Mercodia
Developing diagnostics

Mercodia Rat Glicentin ELISA

Directions for Use

10-1275-01
REAGENTS FOR 96 DETERMINATIONS

For Research Use Only

Manufactured by

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EXPLANATION OF SYMBOLS USED ON LABELS

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

INTENDED USE

Mercodia Rat Glicentin ELISA provides a method for the quantitative determination of rat glicentin in serum and EDTA plasma.

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 includes the sequences of glucagon, mini-glucagon, oxyntomodulin and GRPP, all of which are derived from proglucagon, just like GLP-1 and GLP-2. Reported biological actions of glicentin include stimulation of insulin secretion, inhibition of gastric acid secretion, control of gut motility and stimulation of gut growth.

PRINCIPLE OF THE PROCEDURE

Mercodia Rat 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-rat glicentin antibodies bound to microtitration wells (clone L2B4G) and to a peroxidase-conjugated anti-glicentin antibody (clone RG-OC5). After the 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 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 Rat Glicentin ELISA kit (10-1275-01) contains reagents for 96 wells, sufficient for 42 samples and one Calibrator curve in duplicate and a plate sealer. 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-rat 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 rat glicentin Color coded yellow Concentration stated on vial label. Storage after reconstitution: 2–8°C for 4 weeks.	5 vials	1000 µL	Lyophilized Add 1000 µL redistilled water per vial.
For storage of reconstituted Calibrators for more than 4 weeks, store at -20°C.			
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
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 4 weeks.

SPECIMEN COLLECTION AND HANDLING

Serum or EDTA plasma can be used. However, glicentin in serum or EDTA plasma samples will be sensitive to storage conditions and freeze-thaw cycles.

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.

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 10 µL each of Calibrators, controls and samples into appropriate wells.
4. Add 200 µL enzyme conjugate 1X solution to each well.
5. Incubate (with plate sealer) on a plate shaker (700-900 rpm) for 20 hours (18-22 hours) 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.
   Incubate for 15 minutes on the bench at room temperature (18–25°C).
8. Add 50 µL Stop Solution to each well.
   Place plate on a shaker for approximately 5 seconds to ensure mixing.
9. 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 rat 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 rat glicentin 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 rat glicentin concentration on log-log paper and construct a calibrator curve.
2. Read the concentration of the samples from the calibrator curve.

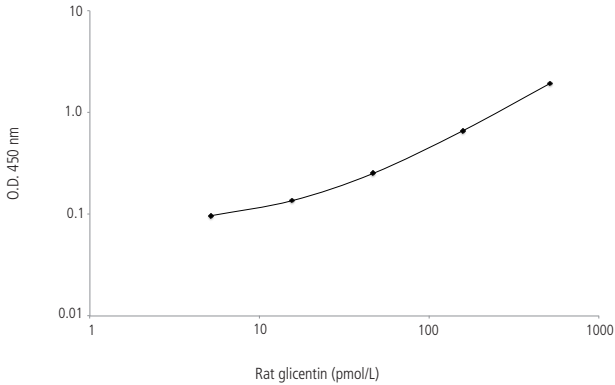
Example of results

Wells	Identity	A_{450}	Mean conc. pmol/L
1A–B	Calibrator 0	0.073/0.073	
1C–D	Calibrator 1*	0.096/0.097	
1E–F	Calibrator 2*	0.137/0.135	
1G–H	Calibrator 3*	0.253/0.250	
2A–B	Calibrator 4*	0.687/0.628	
2C–D	Calibrator 5*	1.988/1.884	
2E–F	Sample 1	0.131/0.133	14.6
2G–H	Sample 2	0.292/0.291	57.5
3A–B	Sample 3	0.506/0.507	116.4

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

The detection limit is 5 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 instead expressed as less than or equal to (≤) the concentration indicated on the vial for Calibrator 1.

Recovery
Recovery upon addition 101-117% (Mean: 110%)
Recovery upon dilution 80-106% (Mean: 96%)

Hook effect
Samples with a concentration up to at least 11,000 pmol/L can be measured without giving falsely low results.

Precision
Each sample was analyzed in 2 replicates on 17 different occasions.

Sample	Mean value pmol/L	Coefficient of variation		
		within assay %	between assay %	total assay %
1	14.9	6.1	13.3	14.0
2	57.2	3.3	6.8	7.2
3	119.8	4.0	7.2	7.7

Specificity

Glucagon	N.D.
Oxyntomodulin	N.D.
Mini-glucagon	N.D.
GLP-1	N.D.
GLP-2	N.D.
Human glicentin	<1.7%
Mouse glicentin	5.2%

N.D. = non detectable

CALIBRATION

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

WARRANTY

The performance data presented here were 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.

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SUMMARY PROTOCOL SHEET

Mercodia Rat Glicentin ELISA

Add Calibrators, controls* and samples	10 µL
Add enzyme conjugate 1X solution	200 µL
Incubate	20 hours 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.

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Version 1.0