Mercodia
Developing diagnostics

Mercodia Porcine C-peptide ELISA

Directions for Use

10-1256-01
REAGENTS FOR 96 DETERMINATIONS

Manufactured by

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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 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 (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

- For research use only. Not for use in diagnostic procedures.
- Not for internal or external use in humans or animals.
- The content 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 0.5M H_2SO_4. Follow routine precautions for handling hazardous chemicals.

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 FOR 1 X 96 KIT

Each Mercodia Porcine C-peptide ELISA kit (10-1256-01) 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-porcine 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 8 weeks.			
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 8 weeks	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 11X, (1+10) in Enzyme Conjugate Buffer according to the table below. When preparing enzyme conjugate 1X solution for the whole plate or if the reagents are to be used within 2 weeks, 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
6 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. 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 (NaN_3) and beta-mercaptoethanol).

Preperation of samples

No dilution is normally required, however, samples containing >1.0 µg/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.

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 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 for 2 hour (700-900 rpm) 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 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 procedure.
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 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! 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, 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.

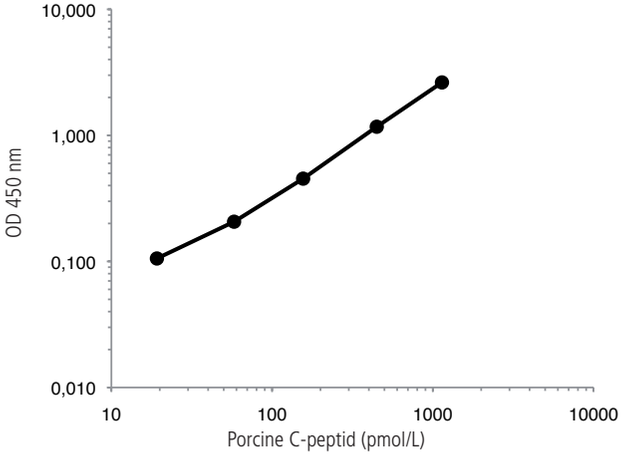
Example of results

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 (≤) 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.

		Coefficient of variation		
Sample	Mean value pmol/L	within assay %	between assay %	total assay %
1	51	3.6	3.4	3.8
2	138	3.2	3.7	4.0
3	398	2.8	3.5	3.8

Specificity
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 1diabetes -Chapter 4: Pre-clinical efficacy and complication data required to justify a clinical trial. *Xenotransplantation* 16:229-238.

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

EXPLANATION OF SYMBOLS USED ON LABELS

 **∑ = 96**	Reagents for 96 determinations
	Expiry date
	Store between 2-8°C
	Lot No.

SUMMARY OF PROTOCOL SHEET

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

*not provided

For full details see Test Procedure