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 (PSA)
#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 (T4)
#1650  Human free triiodothyronine (FT3)  #1700  Human T3 (total)

#1850  Human Cortisol  #1860  Human Progesterone
#1865  Human Pregnenolone  #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)

For In Vitro Research Use Only

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

Alpha Diagnostic Intl.  (www.4adi.com)  040/D150625APage 7
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 useful monitor of average response to meals, measurement of the 24 hour urinary excretion of C-PEPTIDE provides a useful measure of average cell insulin secretion more reliable than the level of insulin itself. C-PEPTIDE is cleaved from the A chain by the kidney. Urine concentrations of C-PEPTIDE are 20-50 times higher than insulin, 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. Elevation 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

<table>
<thead>
<tr>
<th>Sample Serum</th>
<th>Mean (ng/ml)</th>
<th>Intra-assay S.D.</th>
<th>Intra-assay CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>0.04</td>
<td>3.5</td>
</tr>
<tr>
<td>2</td>
<td>4.14</td>
<td>0.09</td>
<td>2.2</td>
</tr>
<tr>
<td>3</td>
<td>12.02</td>
<td>0.55</td>
<td>4.6</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Sample Dilution</th>
<th>C-PEPTIDE Level Expected (ng/ml)</th>
<th>C-PEPTIDE Level Measured (ng/ml)</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undiluted</td>
<td>20</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>1:2</td>
<td>10</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>1:4</td>
<td>5</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>1:8</td>
<td>2.5</td>
<td>2.3</td>
<td>92</td>
</tr>
<tr>
<td>1:20</td>
<td>1</td>
<td>0.85</td>
<td>85</td>
</tr>
</tbody>
</table>

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)

<table>
<thead>
<tr>
<th>C-PEPTIDE</th>
<th>Expected Value</th>
<th>Measured Value</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>3.758</td>
<td>3.8</td>
<td>101</td>
</tr>
<tr>
<td>5</td>
<td>7.5</td>
<td>6.1</td>
<td>81</td>
</tr>
<tr>
<td>10</td>
<td>6.25</td>
<td>4.9</td>
<td>78.4</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>10.6</td>
<td>106</td>
</tr>
</tbody>
</table>

General References:


Alpha Diagnostic Intl. (www.4adi.com) 040/D150625A Page 1
**WORKSHEET OF TYPICAL ASSAY**

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

**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).

**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. If ELISA reader software is being used, we recommend 4-paramter 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), 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**

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 4oC.**

**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.
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, 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.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean (ng/ml)</th>
<th>Abs. range (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>26</td>
<td>0.89</td>
<td>0.24-1.98</td>
</tr>
<tr>
<td>Females</td>
<td>46</td>
<td>1.13</td>
<td>0.15-5.37</td>
</tr>
</tbody>
</table>

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:

<table>
<thead>
<tr>
<th>Peptide/Proteins</th>
<th>% Crossreactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human insulin</td>
<td>0</td>
</tr>
<tr>
<td>Human C-peptide of Insulin</td>
<td>100%</td>
</tr>
</tbody>
</table>

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.