Mercodia
Developing diagnostics

Mercodia Ultrasensitive C-peptide ELISA

ATTENTION!
Updated protocol

Directions for Use

10-1141-01
REAGENTS FOR 96 DETERMINATIONS

For *in vitro* diagnostic use

IVD

CE

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:
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For danske brugsanvisning gå til:
För svensk bruksanvisning gå till:

http://www.mercodia.se/products/directions-for-use.html

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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.
	For *in vitro* diagnostic use

INTENDED USE

Mercodia Ultrasensitive C-peptide ELISA provides a method for the quantitative determination of human C-peptide in serum, plasma or urine.

SUMMARY AND EXPLANATION OF THE TEST

Qualitative and quantitative evaluation of pancreatic ß-cell function is not only of use in the pre- and postdiagnostic study of the natural history of diabetes mellitus, but is also relevant in clinical practice as a guide to the correct choice of treatment. Peripheral insulin levels cannot be used to assess ß-cell function because of a large and variable uptake from the portal circulation into the liver, and because insulin assays cannot distinguish endogenous from exogenous insulin.

Within the pancreatic ß-cell, proinsulin is cleaved into one molecule of C-peptide and one molecule of insulin. C-peptide is subsequently released into the circulation at concentrations equimolar to those of insulin. In contrast to insulin, C-peptide is only minimally extracted by the liver. Peripheral C-peptide concentrations therefore reflect the secretion of ß-cells more accurately than insulin.

PRINCIPLE OF THE PROCEDURE

Mercodia Ultrasensitive C-peptide 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 C-peptide molecule. During incubation C-peptide in the sample reacts with anti-C-peptide antibodies bound to the microtitration well. After washing, peroxidase conjugated anti-C-peptide antibodies are added. After a second incubation and a simple washing step, 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.
- 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 enzyme conjugate 1X solution, Assay Buffer, 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 Ultrasensitive C-peptide ELISA (10-1141-01) kit 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-C-peptide	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, 5 Human C-peptide Color coded yellow Concentration stated on vial label Storage after reconstitution: 2–8°C for 1 week For storage of reconstituted Calibrators for more than 1 week, 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
Assay Buffer Color coded red	1 vial	6 mL	Ready for use
Enzyme Conjugate 21X Peroxidase conjugated mouse monoclonal anti-C-peptide	1 vial	1.2 mL	Preparation, see below
Enzyme Conjugate Buffer Color coded blue	1 vial	24 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, (1+20) in Enzyme Conjugate Buffer or 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. Use within one day.

Number of strips	Enzyme Conjugate 21X	Enzyme Conjugate Buffer
12 strips	1 vial	1 vial
8 strips	700 µL	14 mL
4 strips	350 µL	7 mL

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 3 days. 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 3 days. For longer periods store samples at –20°C. Avoid repeated freezing and thawing.

Urine

Collect a 24 hour urine sample (without preservative). Keep the specimen at 2–8°C between collections. Record the total volume of the specimen and retain a well mixed aliquot for analysis. Store the samples at 2–8°C for a maximum of 24 hours before assay. For longer storage, keep the urine samples frozen at –70°C until assay is performed. Repeated freezing and thawing must be avoided. Cellular debris should be removed before assay, either by filtration or centrifugation.

Preparation of samples

Urine samples should be diluted 1/10 in Calibrator 0 before running the assay. 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 200 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 step 6.
10. Add 200 µL Substrate TMB.
11. Incubate for 30 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 and/or internal serum pools with low, intermediate and high C-peptide 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, 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 C-peptide 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 C-peptide concentration on a log-log paper and construct a calibrator curve.
2. Read the concentration of the samples from the calibrator curve.

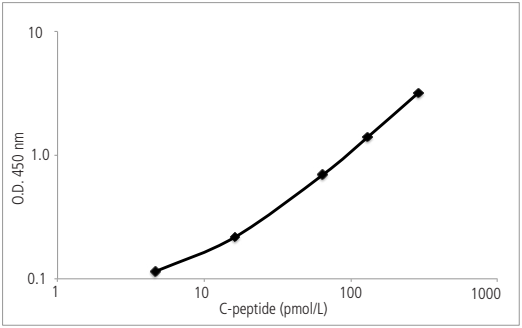
Example of worksheet

Wells	Identity	A_{450}	Mean conc. pmol/L
1A–B	Calibrator 0	0.074/0.075	
1C–D	Calibrator 1*	0.111/0.117	
1E–F	Calibrator 2*	0.210/0.223	
1G–H	Calibrator 3*	0.698/0.688	
2A–B	Calibrator 4*	1.372/1.420	
2C–D	Calibrator 5*	3.173/3.196	
2E–F	Sample 1	0.196/0.210	15
2G–H	Sample 2	0.601/0.585	55
3A–B	Sample 3	1.256/1.259	117

*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. Grossly lipemic, icteric or hemolyzed samples do not interfere in the assay.
Separate 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 serum levels for 136 tested, apparently healthy individuals, yielded a mean of 742 pmol/L (2.2 µg/L), a median of 628 pmol/L (1.9 µg/L) and a range, corresponding to the central 95% of the observations, of 343–1803 pmol/L (1.0–5.4 µg/L).

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 ≤2.5 pmol/L (0.0076 µg/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

Serum:
Recovery upon addition is 87-123% (mean 103%).
Recovery upon dilution is 106-117% (mean 111%)

Hook effect

Samples with a concentration of up to 36 nmol/L can be measured without giving falsely low results.

Precision

Each sample was analyzed in 4 replicates on 14 different occasions.

		Coefficient of variation		
Sample	Mean value pmol/L	within assay %	between assay %	total assay %
1	15	6.2	4.3	5.3
2	54	4.6	3.5	4.2
3	111	3.9	0.0	2.2

Specificity

Proinsulin at a concentration of 105 pmol/L yielded a result of 5 pmol/L. For other peptides, the following cross reactions have been found:

Insulin	<0.0006%
Proinsulin	5 %
Proinsulin Des (31–32)	3 %
Proinsulin Split (32–33)	2 %
Proinsulin Des (64–65)	74 %
Proinsulin Split (65–66)	10 %

CALIBRATION

Mercodia Ultrasensitive C-peptide ELISA kit is calibrated against the International Reference Reagent for C-peptide, IRR C-peptide 84/510.

CONVERSION FACTOR

1 µg/L corresponds to 331 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

Craig ME, Howard NJ, Silink M, Rawlinson WD (2003) Reduced frequency of HLA DRB1*03-DQB1*02 in children with type 1 diabetes associated with enterovirus RNA. *J Infect Dis* 187:1562-1570

Gaines-Das RE and Bristow AF (1988) WHO International reference reagents for human proinsulin and human C-peptide. *J Biol Stand* 16:179-186

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

SUMMARY PROTOCOL SHEET

Mercodia Ultrasensitive C-peptide ELISA

Add Calibrators, controls* and samples	50 µL
Add Assay Buffer	50 µL
Incubate	1 hour at 18–25°C on a shaker 700-900 rpm
Wash with wash buffer 1X solution	700 µL, 6 times
Add enzyme conjugate 1X solution	200 µL
Incubate	1 hour at 18–25°C on a shaker 700-900 rpm
Wash with wash buffer 1X solution	700 µL, 6 times
Add Substrate TMB	200 µL
Incubate	30 minutes at 18-25°C
Add Stop Solution	50 µL Shake for 5 sec to ensure mixing
Measure A_{450}	Evaluate results

*not provided

For full details see page 6