**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*


6203 Woodlake Center Drive • San Antonio• Texas 78244 • USA.

Phone (210) 561-9515 • Fax (210) 561-9544

Toll Free (800) 786-5777

Email: service@4adi.com

Web Site: www.4adi.com

**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

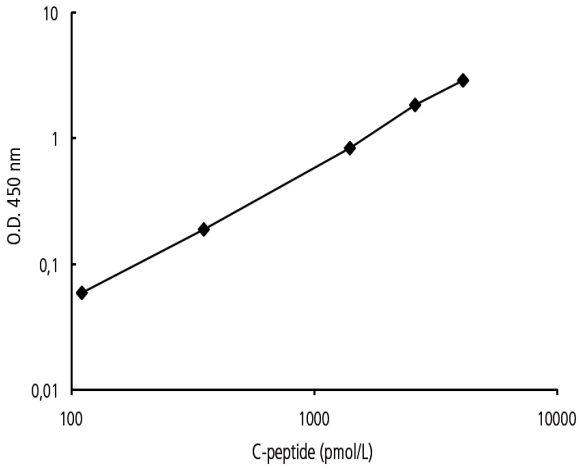
5. Rebsomen L, Pitel S, Boubred F, Buffat C, Feuerstein JM, Raccah D, Vague P and Tsimaratos M (2006) C-peptide replacement improves weight gain and renal function in diabetic rats. Diabetes Metab 32:223-228

# 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.