Invitron C-peptide Assay Kit

IV2-004/104

Rev 01

Introduction

The Invitron C-peptide Assay is an immunometric assay for the quantitative measurement of C-peptide in human plasma samples.

Summary

C-peptide is a product of the metabolic processing of proinsulin to insulin by the pancreatic β-cell. It is produced in equimolar amounts in relation to insulin, though its significantly greater half-life results in relatively high peripheral plasma concentrations. C-peptide is cleared by the kidney. Traditionally, C-peptide assays have been employed in the differential diagnosis of insulinoma, where lack of suppression by insulin-induced hypoglycaemia is indicative of a tumour. Conversely, suppressed circulating levels of C-peptide in patients with high plasma insulin, may indicate factitious hypoglycaemia (caused by administration of exogenous insulin). Though not biologically active in itself, C-peptide is a valuable marker of pancreatic function in insulin resistant syndromes, both in non-insulin dependent as well as insulin dependent diabetics. It is therefore a valuable research tool for the investigation and monitoring of type II diabetes.

The Invitron C-peptide assay is an immunometric method, employing a chemiluminescent end-point, that has been designed to perform well over the extremely wide range of circulating concentrations occurring in both healthy subjects and diabetic patients (type 1 and type 2).

Principle

The Invitron C-peptide Assay is a two-site immunoassay, employing a C-peptide-specific solid phase antibody immobilised on microtitre wells, and a soluble antibody labelled with a chemiluminescent acridinium ester. The plasma sample is incubated simultaneously with the labelled antibody solution in the microtitre well, followed by a wash step to remove unbound labelled antibody before measurement. The bound luminescence is quantified by a microtitre plate luminometer capable of *in situ* reagent addition. The luminescent reaction is a rapid flash type (>95% complete in 1 second) which permits the entire plate to be read in approximately 5 minutes.

Kit Contents

- **Standards:** Synthetic C-peptide in a serum matrix, lyophilised and sealed under vacuum for stability.
- **Labelled Antibody Concentrate:** Chemiluminescent labelled antibody in a protein matrix including preservatives and 0.05% sodium azide.
- **Labelled Antibody Diluent:** For diluting the labelled antibody to its working strength. Protein matrix including preservatives and 0.05% sodium azide.
- **Coated Microtitre Plate:** Microtitre plate coated with a specific polyclonal antibody. The plate is sealed inside a foil pouch with a dessicant to maintain a moisture-free environment.
- **Wash Buffer Concentrate:** (x10) phosphate buffered saline containing a detergent and 0.09% sodium azide.
- **Plate sealers**
- **Product Insert**

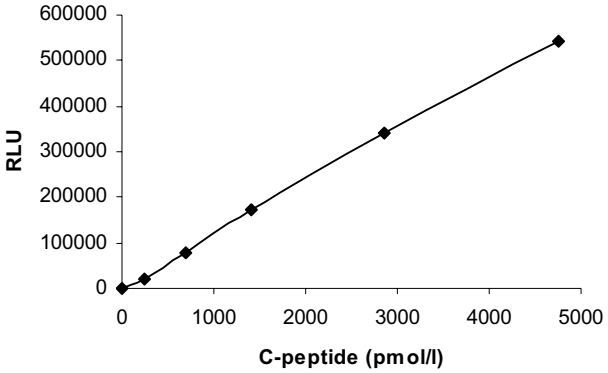
Materials Required But Not Provided

- Deionised water
- Detection reagents (Invitron Catalogue No. IV1-001)
- Uncoated strips

Procedure

1. Bring all kit components and samples to room temperature before use.
2. Reconstitute each of the standards by the addition of 1 ml of deionised water. Allow these to stand for 5 minutes, then mix gently to ensure all solids are dissolved.
3. Pipette 900 µl of labelled antibody concentrate into one bottle of labelled antibody diluent and mix thoroughly.
4. Make up working strength wash buffer by diluting 1 part of wash buffer concentrate with 9 parts of deionised water.
5. Assemble the required number of coated strips in the plate holder. Any strips not used immediately may be stored inside a sealed bag with silica gel dessicant. Make sure to fill remaining spaces in the plate holder with uncoated strips to ensure uniform heat transfer during incubation.
6. Pipette 100 µl labelled antibody solution into each well to be used.
7. Pipette 25 µl of standard or sample into each well as appropriate. It is strongly recommended that samples be run in duplicate.
8. Attach the plate sealer and incubate for 2 hours at 37ºC.
9. Remove the plate sealer and perform 3 wash cycles with working strength wash buffer using an automatic plate washer.
10. Measure the light output from each well in a plate luminometer.

Typical Standard Curve



N.B. This curve is for illustration purposes only, and must not be used for result calculation. RLU = Relative Light Units.

Precision Profile

The precision of duplicate measurements was calculated for 69 patient samples. The mean coefficient of variation for these duplicates, which covered the analytical range 100-2800 pmol/l was 5.2%.

Between Assay Precision

C-peptide (pmol/l)	CV%	n
361	11.3	7
1683	7.7	7

Spiking Recovery

Five plasma samples containing low endogenous C-peptide were spiked with synthetic C-peptide at 3 levels. Recoveries are shown as percentages of the expected result.

Sample	1	2	3	4	5
Spike 5%	103.1	107.2	101.7	105.2	104.8
Spike 10%	102.9	99.8	101.9	101.2	99.5
Spike 15%	101.8	101.6	101.6	104.1	100.5

Mean spiking recovery was 101.9%.

Dilution Recovery

Five plasma samples were diluted with charcoal stripped human plasma by factors of 10%, 20% and 50%. Recoveries of C-peptide are shown as percentages of the expected result.

Sample	1	2	3	4	5
10% dilution	119.9	115.8	107.7	99.7	116.4
20% dilution	120.9	121.8	113.0	98.1	121.2
50% dilution	97.3	92.1	102.2	92.9	104.3

Mean dilution recovery was 108.2%.

Sensitivity

Sensitivity was estimated as two standard deviations from the mean of 20 replicates of a zero standard. Calculated in this way, analytical sensitivity of the C-peptide Assay is 5.0 pmol/l

High Dose Hook Effect

No high dose hook effect has been observed at C-peptide concentrations up to 30,000 pmol/l.

Cross Reactivity

Cross reactivities of related proteins were investigated at concentrations of 100 pM. Results are expressed as percentages of the reactivity of an identical concentration of C-peptide.

Peptide	CR (%)
C-peptide	100
Insulin	0.0
Intact proinsulin	2.0

Range of Standards (Typically)

0-5000 pmol/l

Specimen

Plasma samples should be used for the C-peptide assay.
The assay is not suitable for the measurement of post-mortem or urine samples

Safety Precautions

1. Use good laboratory practice at all times.
2. Wear appropriate protective clothing when using this kit and when handling samples.
3. This kit contains no human-derived material.

Technical Precautions

1. Do not use components after their expiry date.
2. Once components have been opened or reconstituted, they can be used within a two-week period, provided they have been stored at 2-8ºC.
3. Do not mix reagents from different lots.
4. Take care to avoid contamination of any reagent.

This kit is intended for research use only.

For additional information and product support please contact:

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