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 Folicle 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 (tT3)
#1850  Human Cortisol  #1860  Human Progesterone
#1865  Human Pregnanolone  #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)

Human Pancreatic Lipase

ELISA KIT  Cat. No. 1310

For Quantitative Determination of Lipase In Serum

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
Pancreatic Lipase ELISA Kit Cat. No. 1310

For Quantitative Determination of Pancreatic Lipase In Serum

Kit Contents: (reagents for 96 tests)

<table>
<thead>
<tr>
<th>Components</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-h pancreatic Lipase coated microwell strip plate (96 wells). Ready-to-use</td>
<td>1311</td>
</tr>
<tr>
<td>Pancreatic Lipase Sample Diluent 11 ml</td>
<td>SD-1310</td>
</tr>
<tr>
<td>Pancreatic Lipase Std. A, 0.75 ml (0 U/L)</td>
<td>1312</td>
</tr>
<tr>
<td>Pancreatic Lipase Std. B, 0.75 ml (10 U/L)</td>
<td>1313</td>
</tr>
<tr>
<td>Pancreatic Lipase Std. C, 0.75 ml (50 U/L)</td>
<td>1314</td>
</tr>
<tr>
<td>Pancreatic Lipase Std. D, 0.75 ml (100 U/L)</td>
<td>1315</td>
</tr>
<tr>
<td>Pancreatic Lipase Std. E, 0.75 ml (200 U/L)</td>
<td>1316</td>
</tr>
<tr>
<td>Pancreatic Lipase Std. F, 0.75 ml (400 U/L)</td>
<td>1317</td>
</tr>
<tr>
<td>Pancreatic Lipase control serum, 0.75 ml; #C1310 (exact values printed on vial)</td>
<td></td>
</tr>
<tr>
<td>Anti-human Pancreatic Lipase HRP Conjugate, 11 ml (Ready-to-use)</td>
<td>1318</td>
</tr>
<tr>
<td>Wash buffer (100X), 10 ml, <strong>dilute 1:100 with distilled water</strong></td>
<td>W-100</td>
</tr>
<tr>
<td>HRP substrate solution (ready-to-use), 11 ml</td>
<td>TMB1310</td>
</tr>
<tr>
<td>Stop solution (ready-to-use), 10 ml</td>
<td>T-10</td>
</tr>
<tr>
<td>Complete Instruction Manual</td>
<td>1310</td>
</tr>
</tbody>
</table>

Introduction

Lipase (triacylglycerol acylhydrolase EC 3.1.1.3) hydrolyzes preferentially glycerol esters of long chain fatty acid, an enzyme that is anticipated to be specific for pancreas. The serum lipase activity tends to be elevated at about the same time as the elevation of serum amylase in acute pancreatitis. Analyses of pancreatic lipase and amylase in serum have added a new dimension to the laboratory detection and differentiation of pancreatic diseases. The current methodologies for determination of lipase activity using turbidimetric methods are relatively complex. These methods are non-specific and have low sensitivity.

ADI’s human pancreatic lipase ELISA kit provides a direct assay in which pancreatic lipase is specifically recognized both by a solid phase antibody coated on the ELISA plates and the HRP-conjugated antibody. This eliminates the interference of the other isoamylase and the problem derived from non-standardized substrate preparation and conditions, which employed in most other enzymatic assay.

3. RECOVERY

A known amount of pancreatic Lipase (10-100 U/L) was added to three patient sera (with original Lipase concentrations of 22, 39, and 83 U/L) and the total Lipase measured. The assay showed excellent mean recoveries of about 105% (range 98-105%).

4. SPECIFICITY

The test is specific for human pancreatic lipase. The interference of transferin, gamma globulins, bilirubin, triglycerides, hemoglobin, and ascorbic acid were studies were not unusual and expected of any other ELISA test.

5. HIGH DOSE HOOK EFFECT

High pancreatic Lipase concentrations of up to 13200 U/L did not cause any hook effect.

6. Species Reactivity

Human pancreatic lipase test is specific for human samples. We have not tested other species (mouse, rat, monkey etc).

References

2. Tietz NW (1986) Cllin Chem. 32, 301-307
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 1-liter).

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

1. Pipet 25 ul of standards, control, and serum samples into appropriate wells in duplicate. Add 100 ul of Anti-lipase-enzyme conjugate into each well. Mix gently for 5-10 seconds.

2. Cover the plate and incubate at room temp. for 60 minutes.

3. Aspirate and wash the wells 5 times with 300 ul of 1x 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. Cover the plate and incubate at room temp. for 30 minutes. Blue color develops in standards and positive wells.

5. Stop the reaction by adding 50 ul of stop solution to all wells. Mix gently for 5-10 seconds (blue color turns yellow).

6. Measure the Absorbance at 450 nm using an ELISA reader. Color is stable for at least one hr after stopping.

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 wells 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, control, and samples. Draw the standard curve on log-log graph paper by plotting net Absorbance values of standards against appropriate pancreatic Lipase concentrations. Read off the pancreatic Lipase concentrations of the control and patient samples.

Expected Values

It is recommended that each laboratory determine its own normal and abnormal range.

A clinical study of ADI ELISA kit on 36 apparently normal sample yielded values of 26-150 U/L.

Reference range: 56-239 U/L.

PERFORMANCE CHARACTERISTICS

1. DETECTION LIMIT

Based on sixteen replicate determinations of the zero standard, the minimum pancreatic Lipase concentration detectable using this assay is 1.0 U/L. The detection limit is defined as the value deviating by 2 SD from the zero standard.

2. PRECISION

Intra-assay precision:

Three serum samples (mean Lipase concentrations: 44-100 U/L were run in sixteen replicates. The samples showed good intra-assay precision with %CV of 4.9 and S.D. 2.6 U/L.

Inter-assay precision:

Twelve serum samples were run in duplicate in sixteen independent assays. The samples showed good inter-assay precision (7-9 % CV). The actual values were: mean 42.33 U/L, 71.92 U/L, and 137 U/L.
WORKSHEET OF TYPICAL ASSAY

<table>
<thead>
<tr>
<th>Wells</th>
<th>Stds/samples</th>
<th>Net Abs.</th>
<th>Calculated Conc (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1, A2</td>
<td>Std. A (0 U/L)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>B1, B2</td>
<td>Std. B (10 U/L)</td>
<td>0.071</td>
<td></td>
</tr>
<tr>
<td>C1, C2</td>
<td>Std. C (50 U/L)</td>
<td>0.302</td>
<td></td>
</tr>
<tr>
<td>D1, D2</td>
<td>Std. D (100 U/L)</td>
<td>0.580</td>
<td></td>
</tr>
<tr>
<td>E1, E2</td>
<td>Std. E (200 U/L)</td>
<td>1.156</td>
<td></td>
</tr>
<tr>
<td>F1, F2</td>
<td>Std. F (400 U/L)</td>
<td>1.920</td>
<td></td>
</tr>
<tr>
<td>G1, G2</td>
<td>Sample 1</td>
<td>0.437</td>
<td>75</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.

Pancreatic Lipase ELISA kit is based on sequential binding of human pancreatic Lipase 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 colors developed. The enzymatic reaction (color) is directly proportional to the amount of Lipase present in the sample. Adding stopping solution terminates the reaction. Absorbance is measured on a microtiter well ELISA reader at 450 nm. The unknown sample values are then read-off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (20-100 μl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The ADI Pancreatic Lipase ELISA kit is intended for in vitro research use only. The reagents contain thimerosal as preservative; necessary care should be taken when disposing solutions. The Control Serum and Standards have 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), 2N HCl (stop solution), and Proclin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

SPECIMEN COLLECTION AND HANDLING

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

STORAGE AND STABILITY

The microtiter well plate and all other reagents 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 under appropriate storage conditions.

Reagent Preparation:

Dilute wash buffer (1:100) with distilled water (10 ml stock in total of 1-liter). Store at 4oC.