

Mercodia

Porcine C-peptide

ELISA

Directions for Use

10-1256-01

REAGENTS FOR 96 DETERMINATIONS

For Research Use Only

Manufactured by

Mercodia AB

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Sweden

EXPLANATION OF SYMBOLS USED ON LABELS

 $\Sigma = 96$	Reagents for 96 determinations
	Expiry date
	Store between 2-8°C
	Lot No.

INTENDED USE

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

SUMMARY AND EXPLANATION OF THE TEST

Within the pancreatic β -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 β -cells more accurately than insulin (1,2). 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). In islet transplantation studies, determination of C-peptide has become an important method to monitor islet function. In xenotransplantation, determination of porcine insulin can usually not be used to monitor islet function due to crossreaction of porcine insulin with insulin from other species. A key parameter of pre-clinical efficacy of islet xenotransplantation is therefore the specific determination of porcine C-peptide responses in the recipient (4).

PRINCIPLE OF THE PROCEDURE

Mercodia Porcine 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, clone 4B7-E9. After washing, peroxidase-conjugated anti-C-peptide antibodies clone 5G8-G2, 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

- The contents of this kit and their residues must not be allowed to come into contact with ruminating animals or swine.
- All samples should be handled as capable of transmitting infections.
- Not for internal or external use in humans or animals.
- Each well can only be used once.
- For Research Use Only. Not for use in diagnostic procedures.
- 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% reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H-isothiazol-3-one.

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 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 Porcine C-peptide ELISA 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-insulin 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 Porcine C-peptide Color coded yellow Concentration stated on vial label Storage after reconstitution: 2–8°C for 3 weeks. For storage of reconstituted Calibrators for more than 3 weeks, 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
Enzyme Conjugate 11X Mouse monoclonal anti-porcine 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 <i>Note! Light sensitive!</i>	1 vial	22 mL	Ready for use
Stop Solution 0.5 M 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 1X 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	600 µL	6 mL
4 strips	400 µL	4 mL

Storage after dilution: 2-8°C for 3 days.

SPECIMEN COLLECTION AND HANDLING

Serum

Collect blood by venipuncture, allow to clot, and separate the serum by centrifugation. Store samples at below -20°C. Avoid repeated freezing and thawing.

Plasma

Collect blood by venipuncture into tubes containing EDTA as anticoagulant, and separate the plasma fraction by centrifugation. Store samples at below -20°C. Avoid repeated freezing and thawing.

Cell culture medium

Note that different chemicals used in cell culture media can interfere with the assay (such as sodium azide (NaN₃) and beta-mercaptoethanol).

Preparation of samples

No dilution is normally required, however, samples containing $>1.0 \mu\text{g/L}$ should be diluted 1/10 v/v with Calibrator 0. *Note!* Buffers containing sodium azide (NaN_3) can not be used for sample dilution.

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 (according to the table on previous page) and wash buffer 1X solution.
2. Prepare sufficient microplate wells to accommodate Calibrators, controls and samples in duplicate.
3. Pipette 10 μL each of Calibrators, controls and samples into appropriate wells.
4. Add 50 μL of Assay Buffer to each well.
5. Incubate on a plate shaker (700-900 rpm) for 2 hours 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 buffer 1X solution, tap firmly several times against absorbent paper to remove excess liquid. Repeat 5 times. Avoid prolonged soaking during washing.

7. Add 100 μL enzyme conjugate 1X solution to each well.
8. Incubate on a plate shaker for 1 hour (700-900 rpm) at room temperature (18–25°C).
9. Wash as described in step 6.
10. Add 200 μL Substrate TMB.
11. Incubate for 15 minutes 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 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 blank, Calibrators and controls.

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 for 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.

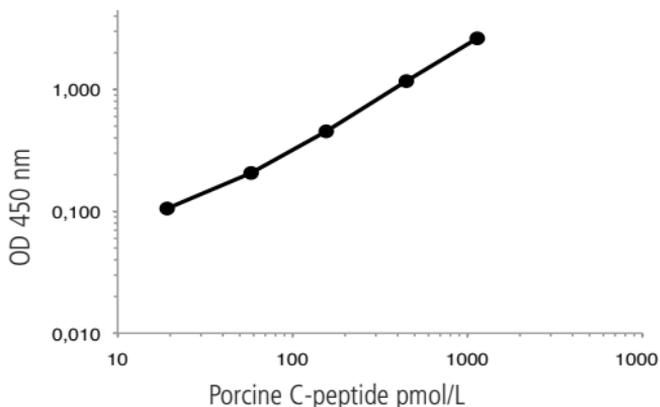
Example of results

Wells	Identity	A ₄₅₀	Mean conc. pmol/L
1A–B	Calibrator 0	0.058/0.061	
1C–D	Calibrator 1*	0.105/0.106	
1E–F	Calibrator 2*	0.212/0.201	
1G–H	Calibrator 3*	0.449/0.458	
2A–B	Calibrator 4*	1.174/1.167	
2C–D	Calibrator 5*	2.634/2.624	
2E–F	Sample 1	0.184/0.179	48
2G–H	Sample 2	0.404/0.401	136
3A–B	Sample 3	1.031/1.042	393

* 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 haemolysed 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 a part of a method validation, rather than the lowest concentration that can be measured.

The detection limit is 10 pmol/L as determined with the methodology described in ISO11843-Part 4.

Concentration of samples with absorbance below Calibrator 1 should not be calculated, instead expressed less or equal to (\leq) the concentration indicated on the vial for Calibrator 1.

Recovery

Recovery upon addition 83%-91% (Mean 87%)

Recovery upon dilution 96%-120% (Mean 107%)

Hook effect

Samples with a concentration up to at least 30000 pmol/L can be measured without giving falsely low results.

Precision

Each sample was analyzed in 4 replicates on 38 different occasions.

Sample	Mean value pmol/L	Coefficient of variation	
		Repeatability %*	Within laboratory %**
1	51	3.6	3.8
2	138	3.2	4.0
3	398	2.8	3.8

*Within assay variation

**Total assay variation

Specificity

The following cross-reactions were found:

Porcine insulin	<0.05%
Porcine proinsulin	<0.02%
Human proinsulin	<0.01%
Human C-peptide	<0.003%
Macaque C-peptide	<0.006%
Mouse proinsulin	<0.002%
Mouse C-peptide	<0.007%
Rat C-peptide	0.08%
Rat insulin	<0.002%
Mouse insulin	<0.13%
Rat proinsulin	<0.002%

CALIBRATION

Mercodia Porcine C-peptide ELISA is calibrated against an in-house reference preparation of porcine C-peptide.

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.

REFERENCES

1. Heding LG and Rasmussen SM (1975) Human C-peptide in normal and diabetic subjects. *Diabetologia* 11:201-6
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. Cooper DKC and Casu A (2009) The International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes -Chapter 4: Pre-clinical efficacy and complication data required to justify a clinical trial. *Xeno-transplantation* 16:229-238.

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

SUMMARY OF PROTOCOL SHEET
Merckodia Porcine C-peptide ELISA

Add Calibrators, controls* and samples	10 µL
Add Assay Buffer	50 µL
Incubate	2 hours 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
Add Stop Solution	50 µL Shake for 5 seconds to ensure mixing
Measure A ₄₅₀	Evaluate results

*not provided

For full details see page 7

For technical support please contact support@merckodia.com.