

Revised 2 Dec. 2010 rm (Vers. 3.0)

Please use only the valid version of the package insert provided with the kit.

INTENDED USE

This C-peptide ELISA provides a method for the quantitative determination of human C-peptide in serum, plasma or urine.

SUMMARY AND EXPLANATION OF THE TEST

Qualitative and quantitative evaluation of pancreatic β -cell function is not only of use in the pre- and postdiagnostic study of the natural history of diabetes mellitus, but is also relevant in clinical practice as a guide to the correct choice of treatment. Peripheral insulin levels cannot be used to assess β -cell function because of a large and variable uptake from the portal circulation into the liver, and because insulin assays cannot distinguish endogenous from exogenous insulin. 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.

Urinary C-peptide excretion is correlated with integrated plasma C-peptide levels but the extraction is highly variable between and within individuals and is, therefore, an imprecise measure of β -cell function.

PRINCIPLE OF THE PROCEDURE

This C-peptide ELISA, is a solid phase two-site enzyme immunoassay. It is based on the direct 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 labelled antibody, the bound conjugate is detected by reaction with 3,3',5,5'-tetramethylbenzidine (TMB). The reaction is stopped by adding acid to give a colorimetric endpoint that is read spectrophotometrically.

WARNINGS AND PRECAUTIONS

- For in vitro diagnostic use.
- The contents 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 0.5 M H₂SO₄. Follow routine precautions for handling hazardous chemicals.
- All patient specimens should be handled as of capable of transmitting infections.

MATERIAL REQUIRED BUT NOT PROVIDED

- Pipettes for 25, 50, 100, 200 and 1000 μ L (repeating pipettes preferred for addition of enzyme conjugate solution, Substrate TMB and Stop Solution)
- Beakers and cylinders for reagent preparation
- Redistilled water

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- Microplate reader with 450 nm filter
 - Plate shaker (The recommended velocity is 700–900 cycles per minute, orbital movement)
 - Microplate washing device

REAGENTS

Each 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	1 plate	96 wells	Ready for Use
Mouse monoclonal anti-C-peptide		8-well strips	
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	5 vials,	1000 µL	Lyophilized
Color coded yellow			Add 1000 µL redistilled water per vial.
Concentration stated on vial label.			
Storage after reconstitution: 2–8°C for 1 week			
For storage of reconstituted Calibrators for more than 1 week, store at -20°C.			
Calibrator 0	1 vial	5 mL,	Ready for use
Colour coded yellow			
Assay Buffer	1 vial	6 mL	Ready for use
Colour coded red			
Enzyme Conjugate 11X	1 vial	1.2 mL	Preparation, see below
Peroxidase conjugated mouse monoclonal Anti-C-peptide			
Enzyme Conjugate Buffer	1 vial	12 mL	Ready for use
Colour coded blue			
Wash Buffer 21X	1 bottle	50 mL	Dilute with 1000 mL redistilled water to make wash buffer 1X solution.
Storage after dilution: 2–8°C for 4 weeks.			
Substrate TMB	1 vial	22 mL	Ready for use
Colourless solution			
<i>Note! Light sensitive!</i>			
Stop Solution	1 vial	7 mL	Ready for use
0.5 M H ₂ SO ₄			

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A. 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, 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	7.0 mL
6 strips	500 µL	5.0 mL
4 strips	350 µL	3.5 mL

Storage after dilution: 2-8°C, use with in 1 week.

SPECIMEN COLLECTION AND HANDLING**Serum**

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

Samples can be stored at 2-8°C up to 3 days.

For longer periods store samples at -20°C. Avoid repeated freezing and thawing.

Plasma

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

Samples can be stored at 2-8°C up to 3 days

For longer periods store samples at -20°C. Avoid repeated freezing and thawing.

Urine

Collect a 24 hour urine (without preservative). Keep the specimen at 2-8°C between collections.

Record the total volume of the specimen and retain a well mixed aliquot for analysis.

Store the samples at 2-8°C for a maximum of 24 hours before assay.

For longer storage, keep the urine samples frozen at -70°C until assay is performed. Repeated freezing and thawing must be avoided. Cellular debris should be removed before assay, either by filtration or centrifugation.

B. Preparation of samplesUrine

Dilute urine samples 1/10 v/v in Calibrator 0 before assay.

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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 and samples in duplicate.
3. Pipette 25 µL each of Calibrators and samples into appropriate wells.
4. Add 50 µL of Assay Buffer to each well.
5. Incubate on a plate shaker for 1 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. 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 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 such as Diabetes Antigen Control (Cat No. CTL-5132) 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.

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CALCULATION OF RESULTS

C. 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.

D. Manual calculation

1. Plot the absorbance values obtained for the Calibrators, except 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.

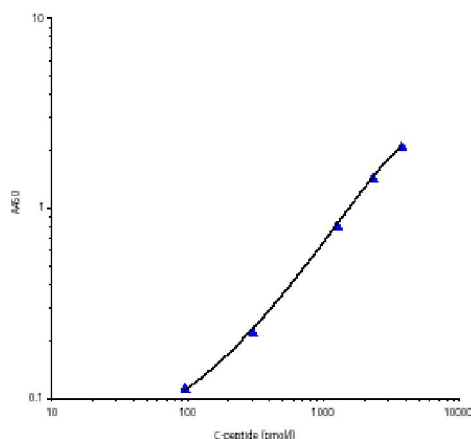
E. Example of worksheet

Wells	Identity	A ₄₅₀	Mean conc. pmol/L
1A-B	Calibrator 0	0.059/0.062	
1C-D	Calibrator 1*	0.113/0.118	
1E-F	Calibrator 2*	0.226/0.224	
1G-H	Calibrator 3*	0.817/0.822	
2A-B	Calibrator 4*	1.476/1.442	
2C-D	Calibrator 5*	2.126/2.157	
2E-F	Sample 1	0.202/0.201	256
2G-H	Sample 2	0.512/0.521	763
3A-B	Sample 3	1.364/1.375	2159

*Concentration stated on vial label

F. Calibration curve

A typical calibration curve is shown here. Do not use this curve to determine actual assay results.



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LIMITATIONS OF THE PROCEDURE

As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical findings have been evaluated. Grossly lipemic, icteric or hemolysed samples do not interfere in the assay.

EXPECTED VALUES

Good practice dictates that each laboratory establishes its own expected range of values. The following results may serve as a guide until the laboratory has gathered sufficient data of its own.

Fasting serum levels for 136 tested, apparently healthy individuals, yielded a mean of 742 pmol/L (2.2 µg/L), a median of 628 pmol/L (1.9 µg/L) and a range, corresponding to the central 95% of the observations, of 343-1803 pmol/L (1.0–5.4 µg/L).

PERFORMANCE CHARACTERISTICS

G. Detection limit

The detection limit is 15 pmol/L (0.045 µg/L) calculated as two standard deviations above the Calibrator 0.

H. Recovery

Serum: Recovery upon addition is 93–113 % (mean 104 %).

Urine: Recovery upon addition is 94–107 % (mean 99 %).

I. Hook effect

Samples with a concentration of up to at least 1 750 000 pmol/L can be measured without giving falsely low results.

J. Precision

Each sample was analyzed in 4 replicates on 7 different occasions.

Sample	Mean value pmol/L	Coefficient of variation		
		within assay %	between assay %	total assay %
1	304	4.8	4.8	6.8
2	818	3.1	4.4	5.4
3	1803	2.9	0.6	3.0

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K. Specificity

Insulin	< 0.0006 %
Proinsulin	< 1.8 %
Proinsulin des (31-32)	74 %
Proinsulin split (32-33)	10 %
Proinsulin des (64-65)	3 %
Proinsulin split (65-66)	2 %

CALIBRATION

C-peptide ELISA (EIA-4262) kit is calibrated against the International Reference Reagent for C-peptide, IRR C-peptide 84/510.

CONVERSION FACTOR

1 µg/L corresponds to 331 pmol/L

WARRANTY

The performance data presented here was obtained using the procedure indicated. Any change or modification in the procedure not recommended by DRG may affect the results, in which event DRG disclaims all warranties expressed, implied or statutory, including the implied warranty of merchantability and fitness for use. DRG and its authorized distributors, in such event, shall not be liable for damages indirect or consequential.

REFERENCES / Literature

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2. Riserus U, Vessby B, Arner P, Zethelius B (2004) Supplementation with trans10cis12-conjugated linoleic acid induces hyperproinsulinemia in obese men: close association with impaired insulin sensitivity. Diabetologia 47:1016-1019
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