Mercodia

Technical Note, No: 34-0144, v.10.0

For Research Use Only. Not for use in diagnostic procedures.

Alternative protocol for increased sensitivity in Ultrasensitive C-peptide ELISA

Advancement of research methods is a prerequisite for advancing research. By using the alternative assay protocol described below extremely low levels of C-peptide can be measured.

Low levels of C-peptide have been detected in type 1 diabetic patients and it has been suggested that preservation of C-peptide levels (> 10 pmol/L) is associated with protection from the onset of diabetes-specific complications. Data suggest that preserved C-peptide levels lead to better glycaemic control as measured with HbA1c.

PLEASE OBSERVE
This protocol requires one extra vial of Assay Buffer and Enzyme Conjugate 21X. Therefore, two kits are required to run 42 samples in duplicates. The kits can be ordered by emailing info-global@mercodia.com or by contacting your local sales representative.

Sensitivity

Table 1: Comparison of detection limit and assay range between the different assay protocols with the Mercodia Ultrasensitive C-peptide ELISA

	Ultrasensitive C-peptide ELISA - alternative protocol	Ultrasensitive C-peptide ELISA - original assay protocol
Detection limit	≤ 1.25 pmol/L ≤ 0.0038 ng/mL	≤ 2.5 pmol/L ≤ 0.0076 ng/mL
Assay range	1.25 - 130 pmol/L 0.0038 - 0.393 ng/mL	5 – 280 pmol/L 0.015 - 0.846 ng/mL

Sample and reagent volumes

The volume of calibrators, controls and samples is 100 µL instead of 50 µL in the standard protocol.
The Assay Buffer volume is 100 µL instead of 50 µl in the standard protocol.

Preparation of enzyme conjugate solution

Pool the two vials of Enzyme Conjugate 21X. Dilute the Enzyme Conjugate 21X 10 times (1+9) in Enzyme Conjugate Buffer. Due to a different dilution factor, the buffer cannot be poured into Enzyme Conjugate 21X vial as suggested in the standard protocol.

Number of strips	Enzyme Conjugate 21X	Enzyme Conjugate Buffer
12 strips	2.25 mL	20.25 mL
8 strips	1.50 mL	13.50 mL
4 strips	0.75 mL	6.75 mL

Preparation of additional calibrators

Calibrator 1 should be diluted ½ and ¼ with Calibrator 0. When running the assay, exclude Calibrator 5 and use Calibrator 0, Calibrator 1 diluted by ¼, Calibrator 1 diluted by ½ and Calibrators 1 to 4 in each run.

Number of replicates	Dilution	
2	¼	75 µL Calibrator 1 + 225 µL Calibrator 0
	½	150 µL Calibrator 1 + 150 µL Calibrator 0
4	¼	125 µL Calibrator 1 + 375 µL Calibrator 0
	½	250 µL Calibrator 1 + 250 µL Calibrator 0

Summary Protocol Sheet

Add calibrators (Calibrator 0, dilutions of Calibrator 1, Calibrators 1-4), controls and samples	**100 µL**
Add Assay Buffer to all wells	100 µL
Incubate	1 hour at 18-25°C on a plate shaker (700-900 rpm)
Wash plate with Wash Buffer 1X solution	6 times*
Add enzyme conjugate solution to all wells	200 µL
Incubate	1 hour at 18-25°C on a plate shaker (700-900 rpm)
Wash plate with Wash Buffer 1X solution	6 times*
Add Substrate TMB	200 µL
Incubate	30 minutes
Add Stop Solution	50 µL Shake plate for 5 seconds to ensure mixing
Measure A450	Evaluate results

* Wash 6 times with 700 µL wash buffer 1X solution per well, using an automatic plate washer with an overflow wash function. After the final wash, invert and tap the plate firmly against absorbent paper.
Do not include a soak step in the washing procedure.
For manual washing, see Technical Note No: 34-0106 Instruction for manual washing procedure for microplates (available online).
Specific details about each step can be found in the Directions for Use for Ultrasensitive C-peptide ELISA 10-1141-01.

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 ≤ 1.25 pmol/L (0.0038 ng/mL) as determined by the methodology described in ISO11843- Part 4. The concentration of samples with an absorbance below the lowest calibrator used should not be calculated, instead expressed as less than or equal to (≤) the concentration indicated on the vial for Calibrator 1 divided by 4.

Recovery

The recovery upon dilution at 1/2 and 1/5 of 6 samples from type 1 diabetes patients was 82 – 114% (mean 100%). The recovery upon addition of 21.4 pmol/L C-peptide to 5 samples from type 1 diabetes patients was 94 – 124% (mean 110%).

Specificity

Insulin	< 0.0006%
Proinsulin	5%

Precision

Four buffer samples for precision studies were prepared by addition of C-peptide. Each sample was analyzed in 2 - 4 replicates on 9 -10 occasions. Analyses were performed by 2 technicians using different ELISA lots. Results are shown in Table 2.

Table 2. Precision data with the alternative protocol for Ultrasensitive C-peptide ELISA.

Sample	Mean value (pmol/L)	Mean value (ng/mL)	Coefficient of variation	
			Repeatability* (%)	Within laboratory** (%)
1	2.0	0.0060	6.4	5.5
2	4.8	0.015	3.9	4.9
3	15	0.046	4.1	3.4
4	54	0.16	4.5	5.3

*Within assay variation

**Total assay variation

Sample Stability

Freeze-Thaw Cycles

Sera from 4 different type 1 diabetes patients were used. Aliquots of samples were frozen (at -20°C) and thawed for 1 to 4 cycles. No trends were observed when using one way anova for evaluation, Figure 1.

Sample storage

Six type 1 diabetes samples stored at -80°C for 8 months showed a recovery of 89 – 107% (mean 102%).

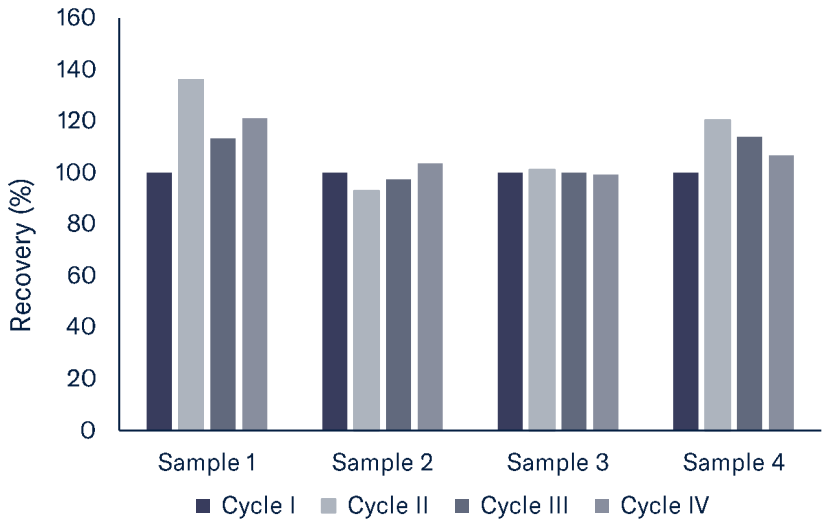

Figure 1. C-peptide concentrations in type 1 diabetics (n=4) after 1 to 4 freeze-thaw cycles.

References

Kuhtreiber WM, Washer SL, Hsu E, Zhao M, Reinhold P, Burger D, Zheng H, Faustman DL. (2015) Low levels of C-peptide have clinical significance for established Type 1 diabetes. Diabet Med.32(10):1346-53.