

**ELISA kits available from ADI (see details at the web site)**

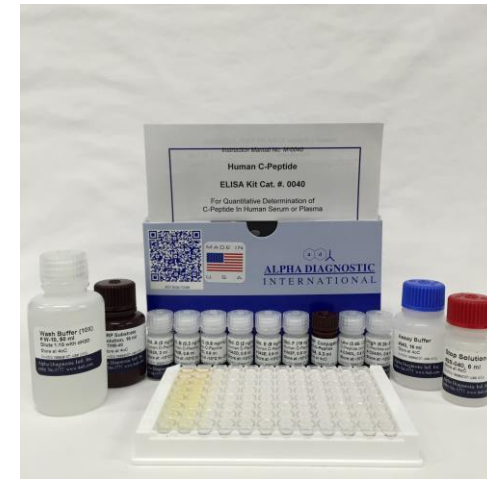
<b>#0010</b>	Human Leptin		
<b>#200-120-AGH</b>	Human globular Adiponectin (gAcrp30)		
<b>#0700</b>	Human Sex Hormone Binding Glob (SHBG)		
<b>#0900</b>	Human IGF-Binding Protein 1 (IGFBP1)		
<b>#1000</b>	Human C-Reactive Protein (CRP)		
<b>#100-110-RSH</b>	Human Resistin /FIZZ3		
<b>#100-140-ADH</b>	Human Adiponectin (Acrp30)		
<b>#100-160-ANH</b>	Human Angiogenin		
<b>#100-180-APH</b>	Human Angiopoietin-2 (Ang-2)		
<b>#100-190-B7H</b>	Human Bone Morphogenic Protein 7 (BMP-7)		
<b>#1190</b>	Human Serum Albumin	<b>#1200</b>	Human Albumin (Urinary)
<b>#1750</b>	Human IgG (total)	<b>#1760</b>	Human IgM
<b>#1800</b>	Human IgE	<b>#1810</b>	Human Ferritin
<b>#1210</b>	Human Transferrin (Tf)	<b>#0020</b>	Beta-2 microglobulin
<b>#1600</b>	Human Growth Hormone (GH)		
<b>#0060</b>	Human Pancreatic Colorectal cancer (CA-242)		
<b>#1820</b>	Human Ovarian Cancer (CA125)	<b>#1830</b>	Human CA153
<b>#1840</b>	Human Pancreatic & GI Cancer (CA199)		
<b>#1310</b>	Human Pancreatic Lipase		
<b>#1400</b>	Human Prostatic Acid Phosphatase (PAP)		
<b>#1500</b>	Human Prostate Specific Antigen (PSA)	<b>#1510</b>	free PSA (fPSA)
<b>#0500</b>	Human Alpha Fetoprotein (AFP)		
<b>#0050</b>	Human Neuron Specific Enolase (NSE)		
<b>#0030</b>	Human Insulin	<b>#0040</b>	Human C-peptide
<b>#0100</b>	Human Luteinizing Hormone (LH)		
<b>#0200</b>	Human Follicle Stimulating Hormone (FSH)		
<b>#0300</b>	Human Prolactin (PRL)		
<b>#0400</b>	Human Chorionic Gonadotropin (HCG)	<b>#0410</b>	HCG-free beta
<b>#0600</b>	Human Thyroid Stimulating Hormone (TSH)		
<b>#1100</b>	Human Total Thyroxine (T4)	<b>#1110</b>	Human Free T4 (ft4)
<b>#1650</b>	Human free triiodothyronine (ft3)	<b>#1700</b>	Human T3 (total)
<b>#1850</b>	Human Cortisol	<b>#1860</b>	Human Progesterone
<b>#1865</b>	Human Pregnlone	<b>#1875</b>	Human Aldosterone
<b>#1880</b>	Human Testosterone	<b>#1885</b>	Human free Testosterone
<b>#1910</b>	Human Androstenedione	<b>#1920</b>	Human Estradiol
<b>#1925</b>	Human Estrone	<b>#1940</b>	Dihydrotestosterone (DHT)
<b>#1950</b>	Human DHEA-sulphate (DHEA-S)		
<b>#3400</b>	Human serum Neopterin		
<b>#3000</b>	Human Rheumatoid Factors IgM (RF)		
<b>#3100</b>	Human anti-dsDNA		
<b>#3200</b>	Anti-Nuclear Antibodies (ANA)		

Instruction Manual No. M-0040

## Human C-Peptide

### ELISA Kit Cat. #. 0040, 96 Tests

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



*For In Vitro Research Use Only*



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

Components	96 tests
Anti-C-Peptide IgG coated microwell strip plate (96 wells)#041	1 Plate
Human C-Peptide <b>Std. A</b> , 0 ng/ml, 2 ml #042A	1 vial
Human C-Peptide <b>Std. B</b> , 0.3 ng/ml, 0.6 ml, #042B	1 vial
Human C-Peptide <b>Std. C</b> , 0.8 ng/ml, , 0.6 ml, #042C	1 vial
Human C-Peptide <b>Std. D</b> , 2 ng/ml, , 0.6 ml, #042BD	1 vial
Human C-Peptide <b>Std. E</b> , 8 ng/ml, , 0.6 ml, #042E	1 vial
Human C-Peptide <b>Std. F</b> , 16 ng/ml, , 0.6 ml, #042F	1 vial
Human C-Peptide, <b>Controls Low &amp; High</b> (exact values printed on vial # C040L-H, 0.6 ml: <b>Note: Standards and Calibrators must be stored frozen in suitable aliquots at -20oC or below upon arrival. Stds values may also be lot specific. Use the values provided on the actual vials.</b>	
Assay Buffer, 15 ml, #043	1 bottle
Anti-C-Peptide-HRP Conjugate(100X), 0.3 ml, #044	1 bottle
HRP substrate <b>Solution</b> , 16 ml, #TMB-40	1 bottle
<b>Wash buffer (10X), 50 ml</b> (dilute 1:10 with distilled water), <b>W - 1 0</b>	1 bottle
Stop solution, 6 ml, #SS-040	1 bottle
Instruction Manual, M - 0 0 4 0	1

## 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<sup>1</sup>. 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<sup>2</sup>. 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	1	0.04	3.5	0.03	2.7
2	4.14	0.09	2.2	0.11	2.7
3	12.02	0.55	4.6	0.62	5.0

### 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:

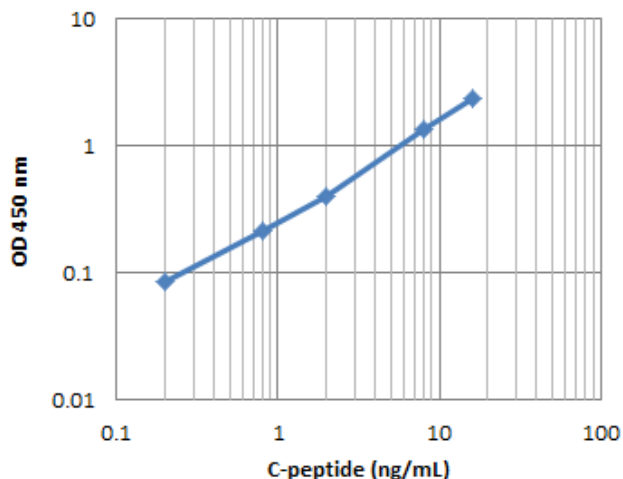
Ashby JP et al (1981) Annals of Clin. Biochem. 18, 125; Yue DK et al (1978) metabolism 27, 1, 1978; Krause UB et al (1981) J Immunol. 2, 33; Horowitz DI (1978) Diabetes 27, 267-271; Beischer W (1978) Diabetes 27, 235-240; Melani F (1970) PNAS 67, 148-155; Myrick JE (1989) Clin. Chme. 35, 1-37

## WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean A <sub>450nm</sub>
A1, A2	Std. A (0 ng/ml)	0.067
B1, B2	Std. B (0.2 ng/ml)	0.087
C1, C2	Std. C (0.8 ng/ml)	0.112
D1, D2	Std. D (2 ng/ml)	0.398
E1, E2	Std. E (8 ng/ml)	1.676
F1, F2	Std. F (16 ng/ml)	2.381
G1, G2	Sample 1	0.302

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).



Kit-spec-XL

### 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 log-log paper by plotting net absorbance values of standards against appropriate protein concentrations. Read off the C-Peptide concentrations of the control and samples. If ELISA reader software is being used, we recommend 4-parameter or 5-parameter curve.

## 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 Alpha Diagnostic Intl., Inc. 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 (1:10) with distilled water (50 ml stock in total of 450 mL). Store at 4°C.**

**HRP Conjugate conc.:** Dilute 1:100 with assay buffer (eg. 20 ul of HRP in 2 ml of assay buffer). Prepare in required amounts only.

## 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 (1:10) with distilled water (50 ml stock in 450 mL). Dilute HRP Conjugate stock 1:100 with assay buffer (eg. 20 ul of HRP in 2 ml of assay buffer

1. Label or mark the microtiter well strips to be used on the plate.
2. Pipet **50 ul of standards** and serum samples into appropriate wells in *duplicate*. Dispense **50 ul of Antibody-Enzyme Conjugate** into each well. Gently mix the samples for 5-10 seconds, cover the plate and incubate on a plate shaker (approx. 200 rpm) at room **temp for 90 minutes**.
3. Wash the plate **3X** 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 **150 ul TMB substrate per well**. Mix gently, cover the plate and **incubate** on a plate shaker **for 15-20 mins at room temp**. Blue color develops into standard and all positive wells. Note: It is possible to change the incubation time  $\pm$  5 mins so as to get the maximum color at around A450=2.0-2.5 or within the range of the reader.
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.

## EXPECTED VALUES

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.

Group	N	Mean (ng/ml)	Abs. range (ng/ml)
Males	26	0.89	0.24-1.98
Females	46	1.13	0.15-5.37

### Reference Range

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

### SPECIFICITY (Crossreactivity)

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

Peptide/Proteins	% Crossreactivity
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 standards, the minimum concentration of human C-Peptide detected using this assay is ~ 0.2 ng/ml. The detection limit is defined as the value deviating by 2 SD from the zero standards.

### 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.