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

#0010	Human Leptin		
#200-120-AGH	Human globular Adiponectin (gAcrp30)		
#0700	Human Sex Hormone Binding Glob (SHBG)		
#0900	Human IGF-Binding Protein 1 (IGFBP1)		
#1000	Human C-Reactive Protein (CRP)		
#100-110-RSH	Human Resistin /FIZZ3		
#100-140-ADH	Human Adiponectin (Acrp30)		
#100-160-ANH	Human Angiogenin		
#100-180-APH	Human Angiopoietin-2 (Ang-2)		
#100-190-B7H	Human Bone Morphogenic Protein 7 (BMP-7)		
#1190	Human Serum Albumin	**#1200**	Human Albumin (Urinary)
#1750	Human IgG (total)	**#1760**	Human IgM
#1800	Human IgE	**#1810**	Human Ferritin
#1210	Human Transferrin (Tf)	**#0020**	Beta-2 microglobulin
#1600	Human Growth Hormone (GH)		

#0060	Human Pancreatic Colorectal cancer (CA-242)		
#1820	Human Ovarian Cancer (CA125)	**#1830**	Human CA153
#1840	Human Pancreatic & GI Cancer (CA199)		
#1310	Human Pancreatic Lipase		
#1400	Human Prostatic Acid Phosphatase (PAP)		
#1500	Human Prostate Specific Antigen (PSA)	**#1510**	free PSA (fPSA)
#0500	Human Alpha Fetoprotein (AFP)		
#0050	Human Neuron Specific Enolase (NSE)		

#0030	Human Insulin	**#0040**	Human C-peptide
#0100	Human Luteinizing Hormone (LH)		
#0200	Human Follicle Stimulating Hormone (FSH)		
#0300	Human Prolactin (PRL)		
#0400	Human Chorionic Gonadotropin (HCG)	**#0410**	HCG-free beta

#0600	Human Thyroid Stimulating Hormone (TSH)		
#1100	Human Total Thyroxine (T4)	**#1110**	Human Free T4 (fT4)
#1650	Human free triiodothyronine (fT3)	**#1700**	Human T3 (total)

#1850	Human Cortisol	**#1860**	Human Progesterone
#1865	Human Pregnolone	**#1875**	Human Aldosterone
#1880	Human Testosterone	**#1885**	Human free Testosterone
#1910	Human Androstenedione	**#1920**	Human Estradiol
#1925	Human Estrone	**#1940**	Dihydrotestosterone (DHT)
#1950	Human DHEA-sulphate (DHEA-S)		
#3400	Human serum Neopterin		

#3000	Human Rheumatoid Factors IgM (RF)
#3100	Human anti-dsDNA
#3200	Anti-Nuclear Antibodies (ANA)

Instruction Manual No. M-0030-70-1

Mouse/Rat C-Peptide

ELISA Kit Cat. # 0030-70-1

For Quantitative Determination of C-Peptide in Mouse/Rat Serum, EDTA-plasma and cell culture medium

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

India Contact:

Life Technologies (India) Pvt. Ltd.
306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi – 110034
Ph: +91-11-42208000, 42208111, 42208222

Mobile: +91-9810521400
Fax: +91-11-42208444
Email: customerservice@atzlabs.com
Web: www.atzlabs.com

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DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHNAGES.

Mouse/Rat C-Peptide ELISA KIT # 0030-70-1, Kit Contents

Components	96 tests
Mouse Mono. Anti-C-Peptide IgG coated microwell **strip plate** (96 wells)#0030-71P	**1 Plate**
C-Peptide **Cal. 0** (color coded yellow), **5 ml ready-to-use,** #030-72A	1 vial
C-Peptide **Cal. 1**, **Lyophilized**, #030-72B	1 vial
C-Peptide **Cal. 2**, **Lyophilized**, #030-72C	1 vial
C-Peptide **Cal. 3**, **Lyophilized**, #030-72D	1 vial
C-Peptide **Cal. 4**, **Lyophilized,** #030-72E	1 vial
C-Peptide **Cal. 5**, **Lyophilized,** #030-72F	1 vial
C-Peptide, **Calibrators (1-5) are in Lyophilized form.** **Note: Lyophilized Calibrators should be reconstituted in 1 ml distilled water. Actual values are lot specific printed on vials.**	
Assay Buffer, (color coded red) 6 ml, #030-70-AB	1 bottle
Anti-rat C-peptide **HRP Conjugate (11X)**, 1.3 ml, #030-73	1 bottle
C-Peptide **Enzyme Conjugate buffer**, 13 ml, # 030-73	1 bottle
HRP **substrate Solution**, 22 ml, #30-70-TMB	1 bottle
Wash buffer (21X), 50 ml (dilute 1:21 with distilled water), 030-70-WB	1 bottle
Stop solution, 7 ml, #30-70-SS	1 bottle
Instruction Manual, M - 0 0 3 0 - 7 0 - 1	1

Intended Use

ADI's Mouse/Rat C-Peptide ELISA kit is a highly sensitive sandwich type assay for the measurement of C-peptide in Mouse/Rat serum EDTA-plasma and cell culture medium. For research use only (RUO), not for diagnostic procedures.

Introduction

C-peptide is formed together with insulin from the cleavage of proinsulin within secretory granules in the s-cell. In most species the insulin gene exists in a single copy. Rats and mice however, have two closely related genes which produce two nonallelic proinsulins (1). The rat proinsulins are cleaved to form two insulins (insulin I and insulin II) and two C-peptides (C-peptide I and C-peptide II): The two C-peptides differ with regard to two amino acids in the middle segment of the molecule. C-peptide is considered to have a longer half-life in circulation than insulin, and is used in humans and animal models as a marker of endogenous insulin production (2). Traditionally C-peptide has been considered to be without biological effects of its own, but in recent years it has been reported that C-peptide treatment may affect renal and nerve dysfunction in type 1 diabetes patients (3). Physiological effects of C-peptide have also been observed in animal models of diabetes (4, 5). Rat C-peptide ELISA calibrators are made from synthetic rat C-peptide I. Both rat C-peptide I and II are measured in the assay.

PERFORMANCE CHARACTERISTICS

Precision:

Each sample was analyzed in 4 replicates on 24 different occasions.

Sample	Mean pmol/L	Within Assay %	%COV	Total assay %
1	**335**	**4.2**	**7.12**	**7.5**
2	**1432**	**2.5**	**3.9**	**4.1**
3	**2831**	**2.0**	**2.2**	**2.4**

INTERNAL QUALITY CONTROL

Commercial controls and/or in-house serum pools with low, intermediate and high rat C-peptide concentrations should routinely be assayed as samples, and results charted from day to day. It is good laboratory practice to record the following data for each assay: kit lot number, preparation dates of kit components, OD values for the blank, Calibrators and controls.

General References:

1. Steiner DF, Chan SJ, Welsh JM and Kwok SC (1985) Structure and evolution of the insulin gene. Annu Rev Genet 19:463-484

2. Faber OK, Hagen C, Binde, C, Markussen J, Naithani, VK, Blix PM, Kuzuya H, Horwitz DL, Rubenstein AH and Rossing N (1978) Kinetics of human connecting peptide in normal and diabetic subjects. J Clin Invest 62:197-203

3. Wahren J, Ekberg K and Jornvall H (2007) C-peptide is a bioactive peptide. Diabetologia 50:503-509

4. Nordquist L, Moe E, Sjoquist M (2007) The C-peptide fragment EVARQ reduces glomerular hyperfiltration in streptozotocin-induced diabetic rats. Diabetes Metab Res Rev 2006 23:400-405

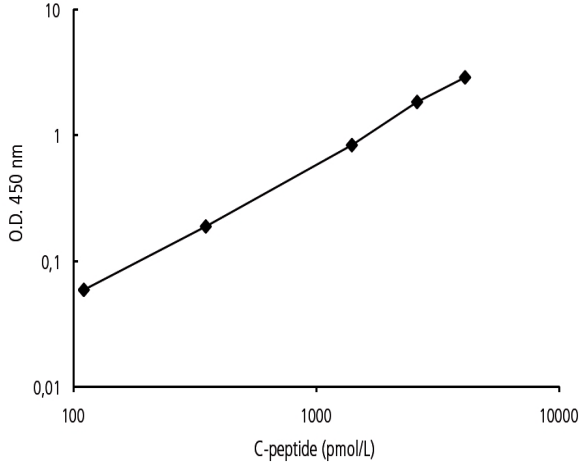
5. Rebsomen L, Pitel S, Boubred F, Buffat C, Feuerstein JM, Raccah D, Vague P and Tsimaratos M (2006) C-peptide replacement improves weight gain and renal function in diabetic rats. Diabetes Metab 32:223-228

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples (pmol/l)	Mean A_{450nm}
A1, A2	**Cal. 0**	0.114
B1, B2	**Cal. 1**	0.172
C1, C2	**Cal. 2**	0.301
D1, D2	**Cal. 3**	0.935
E1, E2	**Cal. 4**	1.947
F1, F2	**Cal. 5**	2.987
G1, G2	**Sample 1**	303.0

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

Subtract the absorbance of the zero standard from the mean absorbance values of calibrators and samples.

Plot the A450 values of the calibrators against the concentration and use cubic spline regression. For manual plots, read the conc from the calibrator curve.

PRINCIPLE OF THE TEST

Mouse/Rat C-Peptide ELISA kit is based on simultaneous binding of rat 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), H2SO4 (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:

Serum: Collect blood by venipuncture, allow to clot and separate the serum by centrifugation.

Plasma: Collect blood by venipuncture into tubes containing EDTA as anticoagulant, and separate the plasma fraction.

Cell culture medium: Note that different chemicals used in cell culture media can interfere with the assay (such as sodium azide (NaN3) and beta-mercaptoethanol).

PREPARATION OF SAMPLES:

No dilution is normally required for serum or plasma. All samples containing rat C-peptide above the highest Calibrator should be diluted with **Calibrator 0**.

Reagent Preparation:

Dilute wash buffer (1:21) with distilled water (50 ml stock in total of 1L). Store at 4oC.

HRP Conjugate (11X): dilute with Enzyme conjugate buffer (100 ul stock conjugate and 1 ml of the buffer)**.** Prepare 1 ml per strip or 11 ml for full plate. Do not keep diluted stock and dilute as needed.

STORAGE AND STABILITY

The microtiter well plate and all other reagents (except the standards) are stable at 2-8^{o}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 –20oC 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 (1:21) with distilled water (50 ml stock in 1-L of distilled water). Dilute Antibody-HRP Conjugate (1:11) with HRP Conjugate buffer in required volume.

Reconstitute Calibrators 1-5 with 1000 µL distilled water per vial.

1. Label or mark the microtiter well strips to be used on the plate.

2. Pipet **10 µl of calibrators** and serum samples into appropriate wells in *duplicate*. Dispense **50 µl** of Assay buffer into each well. Gently mix the samples, cover the plate and incubate at **room temp (18-25 °C) for 1 hrs on a plate shaker (700-900 rpm).** if plate shaker is not available, plates can be manually mixed 3-4 times during the incubation.

3. Wash the plate **6X** with 1x-**wash buffer (350 ul/wash**). 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. Add **100 µL** enzyme conjugate 1X solution into each well, cover the plate and incubate at **room temp (18-25 °C) for 1 hrs on a plate shaker (700-900 rpm).**

5. Wash the plate **6X** with 1x-**wash buffer (350 ul/wash**) as in **step 3**

6. Dispense **200 ul TMB substrate per well.** Mix gently for 5-10 seconds, cover the plate and incubate at room temp for **15 min. Blue color** develops in positive wells.

7. Stop the reaction by adding **50 µL of stop solution** to all wells. 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^{o}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.

LIMITATIONS OF THE PROCEDURE

Grossly lipemic, icteric or haemolyzed samples do not interfere in the assay.

PERFORMANCE CHARACTERISTICS

Detection limit

Detection limit is defined as the Capability of Detection according to ISO11843-Part 1. Capability of Detection should be seen as part of a method validation, rather than the lowest concentration that can be measured. The detection limit is lower than the concentration of Calibrator 1 determined with the methodology described in ISO11843-Part 4.

Concentration of samples with absorbance below Calibrator 1 should not be calculated, instead expressed as less or equal to (≤) the concentration indicated on the vial for Calibrator 1.

Recovery

Recovery upon addition is 97-100% (mean 98%).

Recovery upon dilution is 91-105% (mean 100%).

Hook effect

Samples with a concentration of up to 400 000 pmol/L have been tested without giving falsely low results.

Specificity

The following cross reaction have been found:

Rat Insulin <0.01%

Rat Proinsulin 4.55%

Human C-Peptide <0.001%

CALIBRATION

ADI's Mouse/Rat C-peptide ELISA is calibrated against an in-house reference preparation of rat C-peptide I.