Alternative protocol for increased sensitivity Ultrasensitive C-peptide ELISA

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

This tech note describes an alternative test procedure for use with the Mercodia Ultrasensitive C-peptide ELISA (10-1141-01).

By using this alternative protocol, greater sensitivity can be achieved. The lowest concentration that can be measured is approximately 1.25 pmol/L (0.0038 ng/mL) instead of 5 pmol/L (0.015 ng/mL). The measuring range for this alternative protocol is approximately 1.25 - 130 pmol/L (0.0038 - 0.393 ng/mL). The measuring range for the standard protocol (included in the 10-1141-01 Directions For Use) is approximately 5 - 280 pmol/L (0.015 - 0.846 ng/mL).

Changes to the protocol are detailed in the space below. See page 3 for a protocol summary sheet.

Extra reagents (sold separately)
The Mercodia Ultrasensitive C-peptide ELISA alternative protocol requires two extra components not included in the Ultrasensitive C-peptide (10-1141-01). The components are one extra vial of Assay Buffer, article number 20-6640 (6 mL), and one extra vial of Enzyme Conjugate 21X, article number 20-7100 (1,2 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 and dilute 10 times (1+9) in Enzyme Conjugate Buffer.

Example of enzyme conjugate preparation:

Number of strips	Enzyme Conjugate 21X	Enzyme Conjugate Buffer
12 strips	2,25	20,25 mL
8 strips	1,5 mL	13,5 mL
4 strips	0,75 mL	6,75 mL

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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.

Example of Calibrator 1 dilution:

Number of replicates	Dilution	
2	1/4	75 µL Calibrator 1 + 225 µL Calibrator 0
	1/2	150 µL Calibrator 1 + 150 µL Calibrator 0
4	1/4	125 µL Calibrator 1 + 375 µL Calibrator 0
	1/2	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 viewed in the Directions for Use for 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%).

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.

Sample	Mean value (pmol/L)	Mean value (ng/mL)	Coefficient of variation		
			Within assay (%)	Between assay (%)	Total assay (%)
1	2.0	0.0060	6.4	4.5	5.5
2	4.8	0.015	3.9	4.5	4.9
3	15.2	0.046	4.1	1.8	3.4
4	54.4	0.164	4.5	4.2	5.3

Specificity

Insulin < 0.0006%
Proinsulin < 1.8%

Sample Stability

Storage at -80°C

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

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 can be seen when using one way anova.

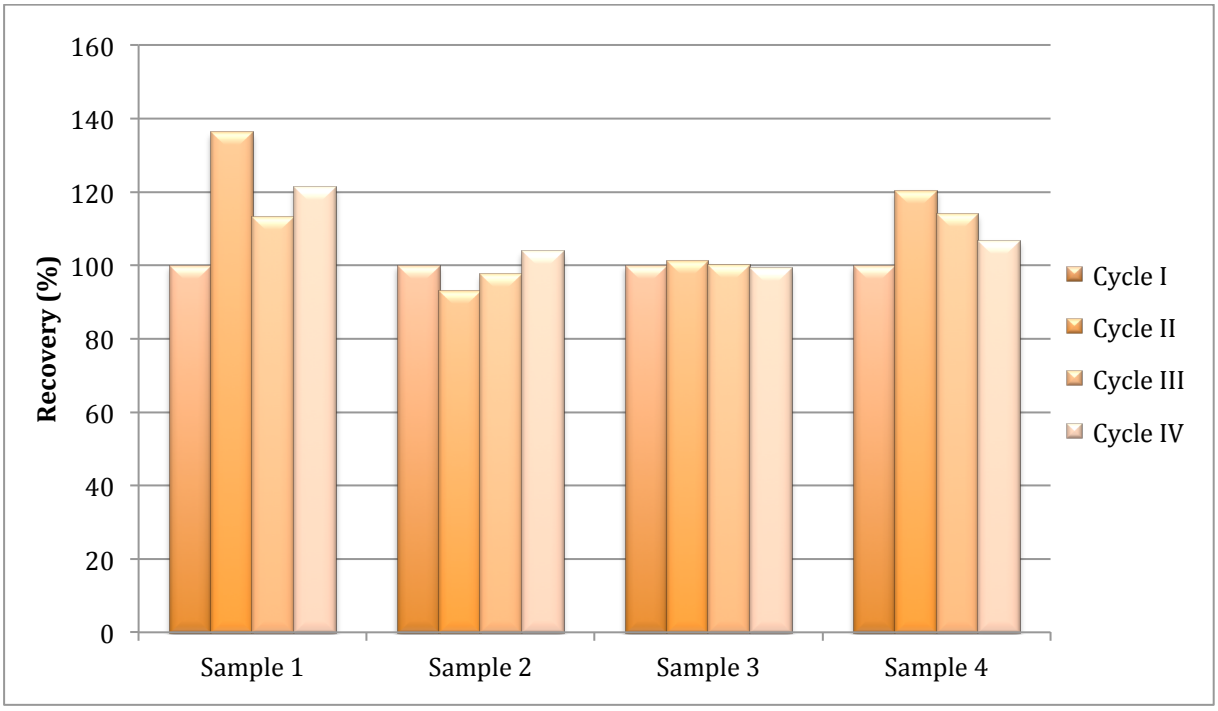

Figure 1. C-peptide concentrations in type 1 diabetics (n=4) after 1 to 4 freeze-thaw cycles.