

C-Peptide

Enzyme immunoassay for the quantitative
determination of C-Peptide in human serum or plasma

Only for in-vitro diagnostic use



Product Number: DNOV112 (96 Determinations)

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1. INTRODUCTION

C-peptide is the abbreviation for connecting peptide; it is a 31-aminoacid peptide. C-peptide of insulin is the C-terminal cleavage product produced during processing of the insulin prohormone to the mature insulin molecule. Proinsulin is cleaved when it is released from the pancreas into the blood - one C-peptide for each insulin molecule. C-Peptide is devoid of any biological activity but appears to be necessary to maintain the structural integrity of Insulin.

In-vitro determination of Insulin and C-Peptide level help in differential diagnosis of liver disease, acromegaly, Cushing syndrome, familial glucose intolerance, Insulinimia, renal failure, ingestion of accidental oral hypoglycaemic drugs or C-peptide induced factitious hypoglycaemia.

Newly diagnosed diabetes patient often get their C-peptide levels measured, to find if they have type 1 diabetes or type 2 diabetes. The pancreas of patients with type 1 diabetes is unable to produce insulin and they will therefore usually have a decreased level of C-peptide, while C-peptide levels in type 2 patients is normal or higher than normal. Measuring C-peptide in patients injecting insulin can help to determine how much of their own natural insulin these patients are still producing.

C-peptide assays may be analytically more sensitive than insulin assays. Measurement of the C-peptide may be useful in evaluating endogenous insulin secretion in a variety of clinical conditions. Insulin and C-Peptide are secreted into portal circulation in equimolar concentrations; fasting levels of C-Peptide are 5 – 10 fold higher than those of Insulin owing to the longer half-life of C-Peptide. The liver does not extract C-Peptide however; it is removed from the circulation by degradation in the kidneys with a fraction passing out unchanged in urine. Hence the urine C-Peptide levels correlate well with fasting C-Peptide levels in serum.

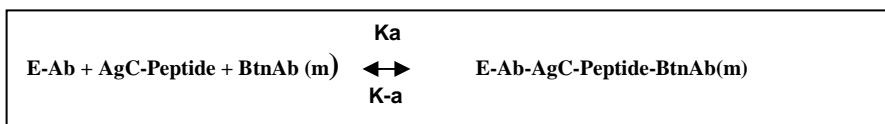
2. INTENDED USE

Immunoenzymatic colorimetric method for quantitative determination of C-Peptide in human serum or plasma.

3. PRINCIPLE OF THE ASSAY

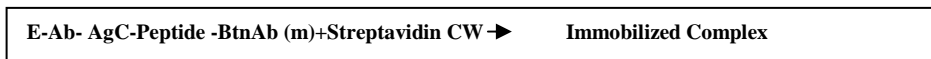
In this method, C-Peptide calibrators, patient specimens and/or controls containing the native antigen are first added to streptavidin coated wells. Biotinylated monoclonal and horseradish peroxidase (HRP) labelled antibodies are added and the reactants are mixed. The different types of antibodies used have high affinity and specificity and are directed against distinct and different epitopes of C-Peptide. Reaction between the various C-Peptide antibodies and native C-Peptide occurs in the microwells without competition or steric hindrance forming a soluble sandwich complex.

The interaction is illustrated by the following equation:



BtAb(m)	Biotinylated Monoclonal Antibody (Excess Quantity)
AgC-Peptide	Native Antigen (Variable Quantity)
E-Ab	enzyme labeled Antibody (Excess Quantity)
HRP-Ab(p)-AgC-Peptide-BtAb(m)	Antigen-Antibodies Sandwich Complex
Ka	Rate Constant of Association
K-a	Rate Constant of Dissociation

Simultaneously, the complex is fixed to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:



Streptavidin CW Streptavidin immobilized on well.
Immobilized Complex Antibodies-Antigen sandwich bound.

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by aspiration. The native antigen concentration is directly proportional to the HRP activity in the antibody-bound fraction. The activity of the conjugated HRP is quantitated by reaction with TMB substrate to produce blue colour. The reaction is terminated by adding stop solution which turns the blue colour into yellow. The absorbance is measured on a plate reader.

4. MATERIALS

4.1. Reagents supplied

- **Coated Mircoplate:** 12 breakapart 8-well snap-off strips coated with streptavidin; in aluminium foil.
- **Conjugate:** 1 bottle containing 13 ml of horseradish peroxidase labelled anti- C-Peptide antibodies and biotinylated monoclonal mouse anti-C-Peptide antibodies.

- **TMB Substrate Solution:** 1 bottle containing 15 ml 3, 3', 5, 5'-tetramethylbenzidine (H₂O₂-TMB 0.26 g/l) (avoid any skin contact).
- **Wash solution 50x conc.:** 1 bottle containing 20 ml (NaCl 45 g/l, Tween-20 55 g/l)
- **Stop Solution:** 1 bottle containing 15 ml sulphuric acid, 0.15 mol/l (avoid any skin contact).
- **Standards:** 6 bottles containing lyophilised standards. The approx. concentrations after reconstitution are:

Standard 0	0 ng/ml
Standard 1	0.2 ng /ml
Standard 2	1.0 ng /ml
Standard 3	2.0 ng /ml
Standard 4	5.0 ng /ml
Standard 5	10.0 ng /ml

4.2. Materials supplied

- 1 Strip holder
- 1 Cover foils
- 1 Test protocol
- 1 Distribution and identification plan

4.3. Materials and Equipment needed

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450 nm
- Manual or automatic equipment for rinsing wells
- Distilled water
- Timer

5. STABILITY AND STORAGE

The closed reagents are stable up to the expiry date stated on the label when stored at 2...8°C in the dark.
 Opened reagents are stable for 60 days when stored at 2...8°C.

6. REAGENT PREPARATION

It is very important to bring all reagents, samples and standards to room temperature (20...28°C) before starting the test run!

6.1. Coated microplate

The ready to use break apart snap-off strips are coated with streptavidin. Store at 2...8°C. Open the bag only when it is at room temperature. *Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8°C.*

6.2. Conjugate

The conjugate is ready to use.

6.3. C-Peptide Standards

The standards are lyophilised. Reconstitute each standard with 2 ml of distilled or deionised water.

Once reconstituted the standards are stable 7 days at 2...8°C.

In order to store for a longer period aliquot the reconstituted standards in vials and store at -20°C (stable for 6 months). Do not freeze thaw more than once.

A preservative has been added.

The standards, human serum based, were calibrated using a reference preparation, which was assayed against the WHO 1st IRR 84/510.

6.4. TMB Substrate Solution

The bottle contains 15 ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at 2...8°C in the dark. *The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.*

6.5. Stop Solution

The bottle contains 15 ml 0.15 M sulphuric acid solution (R 36/38, S 26). This ready to use solution has to be stored at 2...8°C.

6.6 Wash Solution

Dilute the concentrated wash solution to 1000 ml distilled or deionised water. For smaller volumes respect the 1:50 ratio. The diluted wash solution is stable for 30 days at 2...8°C.

7. SPECIMEN COLLECTION AND PREPARATION

Follow Good laboratory procedures for handling blood products.

For accurate comparison to established normal values, a fasting morning serum sample should be obtained.

In order to obtain serum, the blood should be collected in a venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2...8°C for a maximum period of 5 days. If the specimen (s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days.

Avoid repetitive freezing and thawing.

Patient specimens with C-peptide concentrations above 10.0 ng/ml may be diluted (for example 1/10 or higher) with zero standard (C-peptide 0 ng/ml) and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor.

8. ASSAY PROCEDURE

8.1. Test Preparation

Please read the test protocol carefully **before** performing the assay. Result reliability depends on strict adherence to the test protocol as described. Prior to commencing the assay, the distribution and identification plan for all specimens and controls should be carefully established on the result sheet supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than one plate is used, it is recommended to repeat the dose response curve. Please allocate at least:

1 well	e.g. A1)	for blank
2 wells	(e.g. B1+C1)	for standard 0
2 wells	(e.g. D1+E1)	for standard 1
2 wells	(e.g. F1+G1)	for standard 2
2 wells	(e.g. H1+A2)	for standard 3
2 wells	(e.g. B2+C2)	for standard 4
2 wells	(e.g. D2+E2)	for standard 5

It is recommended to determine standards and patient samples in duplicate.

Perform all assay steps in the order given and without any appreciable delays between the steps.

A clean, disposable tip should be used for dispensing each standard and each patient sample.

1. Dispense 50 µl standards and samples (and controls) into their respective wells.
2. Dispense 100 µl conjugate in each well except blank. Cover with a foil.
3. **Incubate for 2 hours at room temperature (22 – 28°C).**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300µl diluted wash solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.

5. Dispense 100 µl TMB Substrate Solution into all wells.
6. **Incubate for 15 min at room temperature (+22...+28°C) in the dark.**
7. Dispense 100 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. Shake the microplate gently.
Any blue colour developed during the incubation turns into yellow.
8. Measure the absorbance of the specimen at 450 nm within 30 min after addition of stop solution against blank.

9. QUALITY CONTROL

Each laboratory should assay controls at levels in the low, medium and high ranges of the dose response curve for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

If computer controlled data reduction is used to calculate the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

10. RESULTS

10.1. Note

The absorbance (OD) of standard 5 should be ≥ 1.0 .

The optical densities (O.D.s) of some standards and samples may be higher than 2.0, in such a case, they could be out of the measurement range of the microplate reader. It is therefore necessary, for O.D.s higher than 2.0, to perform a reading at 405 nm (=wavelength of peak shoulder) in addition to 450 nm (peak wavelength) and 620 (reference filter for the subtraction of interferences due to the plastic).

For microplate readers unable to read the plate at 3 wavelengths at the same time, it is advisable to proceed as follows:

- Read the microplate at 450 nm and at 620 nm.
- Read again the plate at 405 nm and 620 nm.
- Find out the wells whose ODs at 450 nm are higher than 2.0
- Select the corresponding ODs read at 405 nm and multiply these values at 405 nm by the conversion factor 3.0 (where $OD_{450}/OD_{405} = 3.0$), that is: $OD_{450\text{ nm}} = OD_{405\text{ nm}} \times 3.0$.

Warning: The conversion factor 3.0 is suggested only. For better accuracy, the user is advised to calculate the conversion factor specific for its own reader.

10.2. Calculation of results

Calculate the mean absorbance for each point of the standard curve and each sample.

Standard Curve – Automatic method

Use the 4 parameters logistic – preferred – or the smoothed cubic spline function as calculation algorithm.

Standard Curve – Manual method

A dose response curve is used to ascertain the concentration of C-Peptide in unknown specimens.

Record the OD obtained from the printout of the microplate reader. Plot the OD for each duplicate standard versus the corresponding C-Peptide concentration in ng/ml on linear graph paper (do not average the duplicates of the calibrators before plotting).

Draw the best-fit curve through the plotted points.

To determine the concentration of C-Peptide for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated).

10.3. Reference values

C-Peptide values are consistently higher in plasma than in serum; thus, serum is preferred. Based on the clinical data gathered in concordance with the published literature the following ranges have been assigned.

These ranges should be used as guidelines only:

Adult (Normal) 0.7 – 1.9 ng/ml

11. SPECIFIC PERFORMANCE CHARACTERISTICS

11.1. Sensitivity

The lowest detectable concentration of C-Peptide that can be distinguished from the zero standard is 0.01 ng/ml at the 95% confidence limit.

11.2. Specificity

The cross reactivity of the C-peptide ELISA method to selected substances was evaluated by adding the interfering substance. The cross reactivity was calculated by deriving a ratio between dose of interfering substance to dose of C-Peptide needed to produce the same absorbance.

Cross Reagent	Conc. tested	Obtained	Cross Reactivity
C-Peptide	---	---	100 %
Insulin	10000 μ U/ml	N.D.	Not Detected
Proinsulin	1000 ng/ml	N.D.	Not Detected

11.3. Precision

Intra Assay Variation

Within run variation was determined by replicate determination (16x) of three different control sera in one assay. The within assay variability is $\leq 6.2\%$.

Inter Assay Variation

Between run variation was determined by replicate measurements (20x) of three different control sera in different lots. The between assay variability is $\leq 10.0\%$.

11.4. Correlation with RIA

The NovaTec C-Peptide ELISA was compared to another commercially available C-Peptide assay. 194 serum samples were analysed according in both test systems.

The linear regression curve was calculated

$$y = 1.012x + 0.025$$

$$r^2 = 0.991$$

y = C-Peptide Predicate kit

x = C-Peptide NovaTec

12. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values.

13. PRECAUTIONS AND WARNINGS

- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All components of human origin used for the production of these reagents have been tested for anti-HIV 1+2 antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.
- Do not use heavily haemolysed or highly lipemic samples.
- Maximum precision is required for dispensation of the reagents.
- Avoid the exposure of TMB substrate to direct sunlight, metal or oxidants.
- Avoid contact with reagents containing hydrogen peroxide, sulphuric and preservatives, which may be toxic if ingested. Do not pipette by mouth.

WARNING:	Sulphuric acid irritates eyes and skin. Keep out of the reach of children. Upon contact with the eyes, rinse thoroughly with water and consult a doctor!
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13.1. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

14. LITERATURE

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15. ORDERING INFORMATION

Prod. No.:

DNOV112

C-Peptide Determination (96 Determinations)

SCHEME OF THE ASSAY

C-Peptide

Test Preparation

Prepare reagents and samples as described.
Establish the distribution and identification plan for all specimens and controls on the result sheet supplied in the kit.
Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

	Blank	Standard 0 - 5	Sample
Standard 0 - 5	-	50 µl	-
Sample	-	-	50 µl
Conjugate	-	100 µl	100 µl
Cover wells with foil supplied in the kit Incubate for 2 hours at room temperature Wash each well three times with 300 µl diluted Wash Solution			
TMB	100 µl	100 µl	100 µl
Incubate for exactly 15 min at room temperature in the dark			
Stop solution	100 µl	100 µl	100 µl
Shake the microplate gently Photometric measurement at 450 nm			

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DNOV112engl14042011-CR