CATACHEM

LIPASE
MANUAL/AUTOMATED APPLICATIONS
V340-0B

Intended Use
For **IN VITRO quantitative** determination of Lipase in serum using manual or automated applications.

Clinical Significance
The concentration of Lipase activity in blood is useful for the evaluation of pancreatic function. Lipase activity in serum is significantly elevated in cases of acute pancreatitis and obstruction of the pancreatic duct. Elevated values are also observed in intra-abdominal diseases, mumps and bacterial parotitis, but in these latter cases, values are generally lower than those seen in acute pancreatitis. Lower serum Lipase values have been observed in abscesses of the liver, acute hepatocellular damage, cirrhosis, cancer of the liver and bile duct. (1)

Method History
Human pancreatic Lipase catalyzes a two-step reaction: 1, 2 diglyceride is hydrolyzed to 2-monoglyceride and fatty acids. The 2-monoglyceride is further hydrolyzed by monoglyceride Lipase (MGLP) to glycerol and fatty acids. Glycerol is then quantitated using a first order rate GPO-Trinder reaction. Absorbance changes are monitored at 550 nm. The reaction scheme illustrates the reactions that take place in this method.

$$\text{1,2 diglyceride} + H_2O \xrightarrow{\text{Lipase}} \text{2-monoglyceride} + \text{FFA}$$

$$\text{2-monoglyceride} + H_2O \xrightarrow{\text{MGLP}} \text{glycerol} + \text{FFA}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} \text{dihydroxyketone phosphate} + H_2O_2$$

$$H_2O_2 + \text{4-aminoantipyrine} + \text{TOOS} \longrightarrow \text{quinonedimine dye} + 4H_2O$$

Reagent Content
When reconstituted according to the directions, the concentrations of the active ingredients in the reagent will be approximately as follows:

Enzyme Substrate
Each liter contains:

1, 2 diglyceride	0.633g
Monoglyceride Lipase (microbial)	867 U
Glycerol kinase	1,333 U
Glycerol-3-phosphate oxidase (microbial)	40,000 U
TOOS	0.666g
ATP	0.400g
Peroxidase	1,333 U
Co-Lipase	40,000 U
Buffer	

Substrate Diluent
Each liter contains:

Cholic acid	2.166g
Buffer	

Activator Reagent
Each liter contains:

Deoxycholate	14.14 g
4-aminoantipyrine	1.20 g
Buffer	

Standard
Reconstitute each vial of standard/calibrator reagent with 3 ml of deionized water. Mix gently over a ten minute period. Use as directed.

Precautions
Handle the Catachem Lipase reagents following good clinical laboratory practice procedures. Avoid contact with skin and eyes. Should contact occur, wash affected area with plenty of cold water. **DO NOT PIPETTE REAGENTS BY MOUTH.**

Preparation of Working Reagents
Prepare the required number of vials of Catachem Lipase Working Enzyme Substrate Reagent by adding the contents of the 10 ml vial of Substrate Diluent to each Enzyme Substrate vial. Mix well and gently to prevent foaming, until completely in solution. Use the Activator Reagent as supplied. No preparation is required.

Reagent Storage and Stability
Store the Catachem Lipase reagents at 2-8˚C. When stored as directed, these reagents are stable until the expiration date stated on the label. Store the Lipase Working Reagent (liquid) at 2-8˚C. When prepared and stored as directed, the Working Reagent is stable for 21 days at 2˚-8 ˚C.

Specimen Collection and Preparation
Test sera should be fresh, clear, and unhemolyzed. When blood is drawn, it should be processed as soon as possible and the serum should be isolated from the clot without delay. If the assays are not run immediately, the serum samples should be stored at 2-8˚C or at -20˚C.

Quality Control
To monitor the performance of the Working Reagents and the procedure used, we recommend the regular use of normal and abnormal control serum.

Interfering Substances
Several substances have been reported to alter the Lipase activity in serum. (5, 6) Glycerol concentrations greater than 100 mg/dL will produce erroneous results. Specimens with glycerol concentrations greater than 100 mg/dL should be diluted with physiological saline and reassayed.

Expected Values
The range of expected values in humans for this method is 7 - 59 u/L. These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the species under assay and for the area in which it is located.

Procedure
Important: Read entire procedure instructions before proceeding with assay.

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Materials Required (Not Provided)

Spectrophotometer	
Cuvettes	1 cm light path
Timer	to time incubation time
Pipette	0.6 ml for reagent
Pipette	0.010 ml for sample

Materials Provided

Enzyme Substrate
Substrate Diluent
Activator Reagent
Standard:

Analytical Parameters

Wavelength	550 nm
Temperature	37˚C
Pathlength	1 cm
Reaction Mode	Rate
Reaction Time	5 minutes
Reaction Volume (R1)	0.600 ml
Reaction Volume (R2)	0.200 ml
Sample Volume	0.010 ml
Total Volume	0.810 ml
Sample-to-reagent ratio	1:81

Assay Procedure

1. Prepare the required volume of Lipase Enzyme Substrate Working Reagent by following instructions for Working Reagent preparation.
2. Set spectrophotometer wavelength at 550 nm and zero the instrument with the cuvette containing water.
3. Pipette 0.6 ml of Working Reagent into each of two cuvettes marked: "Sample" and "Control".
4. Pipette 0.010 ml of control or sample into their respective cuvettes. Mix all cuvettes well.
5. Incubate cuvettes for 3.0 minutes at 37˚C.
6. Pipette 0.200 ml of Activator Reagent into all cuvettes. Mix all cuvettes well and continuously monitor the change in absorbance for at least 3 minutes.
7. Read the "Control" and "Sample" absorbances.
8. Calculate the Lipase concentration (U/L) in the sample(s), as shown in calculations and results.

Calculations and Results

$$\text{Activity (U/L)} = \frac{\text{Sample (A2 - A1)}}{\text{Calibrator (A2 - A1)}} \times \text{Calibrator/standard (U/L)}$$

Example:	A1	A2
Sample	0.01	0.03
Calibrator	0.01	0.05

Calibrator = 200 U/L

$$\text{Lipase (U/L)} = \frac{0.03 - 0.01}{0.05 - 0.01} \times 200 \text{ U/L}$$

$$= 100 \text{ U/L}$$

Units

One international unit (U/L) is defined as the amount of enzyme that catalyzes the conversion of one micromole of substrate per minute under the defined conditions

Method Performance Characteristics

Sensitivity: The sensitivity of this method is 0.008-0.0120 OD/U/L.

Linear Range: In this method there is no significant nonlinearity over the range of 0-600 U/L.

Precision Study

Lipase	SAMPLE 1	SAMPLE 2
N	15	15
U/L	18.80	72.60
SD	2.10	2.10
CV %	0.92	0.92

Correlation

A comparison of this method using an automated analyzer was carried out against a UV kinetic method. The performance of Catachem's Lipase method was found essentially equivalent to the reference method used. Linear regression analysis produced the following information:

N	=	58
Y	=	1.844 x 7.15
r	=	0.982

References

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