Mercodia
Developing diagnostics

Mercodia Proinsulin ELISA

Directions for Use

10-1118-01
REAGENTS FOR 96 DETERMINATIONS

Gebrauchanweisung in deutsch finden Sie under folgenden Link:
Veuillez trouver le mode d'emploi en français à:
Podrá encontrar las instrucciones de uso en español en:
Le instruzioni per l'uso sono reperibili in italiano all'indirizzo:
For danske brugsanvisning gå til:
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http://www.mercodia.se/products/directions-for-use.html

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CE

Regulatory status in U.S.: For Research use only
Not for use in diagnostic procedures

Manufactured by

Mercodia AB, Sylveniusgatan 8A,
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Sweden

EXPLANATION OF SYMBOLS USED ON LABELS

	Reagents for 96 determinations
	Expiry date
	Store between 2–8°C
	Lot No.
	For *in vitro* diagnostic use

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 principle 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

- For in vitro diagnostic use.
- In USA: For research use only. Not for use in diagnostic procedures.
- 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 0.5 M H_2SO_4. Follow routine precautions for handling hazardous chemicals.
- All samples should be handled as of 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 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 fo 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 For storage of reconstituted Calibrators for more than 1 month, store at -20°C	4 vials	1000 µL	Lyophilized Add 1000 µL redistilled water per vial
Calibrator 0 Color coded yellow	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 with1000 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 normaly required for serum and plasma samples, however, samples with a concentration above Calibrator 5 should be diluted in Calibrator 0.

TEST PROCEDURE

All reagents and samples must be brought to room temperature before use. 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 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! To prevent contamination between the conjugate and substrate, separate pipettes are recommended.

INTERNAL QUALITY CONTROL

Commercial controls such as Mercodia Diabetes Antigen Control (Code No. 10-1134-01/ 10-1164-01) 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 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 proinsulin is obtained by computerized data reduction of the absorbance for the Calibrators, except for Calibrator 0, versus the concentration using cubic spline regression.

Manual calculation

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

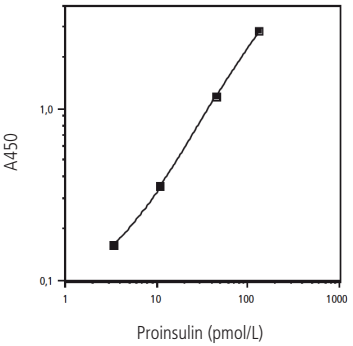
Example of worksheet

Wells	Identity	A_{450}	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 lable.

Calibrator curve

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



LIMITATIONS OF THE PROCEDURE

As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical findings have been evaluated. 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.

Seperate pipettes should be used when pipetting the conjugate and the substrate.

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

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 0.5 pmol/L as determined by 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 97 %.

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	within assay %	between assay %	total assay %
1	7.3	3.2	3.9	5.1
2	20.7	3.2	5.2	6.1
3	65.6	2.5	4.2	5.0

Specificity

The following cross reactions have been found:

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 %

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.

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

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}	Evaluate results

*Not provided

For full details see page 6

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Version 12.0