Mercodia Proinsulin ELISA

Directions for Use

REF 10-1118-03
Reagents for 96 determinations

For Research Use Only
Not for Use in Diagnostic Procedures

Mercodia AB
Sylveniusgatan 8A
SE-754 50 Uppsala
Sweden

31-3205
Version 1.0
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Explanation of symbols used on labels

	Reagents for 96 determinations
	Use-by date
	Temperature limit
	Batch code
	Catalogue number
	Consult electronic Instructions for Use
	Manufacturer

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Intended use

Mercodia Proinsulin ELISA provides a method for the quantitative determination of human proinsulin in serum or plasma.

Summary and explanation of the test

Proinsulin is the precursor of insulin which is the principal hormone responsible for the control of glucose metabolism. It is synthesized in the ß-cells of the Islets of Langerhans and is subsequently processed to form C-peptide and insulin. High proinsulin concentrations are usually noted in patients with benign or malignant ß-cell tumors of the pancreas. Most patients with ß-cell tumors have increased insulin, C-peptide and proinsulin concentrations, but occasionally only proinsulin is elevated. Despite its low biological activity, proinsulin may be increased sufficiently to produce hypoglycemia. Increased proinsulin concentrations may also be detected in patients with renal failure, cirrhosis or hyperthyroidism.

Principle of the procedure

Mercodia 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 microtitration well. After washing, peroxidase-conjugated anti-proinsulin antibodies are added and after the 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 that is read spectrophotometrically.

Warnings and precautions

- All samples should be handled as of capable of transmitting infections.
- Each well can only be used once.
- Do not mix components from different lots.
- Dispose of used components according to local regulations for biohazardous waste.

For safety information, please consult the safety data sheets (SDS) available at our website https://www.mercodia.com/technical-library/.

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

Each Mercodia Proinsulin ELISA kit contains reagents for 96 wells, sufficient for 43 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	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 Recombinant human proinsulin Color coded yellow Concentration stated on vial label Storage after reconstitution: 2–8°C for 1 month	4 vials	1000 µL	Lyophilized Add 1000 µL redistilled water per vial
For storage of reconstituted Calibrators for more than 1 month, store at -20°C			
Calibrator 0 Color coded yellow *Note! Negative control!*	1 vial	5 mL	Ready for use
Assay Buffer Color coded red	1 vial	6 mL	Ready for use
Enzyme Conjugate 21X Peroxidase conjugated mouse monoclonal anti-proinsulin	1 vial	600 µL	Preparation, see below
Enzyme Conjugate Buffer Color coded blue	1 vial	12 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 21X 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 21X vial. Mix gently before use.

Number of strips	Enzyme Conjugate 21X	Enzyme Conjugate Buffer
12 strips	1 vial	1 vial
8 strips	350 µL	7 mL
4 strips	200 µL	4 mL

Storage after dilution: 2–8°C for 2 months.

Specimen collection and handling

Serum

Collect blood by venipuncture, allow to clot, and separate the serum by centrifugation. Samples can be stored at 2–8°C up to 24 hours. For longer periods store samples at –20°C. Avoid repeated freezing and thawing.

Plasma

Collect blood by venipuncture into tubes containing heparin or EDTA as anticoagulant, and separate the plasma fraction by centrifugation. Samples can be stored at 2–8°C up to 24 hours. For longer periods store samples at –20°C. Avoid repeated freezing and thawing.

Preparation of samples

No dilution is normally required for serum and plasma samples, however, samples with a concentration above Calibrator 4 should be diluted in Calibrator 0 (or Mercodia Diabetes Sample Buffer, 10-1195-01).

Test procedure

All reagents and samples 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 enzyme conjugate 1X solution and wash buffer 1X solution.
2. Prepare sufficient microplate wells to accommodate Calibrators 1-4, Calibrator 0 (negative control), positive controls, and samples in duplicate.
3. Pipette 50 µL each of Calibrators, controls and samples into appropriate wells.
4. Add 50 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 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 100 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.
11. Incubate for 15 minutes on the bench at room temperature (18–25°C).
12. Add 50 µL Stop Solution to each well.
    Place plate on a shaker for approximately 5 seconds to ensure mixing.
13. 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 such as Mercodia Diabetes Antigen Control (Code No. 10-1134-01/10-1164-03) 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, preparation date of components, OD values for Calibrators 1-4, and the positive and negative controls.

Calculation of results

The concentration of pronsulin is determined by plotting the optical density (OD) at 450 nm of Calibrators 1-4 against their respective concentrations. Calibrator 0 should be used solely as a negative control and not included in the calibration curve.

It is important to use an appropriate curve fitting model that represents the true dose-response relationship to get accurate results. It is every laboratory's responsibility to validate the functionality of the chosen curve fitting model and software used.

The 10-1118-03 Proinsulin ELISA from Mercodia was validated using cubic spline curve fit.

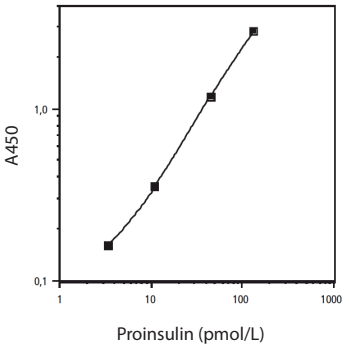
Example of worksheet

Wells	Identity	$A_{450\ nm}$	Mean conc. pmol/L
1A–B	Calibrator 0	0.070/0.069	
1C–D	Calibrator 1*	0.156/0.163	
1E–F	Calibrator 2*	0.347/0.354	
1G–H	Calibrator 3*	1.157/1.176	
2A–B	Calibrator 4*	2.862/2.831	
2C–D	Sample 1	0.208/0.208	5.25
2E–F	Sample 2	0.252/0.254	7.07
2G–H	Sample 3	0.563/0.589	19.8
3A–B	Sample 4	1.592/1.571	63.2

*Concentration stated 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

Application of this test to individuals already undergoing insulin therapy is complicated by formation of anti-insulin antibodies that are capable of interfering in the assay.

Grossly lipemic, icteric or hemolyzed samples do not interfere in the assay. However, hemolysis in serum and plasma samples may result in a degradation of insulin. The degradation is dependent on time, temperature and the hemoglobin concentration. Keep hemolyzed samples cold or on ice to prevent the insulin degradation.

Expected values

Good practice dictates that each laboratory establishes its own expected range of values. The following results may serve as a guide until the laboratory has gathered sufficient data of its own. Fasting levels for 112 tested, apparently healthy individuals, yielded a mean of 10 pmol/L, a median of 7 pmol/L and a range corresponding to the central 95 % of the observations of 3.3-28 pmol/L.

Performance characteristics

Detection limit

The detection limit is 1.7 pmol/L as determined by the methodology described in ISO11843- Part 4.

Capability of Detection should be seen as part of a method validation, rather than the lowest concentration that can be measured. 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 dilution 82%–118% (Mean 98%).
Recovery upon addition 93%–100% (Mean 95%).

Hook effect

Samples with a concentration of up to 80 000 pmol/L can be measured without giving falsely low results.

Precision

Each sample was analyzed in 4 replicates on 7 different occasions.

		Coefficient of variation	
Sample	Mean value pmol/L	Repeatability %*	Within laboratory %**
1	7.3	3.2	5.1
2	20.7	3.2	6.2
3	65.7	2.6	5.0

*Within assay variation
**Total Assay variation

Specificity

The following cross reactions have been found:

	Crossreaction
Insulin	<0.03 %
C-peptide	<0.006 %
Proinsulin Des (64–65)	84 %
Proinsulin Split (65–66)	90 %
Proinsulin Des (31–32)	95 %
Proinsulin Split (32–33)	95 %
Porcine Proinsulin	4 %
Mouse Proinsulin I	3 %
Mouse Proinsulin II	16 %
Rat Proinsulin I	13 %
Rat Proinsulin II	10 %

Calibration

Mercodia Proinsulin ELISA kit is calibrated against the International Reference Reagent for human proinsulin, IRR 84/611.

Conversion factor

1 µg/L corresponds to 110 pmol/L

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.

References

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

Notes

Notes

	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 of protocol sheet
Mercodia Proinsulin ELISA

Add Calibrators, controls* and samples	50 µL
Add Assay Buffer	50 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	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 Substrate TMB	200 µL
Incubate	15 minutes at 18–25 °C
Add Stop Solution	50 µL Shake for 5 seconds to ensure mixing
Measure $A_{450\ nm}$	Evaluate results

*not included

For full details see page 6

For technical support please contact: support@mercodia.com

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