

*Instruction Manual No. M-0040*

## **Human C-Peptide**

**ELISA Kit Cat. #. 0040**

For Quantitative Determination of  
C-Peptide In Human Serum or Plasma

*For In Vitro Research Use Only*

## Human C-Peptide ELISA KIT # 040, Kit Contents

Components	96 tests
Anti-C-Peptide IgG coated microwell <b>strip plate</b> (96 wells)	<b>Cat. # 041</b>
Human C-Peptide <b>Std. A</b> (sample diluent), 11 ml, 0 ng/ml,	<b>Cat. # 042</b>
Human C-Peptide <b>Std. B</b> , 0.5 ng/ml, 0.75 ml	<b>Cat. # 043</b>
Human C-Peptide <b>Std. C</b> , 2.5 ng/ml, 0.75 ml	<b>Cat. # 044</b>
Human C-Peptide <b>Std. D</b> , 5.0 ng/ml, 0.75 ml	<b>Cat. # 045</b>
Human C-Peptide <b>Std. E</b> , 10 ng/ml, 0.75 ml	<b>Cat. # 046</b>
Human C-Peptide <b>Std. F</b> , 20 ng/ml, 0.75 ml	<b>Cat. # 047</b>
Human C-Peptide, <b>Control</b> (exact values printed on vial <b>Cat. # C040</b> )	
<b>Note:</b> the Stds are not stable and should be stored in suitable aliquot at -20oC for extended storage. Once vial is opened, the stds are stable for 5 days at 4oC.	
Anti-C-Peptide- <b>HRP Conjugate</b> , 11 ml,	<b>Cat. # 048</b>
HRP substrate <b>Solution</b> , 11 ml	<b>Cat. # TMB-40</b>
<b>Wash buffer (100X)</b> , 10 ml (dilute 1:100 with distilled water)	<b>W - 1 0 0</b>
Stop solution, 10 ml	<b>Cat. # T-10</b>
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## Introduction

Human insulin and C-PEPTIDE originate as a single polypeptide chain known as proinsulin (M.Wt 9000) in the pancreatic cell. Proinsulin is cleaved proteolytically to form equimolar amounts of mature insulin and C-PEPTIDE that are released into the portal vein. So called because it connects the A and B chains of insulin in the proinsulin molecule. C-PEPTIDE is a single chain of 31 amino acid (Mol. Wt 3020). Unlike insulin has no known physiological function. Because C-PEPTIDE has a longer half-life than insulin (2-5 times longer), high concentrations of C-PEPTIDE persist in the peripheral circulation and these level fluctuate less insulin. For these reasons, in plasma C-Peptide concentrations may reflect pancreatic insulin secretion more reliable than the level of insulin itself. C-PEPTIDE is cleaved from the body by the kidney. Urine concentrations of C-PEPTIDE are 20-50 times higher than in plasma, unlike plasma insulin levels, which fluctuate in response to meals, measurement of the 24 hour urinary excretion of C-PEPTIDE provides a useful monitor of average cell insulin secretion. C- PEPTIDE measurements are useful in insulinoma diagnosis, especially in patients treated with insulin. Elevated C-PEPTIDE levels are indicative of insulinoma. C-PEPTIDE measurements are useful in the need for progression to insulin therapy in non-insulin dependent diabetics (NIDDM). C-PEPTIDE measurements are useful as a marker for residual pancreatic tissue after pancreatectomy. It may also be used to monitor the progress of pancreas or islet cell transplantation. C-PEPTIDE measurements are useful in the diagnosis of hypoglycemia brought on by surreptitious insulin administration.

## PERFORMANCE CHARACTERISTICS

### Precision:

Intra-assay: three pool sera were assayed of 8 in a single run

Inter-assay: three pool sera were assayed in duplicate in three days

Serum Sample	Mean (ng/ml)	Intra-assay		Inter-assay	
		S.D.	CV%	S.D.	CV%
1	0.87	0.04	4.7	0.04	4.74
2	4.11	0.22	5.39	0.32	8.28
3	7.02	0.22	3.07	0.59	7.75

### Accuracy

A serum containing 20 ng/ml of C-Peptide was diluted with series of C-Peptide free serum. The dilutions were tested and the C-Peptide recoveries were compared with the expected concentrations.

Sample Dilution	C-Peptide Level Expected (ng/ml)	C-Peptide Level Measured (ng/ml)	Recovery %
Undiluted	20		
1:2	10	10	100
1:4	5	5	100
1:8	2.5	2.3	92
1:20	1	0.85	85

Known C-Peptide samples were spiked with different concentrations of C-Peptide. Samples were then tested and the C-Peptide recoveries compared with the expected concentrations as illustrated: (Unit ng/ml)

C-Peptide	Expected Value	Measured Value	Recovery %
5	3.758	3.8	101
5	7.5	6.1	81
10	6.25	4.9	78.4
10	10	10.6	106

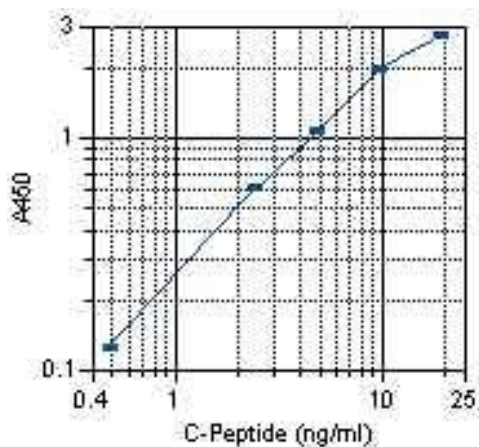
### General References:

Ashby JP et al (1981) Annals of Clin. Biochem. 18, 125; Yue DK et al (1978) metabolism 27, 1, 1978; Krause UB et al (1981) J Immunol. 2, 33

## WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean A <sub>450nm</sub>	Calculated Conc. (ng/ml)
A1, A2	<b>Std. A</b> (0 ng/ml)	0.033	
B1, B2	<b>Std. B</b> (0.5 ng/ml)	0.113	
C1, C2	<b>Std. C</b> (2.5 ng/ml)	0.428	
D1, D2	<b>Std. D</b> (5.0 ng/ml)	0.761	
E1, E2	<b>Std. E</b> (10 ng/ml)	1.425	
F1, F2	<b>Std. F</b> (20 ng/ml)	2.472	
G1, G2	<b>Sample 1</b>	0.136	9.5

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



A typical std. assay curve (do not use this for calculating sample values)

## PRINCIPLE OF THE TEST

Human C-Peptide ELISA kit is based on simultaneous binding of human C-Peptide from samples to two antibodies, one immobilized on microtiter well plates, and other conjugated to the enzyme horseradish peroxidase. After a washing step, chromogenic substrate is added and color developed. The enzymatic reaction (color) is directly proportional to the amount of C-Peptide present in the sample. Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm and the concentration of C-Peptide in samples and control is read off the standard curve.

## MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5-100 ul) and Multichannel pipet with disposable plastic tips. Reagent troughs, Plate washer (recommended) and ELISA plate Reader.

## PRECAUTIONS

The C-Peptide ELISA test is intended for *in vitro* research use only. The reagents contain Proclin-300 as preservative; necessary care should be taken when disposing solutions. The Control Serum has been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site.

TMB (substrate), H<sub>2</sub>SO<sub>4</sub> (stop solution), and Proclin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).

## SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow clotting, and separating the serum by centrifugation at room temperature. Do not heat inactivate the serum. If sera cannot be immediately assayed, these could be stored at -20°C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

## Reagent Preparation:

**Dilute wash buffer (1:100) with distilled water (10 ml stock in total of 990 mL).** Store at 4°C.

## STORAGE AND STABILITY

The microtiter well plate and all other reagents (except the standards) are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping. Standards should be kept at -20°C for extended storage. The unused portions of the standards can be frozen in suitable aliquots for long-term use. Repeated freezing and thawing is not recommended.

**TEST PROCEDURE** (ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE). Dilute wash buffer (1:100) with distilled water (10 ml stock in total of 990 mL).

1. Label or mark the microtiter well strips to be used on the plate. Dispense 200-300 ul of wash buffer or water to all wells. Mix for 5 seconds and discard or aspirate the solution. The step should be done just before adding the samples, do not allow the wells to dry at any time during the assay.
2. Pipet **25 ul of standards** and serum samples into appropriate wells in *duplicate*. Dispense **100 ul of Antibody-Enzyme** Conjugate into each well. Gently mix the samples for 5-10 seconds, cover the plate and incubate at room temp for **30 min**.
3. Wash the plate **5X** with diluted wash buffer. We recommend using an automated ELISA plate Washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing
4. Dispense **100 ul TMB substrate per well**. Mix gently, cover the plate and **incubate for 15 min at room temp**. Blue color develops into standard and all positive wells.
5. Stop the reaction by adding **50 ul of stop solution** to all wells at the same timed intervals as in step 4. Mix gently for 5-10 seconds. Blue color turns yellow. Measure the **absorbance at 450 nm** using an ELISA reader within 30 min.

**NOTES-** Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4°C.

Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

## CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Subtract the absorbance of the zero standard from the mean absorbance values of standards and samples. Draw the standard curve on a graph paper by plotting net absorbance values of standards against appropriate protein concentrations. Read off the C-Peptide concentrations of the control and samples.

## EXPECTED VALUES

A limited testing of 100 serum samples –range 0.2-13.2 ng/ml  
It is recommended that each laboratory determine its own normal and abnormal range.

Since the kidney is the major sites for C-peptide metabolism, patients with severe renal insufficiency may have abnormally high circulating C-peptide levels.

## Reference Range

Fasting: 1.0-3.0 ng/ml (To convert nmol/L=ng/ml x1/3)

## Testing of other Biological Fluids Species Crossreactivity

This kit is primarily designed to test human serum samples. It is possible to use the plasma and other biological fluids. However, the sample volume and dilutions must be adjusted according to the expected concentrations or unknown samples be tested at several dilutions to determine the optimum range.

Crossreactivity of human C-Peptide antibodies used in the kit with C-Peptide from other species (mouse, rat, and monkey) has not been established.

## SPECIFICITY

ADI C-peptide ELISA kit was tested with the following:

Peptide/Proteins	% Crossreactivity
Human Pro-insulin23-33 split	63%
Human Pro-insulin 64-65 split	87%
Human insulin	0
Human C-peptide of Insulin	100%

Fasting concentration of intact and split pro-insulin are typically only 1-2% of C-peptide. Crossreactivity with these molecules is not clinically significant.

**DETECTION LIMIT** - Based on sixteen replicates determinations of the zero standard, the minimum concentration of human C-Peptide detected using this assay is ~ 0.1 ng/ml. The detection limit is defined as the value deviating by 2 SD from the zero standards. The **sensitivity of the assay is 0.3 ng/ml**.