Mercodia
Developing diagnostics

Mercodia Rat/Mouse Proinsulin ELISA

Directions for Use

10-1232-01
REAGENTS FOR 96 DETERMINATIONS

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 Rat/Mouse Proinsulin ELISA provides a method for the quantitative determination of proinsulin in rat and mouse serum, plasma, cell culture medium and cellular extracts.

SUMMARY AND EXPLANATION OF THE TEST

Proinsulin is the precursor of insulin, which is the principle hormone responsible for the control of glucose metabolism. Proinsulin is synthesized in the ß-cells of the islets of Langerhans and is subsequently processed to form C-peptide and insulin. In most species the insulin gene exists in a single copy. Rats and mice however, have two closely related genes which produce two nonallelic insulins, insulin I and insulin II (1). In rats, insulin I is more abundant than insulin II, whereas the opposite is found in mice (2). It is suggested that the difference in ratio between insulin I/insulin II may depend both on differences in expression of proinsulin I and proinsulin II, and in the rates of conversion to insulin (3). Increased proinsulin over insulin ratio may be found in rat and mouse models of hyperglycemia (4,5), or after manipulation of proinsulin-processing enzymes (6,7). The Mercodia Rat/Mouse Proinsulin ELISA is specific for proinsulin and does not cross-react with insulin or c-peptide in rat or mouse.

PRINCIPLE OF THE PROCEDURE

Mercodia Rat/Mouse Proinsulin ELISA is a solid phase two-site enzyme immunoassay. It is based on the sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the proinsulin molecule. During incubation, proinsulin in the sample reacts with anti-proinsulin antibodies bound to the microtitration well and peroxidase-conjugated anti-proinsulin antibodies simultaneously. After a simple washing step that removes unbound sample and enzyme labelled antibody, the bound conjugate is detected by reaction with 3,3´-5,5´-tetramethylbenzidine (TMB). The reaction is stopped by the addition of acid, giving a colorimetric endpoint that can be read spectrophotometrically.

WARNINGS AND PRECAUTIONS

- 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.
- 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 and Wash 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 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

Each Rat/Mouse Proinsulin ELISA kit (10-1232-01) 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-proinsulin) For unused microtitration strips, reseal the bag using adhesive tape, store at 2-8°C and use within 2 months.	1 plate	96 wells 8-well strips	Ready for Use
Calibrators 1, 2, 3, 4, 5 Color coded yellow Concentration indicated 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 (Peroxidase conjugated mouse monoclonal anti-proinsulin)	1 vial	700 µl	Preparation, see below
Enzyme Conjugate Buffer Color coded blue.	1 vial	7 ml	Ready for use
Wash Buffer 21X Storage after dilution: 2-8°C for 1 month.	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 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. When preparing enzyme conjugate 1X solution for the whole plate, pour all of the Enzyme Conjugate Buffer into the Enzyme Conjugate 11X vial. Mix gently.

Number of strips	Enzyme Conjugate 11X	Enzyme Conjugate Buffer
12 strips	1 vial	1 vial
8 strips	0.4 ml	4 ml
4 strips	0.2 ml	2 ml

Storage after dilution: 2-8°C for 1 week.

SPECIMEN COLLECTION AND HANDLING

Serum and plasma

To prepare serum collect blood, allow to clot and separate the serum by centrifugation. To prepare plasma collect blood in tubes containing EDTA, heparin or citrate as anticoagulant, and separate the plasma fraction by centrifugation. Store samples below -20°C and avoid repeated freezing and thawing.

No dilution is normally required, however, samples above the obtained value of Calibrator 5 should be diluted with Calibrator 0.

Note!
There is a continuous degradation of proinsulin in serum and plasma samples at room temperature. Make sure to keep the samples on ice as much as possible during sample collection and handling. Also make sure that all samples used in a study are collected according to the same sample preparation protocol.

Cell culture media and other sample types

Most common cell culture media can be analyzed in the assay. Cellular extracts containing acid ethanol may be analyzed in the assay after dilution of at least 100 times.

Extra dilution buffer may be needed when analyzing cell culture media or cellular extracts. For this please use Mercodia Diabetes Sample Buffer, Catalog no. 10-1195-01.

TEST PROCEDURE

All reagents must be brought to room temperature before use. Prepare a calibrator curve for each assay run. The product has been optimized and validated without plate sealer.

1. Prepare Calibrators, enzyme conjugate 1X solution and wash buffer 1X solution.
2. Prepare sufficient microplate wells to accommodate Calibrators, controls and samples in duplicate.
3. Add 50 µl of enzyme conjugate 1X solution to each well.
4. Pipette 25 µl each of Calibrator, control and sample to appropriate wells.
5. Incubate on a plate shaker (700-900 rpm) for 2 hours 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. Do not include soak step in washing procedure.
   Or manually,
   discard the reaction volume by inverting the microplate over a sink. Add 350µl wash buffer 1X solution to each well. Discard the wash 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 to each well.
8. Incubate on the bench for 30 minutes at room temperature (18-25°C).
9. Add 50 µl Stop Solution to each well.
   Place the plate on the shaker for approximately 5 seconds to ensure mixing.
10. 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 proinsulin 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, reconstitution dates of kit components, OD values for the blank, Calibrators and controls.

CALCULATION OF RESULTS

Computerized calculation

The concentration of proinsulin is obtained by computerized data reduction of the absorbance for the Calibrators, except Calibrator 0, versus the concentration using cubic spline regression analyzis.

Manual calculation

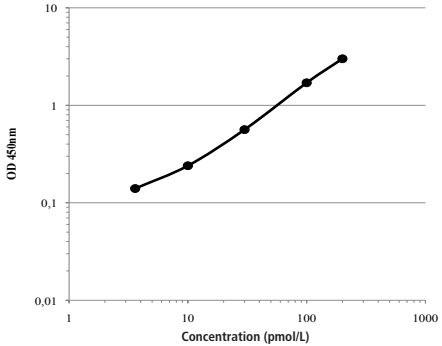
1. Plot the absorbance values obtained for the Calibrators, except Calibrator 0, against the rat/mouse proinsulin concentration on a log-log paper and construct a calibrator curve.
2. Read the concentration of the unknown samples from the calibrator curve.
   If sample has been diluted, multiply the concentration with the dilution factor.

Wells	Identity	A_{450}	Conc. pmol/L
1 A-B	Calibrator 0	0.075/0.078	
2 C-D	Calibrator 1*	0.137/0.142	
3 E-F	Calibrator 2*	0.240/0.241	
4 G-H	Calibrator 3*	0.562/0.564	
2 A-B	Calibrator 4*	1.718/1.686	
2 C-D	Calibrator 5*	2.963/3.039	
2 E-F	Sample 1	0.469/0.459	24
2 G-H	Sample 2	1.393/1.343	79
3 A-B	Sample 3	2.913/2.886	191

*Concentration indicated on vial label.

Example of calibrator curve

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



LIMITATIONS OF THE PROCEDURE

Performance limitations

Grossly lipemic, icteric or haemolyzed 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 ≤ 3 pmol/L 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 is 86-109% (mean 98%).
Recovery upon dilution is 85-107% (mean 97%).

Hook effect

Samples with a concentration of up to 90 000 pmol/L can be measured without giving falsely low results.

Precision

Each sample was analyzed in 4 replicates on 22 different occasions.

		Coefficient of variation		
Sample	Mean value pmol/L	within assay %	between assay %	total assay %
1	18	2.5	6.3	6.5
2	70	3.6	1.8	2.6
3	162	2.7	2.0	2.4

Specificity

The following crossreactions have been found:

	Crossreaction	Highest concentrations tested
Rat insulin	n.d.	90 µmol/L
Rat C-peptide	n.d.	3600 µmol/L
Rat proinsulin I	100%	90 pmol/L
Rat proinsulin II	145 %	90 pmol/L
Mouse insulin	n.d.	1.2 µmol/L
Mouse C-peptide	n.d.	5.1 µmol/L
Mouse proinsulin I	234 %	90 pmol/L
Mouse proinsulin II	100 %	90 pmol/L
Human insulin	n.d.	3600 µmol/L
Human C-peptide	n.d.	360 µmol/L
Human proinsulin	0.5 %	13.3 nmol/L

CALIBRATION

Rat/Mouse Proinsulin ELISA is calibrated against an in-house reference preparation of Rat Proinsulin I.

CONVERSION FACTOR

1 pmol corresponds to approximately 9.5 ng proinsulin.

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 of consequential.

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Further references can be found on our website: www.mercodia.com

SUMMARY OF PROTOCOL SHEET

Rat/Mouse Proinsulin ELISA

Add enzyme conjugate 1X solution	50 µL
Add Calibrators, controls* and samples	25 µL
Incubate	2 hours at 18-25°C on a plate shaker 700-900 rpm
Wash plate with wash buffer 1X solution	700µL, 6 times
Add Substrate TMB	200 µL
Incubate	30 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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