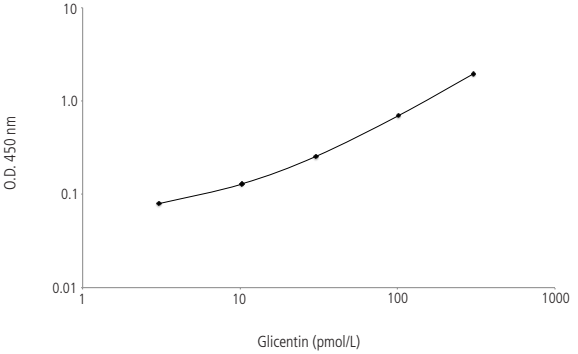
Example of results

Wells	Identity	A_{450}	Mean conc. pmol/L
1A–B	Calibrator 0	0.065/0.064	
1C–D	Calibrator 1*	0.079/0.078	
1E–F	Calibrator 2*	0.128/0.129	
1G–H	Calibrator 3*	0.258/0.251	
2A–B	Calibrator 4*	0.700/0.701	
2C–D	Calibrator 5*	1.955/1.968	
2E–F	Sample 1	0.132/0.119	9.9
2G–H	Sample 2	0.220/0.214	24.2
3A–B	Sample 3	0.373/0.368	49.1

* 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 haemolysed samples do not interfere in the assay.

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 a part of a method validation, rather than the lowest concentration that can be measured.

The detection limit is 3 pmol/L as determined with the methodology described in ISO11843-Part 4.

Concentration of samples with absorbance below Calibrator 1 should not be calculated, instead expressed as less or equal to (≤) the concentration indicated on the vial for Calibrator 1.

Recovery
Recovery upon addition 94-101% (Mean: 98%)
Recovery upon dilution 93-112% (Mean: 99%)

Hook effect
Samples with a concentration up to at least 100 nmol/L can be measured without giving falsely low results.

Precision
Each sample was analyzed in 2 replicates on 20 different occasions.

Sample	Mean value pmol/L	Coefficient of variation	
		Repeatability %*	Within laboratory %**
1	9.06	8.0	12.2
2	22.7	4.0	10.5
3	45.0	2.7	6.2

*Within assay variation
**Total assay variation

Specificity

Glucagon	N.D.
Oxyntomodulin	N.D.
Mini-glucagon	N.D.
GLP-1	N.D.
GLP-2	N.D.
Rat glicentin	0.9%
Mouse glicentin	N.D.

N.D.=Not Detected

CALIBRATION

Mercodia Glicentin ELISA is calibrated against an in-house preparation of glicentin.

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 authorised distributors, in such event, shall not be liable for damages indirect or consequential.

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Orskov C *et al.* (1986) Glucagon-like peptides GLP-1 and GLP-2, predicted products of the glucagon gene, all secreterd separately from pig small intestine, but not pancreas. *Endocrinology* 119:1467-1475.

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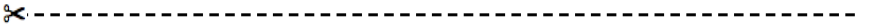

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Experiment: Date: KIT LOT#:

SUMMARY PROTOCOL SHEET

Mercodia Glicentin ELISA

Add Calibrators, controls* and samples	25 µL
Add Assay Buffer	100 µL
Incubate	1 hour at 18–25°C on a plate shaker, 700-900 rpm
Wash plate with wash buffer 1X solution	700 µL, 6 times
Add enzyme conjugate 1X solution	200 µL
Incubate	1 hour at 18–25°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 7.

For technical support please contact support@mercodia.com

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