

IV2-004

*English*

## MLT™ C-Peptide Assay Kit

For in-vitro diagnostic use only

MLT™ (*Molecular Light Technology*) Chemiluminescence assay for the measurement of C-Peptide in human samples



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## Definitions



Instructions for use



Catalogue number



Use by



Lot/Batch Code



Storage temperature limitations



In vitro diagnostic medical device



Manufactured by



Contains sufficient for <N> tests



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# MLT C-Peptide Kit

## Intended Use

Invitron's MLT C-peptide Assay is an immunometric assay for the quantitative measurement of C-peptide in human plasma samples. Measurements of C-peptide are useful in the investigation and management of patients with diabetes.

## Summary and Explanation

C-peptide is a product of the metabolic processing of proinsulin to insulin by the pancreatic  $\beta$ -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. C-peptide assays can be used 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 deficient and insulin resistant syndromes. It is therefore a valuable research tool for the investigation and monitoring of both type 1 and type 2 diabetes.

## 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.

## Materials Provided

- Coated Microtitre Plate  
(5 x 96 wells) stripwells coated with a specific polyclonal antibody. The plate is sealed inside a foil pouch with a dessicant to maintain a moisture-free environment.
- Labelled Antibody Concentrate  
(1 x 5.5 ml) Chemiluminescent labelled antibody in a protein matrix including preservatives and 0.05% sodium azide.
- Labelled Antibody Diluent  
(5 x 14.1 ml) Ready to use for diluting the labelled antibody to its working strength. Protein matrix including preservatives and 0.05% sodium azide.
- Standards  
(6 x 1 m lyophilized) of 6 concentrations – (typically) 0.0; 215; 880; 1900; 3000; 5000 pmol/l of synthetic C-peptide in a serum matrix, lyophilised and sealed under vacuum for stability. See label for each lot of kits for actual concentrations. ***The standards are calibrated against WHO International Standard for C-Peptide (IRP 84/510).***
- Wash Buffer Concentrate  
(1 x 50 ml) Phosphate buffered saline containing a detergent and preservative.
- Plate Sealers – 5 each
- Product Insert

## Materials Required But Not Provided

- Detection reagents. Invitron Cat. No. IV1-001.
- Deionised water
- Uncoated strips
- Microtitre plate Luminometer capable of direct injection and of measuring flash kinetics.
- Calibrated Precision Micropipettes with disposable tips.

## Warnings and Precautions

- For *in-vitro* diagnostic use only. For professional use only.
- For information on hazardous substances included in the kit please refer to Material Safety Data Sheets.
- Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- Wear disposable latex gloves and appropriate protective clothing when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
- Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- Do not use reagents beyond expiry date as shown on the kit labels.
- Once components have been opened or reconstituted, they can be used within a two-week period, provided they have been stored at 2-8°C.
- Optimal test results are only obtained when using calibrated pipettes and luminometer.
- Do not mix or use components from kits with different lot numbers.
- This kit contains no human-derived material.

## Preparation, Storage & Stability of Reagents

When stored at 2-8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date. Opened reagents must be stored at 2-8°C. Microtitre wells must be stored at 2-8°C. Once the foil bag has been opened, care should be taken to close it tightly again. Opened kits retain activity for two months if stored as described above. Reconstituted/diluted reagents are stable for 2 weeks when stored at 2-8°C.

### **Standards**

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. Stability of the reconstituted Standards is two (2) weeks when stored at 2-8°C.

### **Labelled Antibody Concentrate**

Pipette 900 µl of labelled antibody concentrate into one bottle of Labelled Antibody Diluent and mix thoroughly. Diluted Labelled Antibody is stable for two (2) weeks when stored at 2-8°C.

### **Wash Buffer**

Make up working strength Wash Buffer by diluting 1 part of Wash Buffer concentrate with 29 parts of deionised water.

## Luminometer Set-up

The microtitre plate luminometer must be fitted with 2 injectors and it is important to check that the instrument is capable of measuring "flash" type kinetics. The measurement protocol should be set as follows:

1. Set injector 1 to deliver 100 µl of Detection Reagent 1
2. Set injector 2 to deliver 100 µl of Detection Reagent 2
3. Set a delay of 2 seconds between injection 1 and injection 2.
4. Light measurement must start at the time of the second injection (i.e. there is no delay between injection 2 and measurement).
5. Measurement time is 1 second.

## Specimen Collection & Storage

Heparin or EDTA Plasma can be used in this assay. Do not use severely haemolysed specimens.

### **Specimen Collection**

**Plasma:** Whole blood should be collected into a tube containing EDTA or heparin anticoagulant and centrifuged immediately after collection.

### **Specimen Storage**

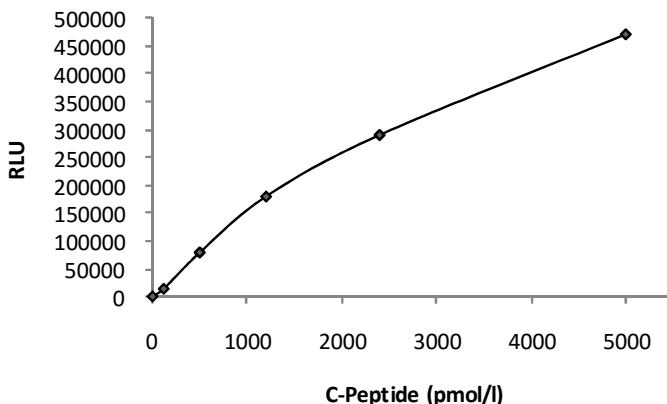
Specimens should be capped and may be stored for up to 24 hours at 2-8°C prior to assaying. Specimens held for a longer time should be stored frozen at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

## Assay Procedure

1. Bring all kit components and samples to room temperature before use.
2. Assemble the required number of coated strips in the plate holder. Any strips not used immediately may be stored inside a sealed polythene bag with silica gel desiccant. Make sure to fill remaining spaces in the plate holder with uncoated strips to ensure uniform heat transfer during incubation.
3. Pipette **100 µl labelled antibody** solution into each well to be used.
4. Pipette **25 µl of standard or sample** into each well as appropriate. Standards must be run in duplicate.
5. Attach the plate sealer and incubate for **2 hours at 37°C**.
6. Remove the plate sealer and perform **3 wash cycles** with working strength Wash Buffer (300µl each cycle) using an automatic plate washer.
7. Measure the light output from each well in a plate luminometer within 15 minutes.

## Typical Standard Curve

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



## Calculation of Results

The results may be calculated automatically using a cubic spline curve fit. Other data reduction functions may give slightly different results. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard should be further diluted. For the calculation of the concentrations this dilution factor has to be taken into account.

## **Expected Values**

It is strongly recommended that each laboratory determines its own normal and abnormal values.

## Quality Control

The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels. It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results. Employ appropriate statistical methods for analyzing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices; luminometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods. After checking the above mentioned items without finding any error contact your distributor directly.

## Limitations

- The values obtained from this assay are intended to aid in diagnosis only. As with all serological tests, interpretation of results obtained with this test must be used in conjunction with the patient's clinical symptoms, medical history and other clinical and/or laboratory findings.
- Only if test instructions are rigidly followed will optimum results be achieved.
- Use fresh plasma or specimens frozen and thawed no more than twice. Specimens that are improperly stored or are subjected to multiple freeze-thaw cycles may yield spurious results.
- Reproducible results depend on careful pipetting, observation of incubation periods and temperature, as well as thorough mixing of all prepared solutions.
- While rinsing, check that all wells are filled evenly with Washing Solution, and that there are no residues in the wells.
- Instructions for using appropriate luminometers are to be observed. Check that the instrument has the correct measurement protocol installed.



## Interfering Substances

Interferences were studied in accordance with CLSI recommendations (CLSI EP7-A2). To study the effect of lipaemia, test pools were prepared by spiking plasma samples with a commercial lipid emulsion (Intralipid, Sigma). Test samples for investigating the effect of haemolysis were obtained by osmotic shock. Icteric samples were prepared by spiking plasma samples with commercial bilirubin (Sigma).

No effect of lipaemia was found up to a lipaemic index of 668. Interference due to haemolysis was not apparent at a haemolysis index up to 98. Bilirubin produced no apparent interference up to an icterus index of 705. It is recommended that haemolysed specimens should not be used for analysis.

## Performance Characteristics

### **Between Assay Precision**

Three plasma pools were measured in duplicate in 5 individual assays. The following results were obtained.

C-peptide (pmol/l)	CV%	n
268	7.8	5
990	4.3	5
1862	6.7	5

### **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. The dynamic range of this assay is 5.0 – 5000 pmol/L.

### **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 102.5%.

## Linearity

Five patient 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.

Recovery (%)					
Dilution Factor	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
10% dilution	119.9	115.8	107.7	99.7	116.5
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%

## 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

## For additional information and product support please contact:

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