
Please use only the valid version of the package insert provided with the kit.

1 NAME AND INTENDED USE

The Lipase ELISA is a quantitative solid phase enzyme linked immunosorbent assay. This test provides quantitative measurement of human pancreatic lipase (h-p-Lipase) in serum to aid in the management of patients with pancreatic diseases.

For Professional Use Only.

2 SUMMARY AND EXPLANATION OF TEST

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 pancrease^{1,2}. The serum lipase activity tends to be elevated at about the same time as the elevation of serum amylase in acute pancreatitis³. Analyses for pancreatic lipase and amylase in serum have added a new dimension to the laboratory detection and differentiation of pancreatic diseases⁴.

However the current methodologies for detection of lipase activity using turbidimetric methods are relatively complex. These methods are non-specific and have low sensitivity^{5,6}.

Lipase ELISA provides an enzyme immunoassay system for specific determination of pancreatic lipase in serum with high sensitivity to aid in the management of patients with pancreatic diseases.

3 PRINCIPLE OF THE ASSAY

The Lipase ELISA is a solid phase enzyme linked immunosorbent assay (ELISA) based on the sandwich principle. The wells are coated with specific anti-h-p-Lipase antibodies. The samples are incubated in the wells with enzyme conjugate which is another anti-h-p-Lipase antibodies chemically conjugated with horseradish peroxidase. Unbound enzyme conjugate is washed off and the amount of bound peroxidase is proportional to the concentration of the h-p-Lipase present in the sample, standards and controls. Upon addition of TMB substrate, the intensity of color developed is proportional to the concentration of h-p-Lipase in the samples.

4 WARNINGS AND PRECAUTIONS

1. Lipase ELISA is designed for in vitro diagnostic use only
2. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
3. Warning potential bio-hazardous material: The matrix of Negative and Positive controls is human serum. The serum found negative for HBsAg, HIV and HCV antibodies when tested with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HBsAg, HIV, HCV, or other infectious agents are absent, these reagents should be handled at Bio-safety level 2, as recommended for any potentially infectious human serum or blood specimen in the Center for Disease Control/National Institutes of Health Manual, "Bio-safety in Microbiological and Biomedical Laboratories" 1984.

5 STORAGE AND STABILITY

1. Store the kit at 2-8 °C in a refrigerator. Keep micro-wells sealed in dry bag with desiccants.
2. The unopened reagents are stable until expiration of the kit.
3. TMB Solution should be colorless; if the solution turns blue, it must be replaced. Do not expose test reagents to strong light during storage or usage.

6 SAFETY INSTRUCTIONS

1. Positive Control is made from human origin and found to be negative from HBsAg, HIV and HCV antibodies. However, for safety, it must be treated as infectious materials.
2. Do not smoke or eat in areas where specimens or reagent kits are handled.
3. Do not mouth pipette. Wear PVC gloves when handling reagent kits or specimens, and wash hands thoroughly afterwards.
4. Infectious specimens and non-acid-containing spills should be wiped up thoroughly with 5% sodium hypochlorite solution.
5. All waste material should be properly disinfected before disposal. Both liquid and solid waste can be autoclaved for at least one hour at 121.5°C. Solid waste can also be incinerated. Non-acidic liquid waste requires neutralization before similar treatment and should stand for 30 minutes to obtain effective disinfection.
6. Avoid contact of hydrochloric acid with skin and mucous membranes.

7 MATERIALS PROVIDED

1. **Micro-wells strips** (96 wells):
Anti-h-p-Lipase antibodies coated wells. 8 x 12 strips
2. **Sample Diluent or zero standard** (11 mL):
1 bottle
3. **Enzyme conjugate** (11 mL):
Anti-h-p- Lipase antibody conjugated to horseradish peroxidase.
4. **Reference Standard Set** (0.75 mL each vial)
The concentrations are 10, 50, 100, 200 and 400 U/L.
5. **Control** (0.75 mL)
Value as indicated on the vial.
6. **TMB Solution** (11 mL):
Buffer solution containing hydrogen peroxide and TMB.
7. **Washing buffer concentrate** (10 mL):
Prepare working washing buffer by adding 10 mL washing buffer concentrate into 990 mL distilled water.
8. **Stop Solution:**
2 N HCl solution.

9. Well holder:

For securing individual wells.

8 MATERIALS REQUIRED BUT NOT PROVIDED

1. Micro-well reader with working wavelength at 450 nm.
2. Pipetor with tips for 25 µL, 50 µL & 100 µL
3. 1 L washing bottle.

9 SAMPLE COLLECTION AND HANDLING

Collect blood aseptically by venipuncture, allow to clot. Separate the serum by centrifugation at room temperature, and store in sterile tubes.

If sera cannot be assayed immediately, they can be stored at 2-8 °C for a week or frozen at -20 °C for up to 6 months. Repeated freezing and thawing is not recommended. Do not store in self-defrosting freezer. Do not use hyperlipemic, hemolyzed, contaminated or heat inactivated sample as they may cause erroneous results.

10 PREPARATION FOR ASSAY

1. Before beginning the test, bring all samples and reagents to room temperature (24 ± 30 °C) and mix each gently.
2. Have all reagents and samples ready before the start of the assay. Once the test has begun it must be performed without any interruption to get the most reliable and consistent results
3. Use new disposable tips for each sample.

11 ASSAY PROCEDURE

1. Secure the desired number of coated wells in holder. Mark data sheet with sample identification.
2. Dispense **25 µL of standards, control and serum samples**, in duplicate into appropriate well.
3. Dispense **100 µL of enzyme conjugate** into each well and mix for 5 seconds and incubated at room temperature for 30 minutes.
4. Remove mixture and **rinse the wells 5 times with washing buffer solution**. (300 µL/well/each rinse)
Be sure to wash the wells thoroughly and completely dry the wells.
Improper wash may cause false positive results.
5. Dispense **100 µL of TMB Solution** into each well including blank well. Mix for 5 seconds and incubated in the dark for 15 minutes.
6. Stop reaction by adding **50 µL of stop solution** to each well and read at 450 nm with microwell reader against Blank well (only TMB Solution and Stop Solution).

12 QUALITY CONTROL

Lipase ELISA kit contains only one Control. Each laboratory should utilize internal controls several levels to monitor assay performance. The controls should be treated as unknown. Results obtained should be in agreement with the assigned values of the Control. The control should contain no sodium azide.

13 PROCEDURAL NOTE

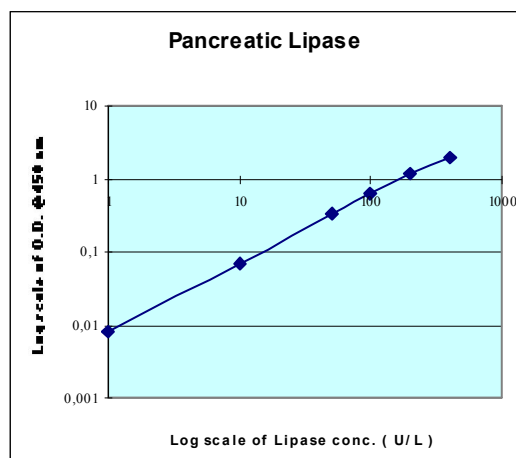
1. It is very important to wash the microwells thoroughly and remove the last droplets of water to achieve the best results.
2. Pipet all reagents and samples into the bottom of well. Avoid scratching the well. Vortex- mixing or shaking of wells is not required.
3. Absorbance is function of the time and temperature of incubations. It is recommended to have all reagents and samples caps removed and all needed wells secured in holder and assigned. This will ensure the equal elapsed time for each pipetting without interruption.
4. For the same reason, the size of the assay run should be limited. It is suggested to run no more than 20 patients with a set of Reference Standards in duplicate.
5. If in the assay, a serum specimen has been found to contain greater than 400 U/L of h-p-Lipase, the sample must be further diluted with sample diluents and reassayed as described in Assay procedures.

14 CALCULATION OF RESULTS

Any microwell reader capable of determining absorbance at 450 nm may be used. The h-p--Lipase value of patient is obtained as follows:

1. Plot the concentration (X) of each Reference Standards against its absorbance (Y) on logarithmic graph paper.
2. Obtain the h-p-Lipase values of samples by reference to the standard curve. The table and the figure are the example. The data is for demonstration purpose and must not be used in place of data generated for each assay.

Well No. Description	Absorbance 450 nm	Value from STD curve
A1 0 U/L	0.000	
B1	0.000	
A2 10 U/L	0.071	
B2	0.068	
A3 50 U/L	0.302	
B3	0.349	
A4 100 U/L	0.580	
B4	0.651	
A5 200 U/L	1.156	
B5	1.202	
A6 400 U/L	1.920	
B6	1.975	
A7 Patient A	0.437	75 U/L
B7	0.513	



15 EXPECTED VALUE

It is recommended that each laboratory should determine its own normal and abnormal ranges as to account for its environmental factor such as diet, climate etc.

A clinical study of Lipase ELISA (EIA-4459) on 36 apparently normal serum samples: The central 95 percentiles of results obtained encompassed 26-150 U/L

REFERENCE RANGE: 56 - 239 U/L

16 LIMITATIONS

For diagnostic purpose, the h-p-Lipase values should be used as an adjunct to other data available to the physician. The Lipase ELISA kit has been designed to avoid “hook effect” at the concentration of h-p-Lipase up to 13,200 U/L. Samples with h-p-Lipase level above 400 U/L should be diluted to obtain an accurate value.

17 PERFORMANCE CHARACTERISTICS**17.1 Accuracy**

Recovery studies were performed by mixing an aliquot of pooled serum and h-p-Lipase standard. The h-p-Lipase values were measured and percentage of recovery were determined.

Initial Value (U/L)	Expected Value (U/L)	Observed Value (U/L)	Recovery (%)
10	30	31	103
50	75	75	100
100	250	260	104

17.2 Precision

Intra-assay: Ten samples each from three pooled sera were assayed in a single run. **Inter-assay:** Three pooled sera were assayed in duplicate in four days.

Inter-Assay	Pool A	Pool B	Pool C
N	12	12	12
Mean(U/L)	42.33	71.92	137.08
S.D. (U/L)	3.08	7.56	12.76
C.V. (%)	7.29	10.51	9.31

Intra-assay	Pool A	Pool B	Pool C
N	10	10	10
Mean(U/L)	44	72	100
S.D. (U/L)	2.16	2.20	2.49
C.V. (%)	4.91	2.8	2.49

17.3 Hook Effect

No hook effect is found until patient sample Lipase concentration reaches 13,200 U/L

17.4 Sensitivity

A linearity study was performed to assess the sensitivity of the assay.

Dilution	Expected Value (U/L)	Observed Value (U/L)	Recovery (%)
1:2	200	210.1	105
1:5	80	79.3	99
1:8	50	52.3	105
1:16	25	26	104
1:40	10	10.4	104
1:80	5	5.8	116

17.5 Specificity

The test is specific for human pancreatic lipase.

The interference of transferrin, gamma globulins, bilirubin, triglycerides, hemoglobin and ascorbic acid were studied. No significant interference was observed at levels encountered in routine testing.

17.6 Minimal detectable Concentration

The minimal detectable concentration of lipase is estimated to be 1 U/L.

The minimal detectable concentration is defined as that concentration of lipase which corresponds to the absorbance value that is two standard deviation greater than the mean absorbance value of twenty replicate determinations of the zero diluent.

18 REFERENCES / LITERATURE

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