

Mercodia

Rat/Mouse Proinsulin

ELISA

Enzyme immunoassay

Directions for Use





10-1232-01
REAGENTS FOR 96 DETERMINATIONS

Manufactured by

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Mercodia 

EXPLANATION OF SYMBOLS USED ON LABELS

 $\Sigma = 96$	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 β -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 content of this kit and their residues must not be allowed to come into contact with ruminating animal or swine.
- The Stop Solution in this kit contains 0.5 M H_2SO_4 . Follow routine precautions for handling hazardous chemicals.
- All samples should be handled as capable of transmitting infections.

MATERIAL REQUIRED BUT NOT PROVIDED

- Pipettes for 25, 50, 200 and 1000 μ l (repeating pipettes preferred for addition of enzyme conjugate solution, Substrate TMB and Stop Solution)
- Beakers and cylinders for reagent preparation
- Redistilled water
- Microplate reader (450 nm filter)
- Plate shaker (the recommended velocity is 700-900 cycles per minute, orbital movement)
- Microplate washing device

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 8 weeks.	1 plate	96 wells 8-well strips	Ready for Use
Calibrators 1, 2, 3, 4, 5 Concentration indicated on vial label. Storage after reconstitution: 2-8°C for 4 weeks. For storage of reconstituted Calibrators for more than 4 weeks, store at -20°C	5 vials	1000 μ l	Lyophilized Add 1000 μ l 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 μ l	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 4 weeks.	1 bottle	50 ml	Dilute with 1000 ml redistilled water to make wash buffer 1X solution.
Substrate TMB Colorless solution <i>Note! Light sensitive!</i>	1 bottle	22 ml	Ready for Use
Stop Solution 0.5 H ₂ SO 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 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
6 strips	0.3 ml	3 ml
4 strips	0.2 ml	2 ml

Storage after dilution: 2-8°C for 7 days.

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 for serum and plasma samples.

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.

1. Prepare Calibrators, enzyme conjugate 1X solution and wash buffer 1X solution.
2. Prepare sufficient microplate wells to accommodate Calibrators and samples in duplicate.
3. Add 50 μ l of enzyme conjugate 1X solution to each well.
4. Pipette 25 μ l each of Calibrator 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 μ l 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 350 μ l 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 μ l Substrate TMB to each well.
8. Incubate for 30 minutes at room temperature (18-25°C).
9. Add 50 μ l 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! 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 proinsulin concentrations should routinely be assayed as unknowns, 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 analysis.

Manual calculation

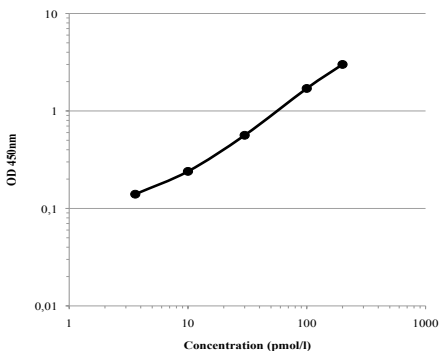
1. Plot the absorbance values obtained for the Calibrators, except Calibrator 0, against the rat/mouse proinsulin concentration on a log-log paper and construct a calibrator curve.
2. Read the concentration of the unknown samples from the calibrator curve.
3. If sample has been diluted, multiply the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

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 (\leq) 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.

Sample	Mean value pmol/l	Coefficient of variation		
		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:

Rat insulin	<0.000004%
Rat C-peptide	<00000001%
Mouse insulin	<0.0003%
Mouse C-peptide	<0.00007%
Human insulin	<0.0000001%
Human C-peptide	<0.000001%
Human proinsulin	2-10% (mean 5%) Range tested: 0.2-1.3 nM

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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SUMMARY OF PROTOCOL SHEET

X-O Graf Tryckeri AB

Add enzyme conjugate 1X solution	50 μ l
Add Calibrators and samples	25 μ l
Incubate	2 hour at 18-25°C on a plate shaker
Wash plate with wash buffer 1X solution	6 times
Add Substrate TMB	200 μ l
Incubate	30 minutes at 18-25°C
Add Stop Solution	50 μ l Shake for 5 seconds to ensure mixing
Measure A_{450}	Evaluate results