Mercodia Rat C-peptide ELISA

Directions for Use

10-1172-01
Reagents for 96 determinations

For Research Use Only

Manufactured by

Mercodia AB
Sylveniusgatan 8A
SE-754 50 Uppsala
Sweden

Explanation of symbols used on labels

Symbol	Meaning
Σ = 96	Reagents for 96 determinations
	Expiry date
	Store between 2–8°C
LOT	Lot No.

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Intended Use

Mercodia Rat C-peptide ELISA provides a method for the quantitative determination of rat C-peptide in serum, EDTA-plasma and cell culture medium.

Summary and explanation of the test

C-peptide is formed together with insulin from the cleavage of proinsulin within secretory granules in the ß-cell. In most species the insulin gene exists in a single copy. Rats and mice however, have two closely related genes which produce two nonallelic proinsulins [1]. The rat proinsulins are cleaved to form two insulins (insulin I and insulin II) and two C-peptides (C-peptide I and C-peptide II):
The two C-peptides differ with regard to two amino acids in the middle segment of the molecule. C-peptide is considered to have a longer half-life in circulation than insulin, and is used in humans and animal models as a marker of endogenous insulin production [2]. Traditionally C-peptide has been considered to be without biological effects of its own, but in recent years it has been reported that C-peptide treatment may affect renal and nerve dysfunction in type 1 diabetes patients [3]. Physiological effects of C-peptide have also been observed in animal models of diabetes [4,5]. Mercodia Rat C-peptide ELISA calibrators are made from syntetic rat C-peptide I. Both rat C-peptide I and II are measured in the assay.

Principle of the procedure

Mercodia Rat C-peptide 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 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 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 the addition of acid, giving a colorimetric endpoint that can be read spectrophotometrically.

Warnings and precautions

- For research use only.
- Not for internal or external use in humans or animals.
- 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 water. 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, Wash Buffer and Assay 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, Wash Buffer and Assay 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 Assay Buffer, enzyme conjugate 1X solution, Substrate TMB and Stop Solution)
- Tubes, beakers and cylinders for reagent preparations
- Magnetic stirrer
- Vortex mixer
- Redistilled water
- Microplate shaker (700–900 cycles per minute, orbital movement)
- Microplate washing devise with overflow function (recommended but not required)
- Microplate absorbance reader (450 nm filter)

Reagents

Each Mercodia Rat C-peptide ELISA (10-1172-01) 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-rat C-peptide For unused microplate 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 Synthetic rat C-peptide I Color coded yellow Concentration stated on vial label Storage after reconstitution: 2–8°C for 1 month.	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-rat C-peptide	1 vial	1.3 mL	Preparation, see below
Enzyme Conjugate Buffer Color coded blue	1 vial	13 mL	Ready for Use
Assay Buffer Color coded red	1 vial	6 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 11X (1+10) in Enzyme Conjugate Buffer or according to the table below. When preparing enzyme conjugate 1X solution for the whole plate or if the reagents are to be used within 1 week, 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	700 µL	7 mL
4 strips	400 µL	4 mL

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

Specimen collection and handling

Serum

Collect blood by venipuncture, allow to clot and separate the serum by centrifugation.

Plasma

Collect blood by venipuncture into tubes containing EDTA as anticoagulant, and separate the plasma fraction by centrifugation.

Cell culture medium

Note that different chemicals used in cell culture media can interfere with the assay (such as sodium azide (NaN_3) and beta-mercaptoethanol).

Preparation of samples

No dilution is normally required for serum and plasma samples, however, samples with a concentration above Calibrator 5 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. Perform each determination in duplicate for calibrators and samples. Prepare a calibrator curve for each assay run. The product has been optimized and validated without plate sealer.

1. Reconstitute Calibrator 1–5 with 1000 µL redistilled water per vial.
2. Prepare enzyme conjugate 1X solution (according to table on previous page) and wash buffer 1X solution.
3. Prepare sufficient microplate wells to accommodate Calibrators, controls and samples in duplicate.
4. Pipette 10 µL each of Calibrators, controls and samples into appropriate wells.
5. Add 50 µL of Assay Buffer to each well.
6. Incubate on a plate shaker (700–900 rpm) for 1 hour at room temperature (18–25°C).
7. 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.
8. Add 100 µL enzyme conjugate 1X solution into each well.
9. Incubate on a plate shaker (700–900 rpm) for 1 hour at room temperature (18–25°C).
10. Wash as described in 7.
11. Add 200 µL Substrate TMB into each well.
12. Incubate on the bench for 15 minutes at room temperature (18–25°C).
13. Add 50 µL Stop Solution to each well.
    Place the plate on the shaker for approximately 5 seconds to ensure mixing.
14. 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 Rat and Mouse, Low, Medium, and High (10-1220-01) 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, dilution and/or reconstitution dates of kit components, OD values for the Calibrator 0, Calibrators and Controls.

Calculation of results

The concentration of rat C-peptide is obtained by computerized data reduction of the absorbance for the Calibrators, except Calibrator 0, versus the concentration using cubic spline regression.

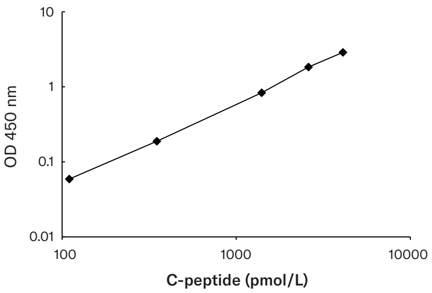
Example of results

Wells	Identity	$A_{450\ nm}$	Mean conc. pmol/L
1 A–B	Calibrator 0	0.114/0.113	
1 C–D	Calibrator 1*	0.176/0.168	
1 E–F	Calibrator 2*	0.301/0.301	
1 G–H	Calibrator 3*	0.969/0.923	
2 A–B	Calibrator 4*	1.958/1.936	
2 C–D	Calibrator 5*	2.983/2.991	
2 E–F	Sample 1	0.275/0.276	303.0
2 G–H	Sample 2	1.086/1.100	1557.2
3 A–B	Sample 3	2.134/2.120	2840.6

*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

Grossly lipemic, icteric or hemolysed samples do not interfere with 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 lower than the concentration of Calibrator 1 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 97%–100% (mean 98%).
Recovery upon dilution is 91%–105% (mean 100%).

Hook effect

Samples with a concentration of up to 400 000 pmol/L have been tested without giving falsely low results.

Precision

Each sample was analyzed in 4 replicates on 24 different occasions.

		Coefficient of variation	
Sample	Mean value pmol/L	Repeatability %	Within laboratory %
1	335	4.2	7.5
2	1432	2.5	4.1
3	2831	2.0	2.4

*Within assay variation
**Total assay variation

Specificity

The following crossreactions have been found:

	Crossreaction	Highest concentrations tested
Rat Insulin	n.d.	800 nmol/L
Rat Proinsulin	4.6 %	50 nmol/L
Human C-peptide	n.d.	14.3 µmol/L
Human Proinsulin	n.d.	21.1 µmol/L
Human Insulin	n.d.	1.1 µmol/L
Mouse C-peptide	6 %	30 nmol/L
Mouse Proinsulin	0.03 %	6.4 µmol/L
Mouse Insulin	n.d.	16 nmol/L
Porcine C-peptide	n.d.	8 µmol/L
Porcine Proinsulin	n.d.	103 nmol/L
Porcine Insulin	n.d.	40 nmol/L
Maqacue C-peptide	n.d.	315 nmol/L

n.d. = not detectable

Calibration

Mercodia Rat C-peptide ELISA is calibrated against an in-house reference preparation of rat C-peptide I.

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 or consequential.

References

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2. Faber OK, Hagen C, Binde, C, Markussen J, Naithani, VK, Blix PM, Kuzuya H, Horwitz DL, Rubenstein AH and Rossing N (1978) Kinetics of human connecting peptide in normal and diabetic subjects. *J Clin Invest* 62:197-203

3. Wahren J, Ekberg K and Jornvall H (2007) C-peptide is a bioactive peptide. *Diabetologia* 50:503-509

4. Nordquist L, Moe E, Sjöquist M (2007) The C-peptide fragment EVARQ reduces glomerular hyperfiltration in streptozotocin-induced diabetic rats. *Diabetes Metab Res Rev* 2006 23:400-405

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

Notes

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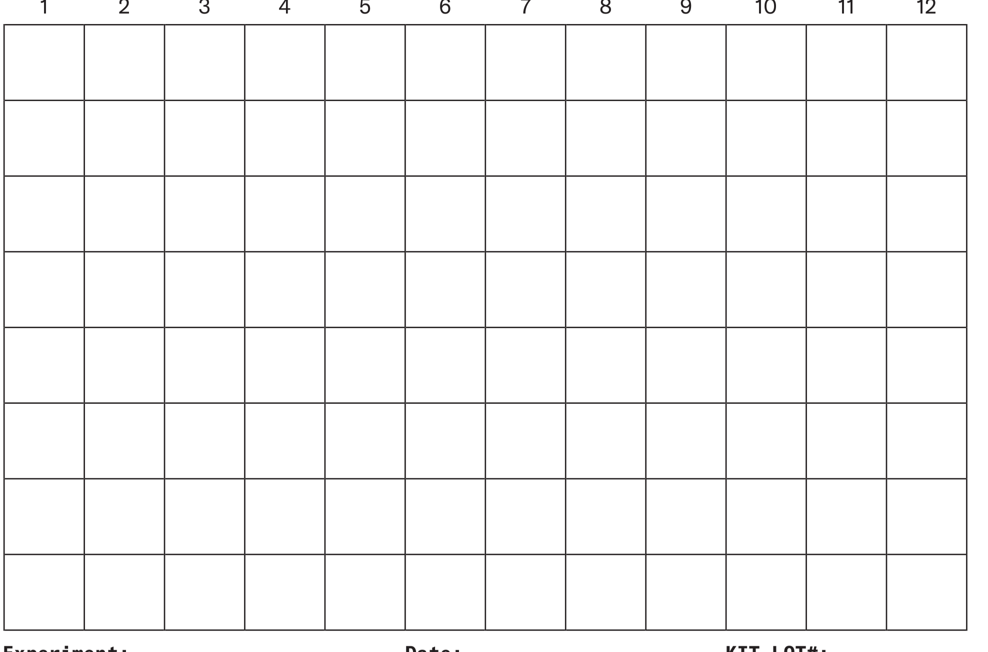
Notes



Experiment: **Date:** **KIT LOT#:**

Summary of protocol sheet
Mercodia Rat C-peptide ELISA

Add Calibrators, controls* and samples	10 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 7

For technical support please contact: support@mercodia.com

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X-O Graf Tryckeri AB