**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 (toal) |

| | | | |
|---|---|---|---|
| **#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-1310*

# Human Pancreatic Lipase

**ELISA KIT Cat. No. 1310**

**For Quantitative Determination of Lipase In Serum**

*For In Vitro Research Use Only*

ALPHA DIAGNOSTIC INTERNATIONAL

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

**Pancreatic Lipase ELISA Kit Cat. No. 1310**

For Quantitative Determination of Pancreatic Lipase In Serum

Kit Contents: (reagents for 96 tests)

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

**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**

1. Lott JA et al (1986) Clin Chem. 32, 1290;
   Lott JA et al (1986) Clin Enzymology, 245
2. Tietz NW (1986) Cllin Chem. 32, 301-307
3. Panteghini M et al (1989) Clin Chem. 35, 417
4. Tietz NW et al (1987) Clin Chem. 33, 1624
5. Verduin PA (1973) Clin Chi Acta 46, 11-19

**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 $4^{o}C$.

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 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 won 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.

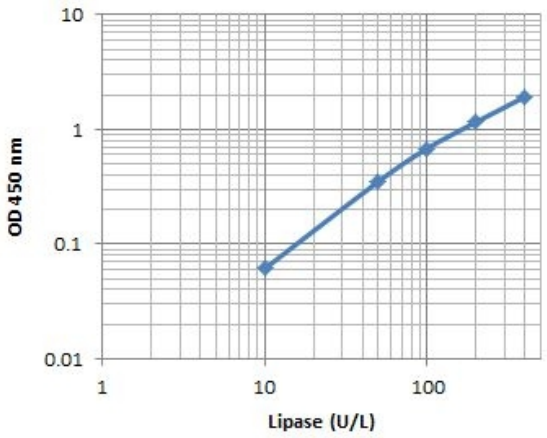
*Inter-assay precision:*

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

| Wells | Stds/samples | Net Abs. | Calculated Concn (U/L) |
|---|---|---|---|
| A1, A2 | **Std. A** (0 U/L) | - | |
| B1, B2 | **Std. B** (10 U/L) | 0.071 | |
| C1, C2 | **Std. C** (50 U/L) | 0.302 | |
| D1, D2 | **Std. D** (100 U/L) | 0.580 | |
| E1, E2 | **Std. E** (200 U/L) | 1.156 | |
| F1, F2 | **Std. F** (400 U/L) | 1.920 | 22 |
| G1, G2 | **Sample 1** | 0.437 | 75 |

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)



## PRINCIPLE OF THE TEST

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 Prolcin-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 could at -20$^{o}$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$^{o}$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.