Lipase

En

REF 08V5125

REF 08V5120

Alinity c

Diagnostic reagent for the determination of Lipase concentration.
Liquid. Dual reagent (Ratio: R1/R2: 4/1). Store at +2/+8°C. For In Vitro Diagnostic use. **Do not freeze.**
The products with 08V5125/08V5120 reference numbers provided are compatible for use with Abbott Alinity biochemistry autoanalyzer series.

Changes made in the instructions for use are marked as gray.

The instructions must be followed carefully. The reliability of the test results cannot be guaranteed unless these instructions are followed.

NAME
Lipase

INTENDED USE
Lipase assay is used for the quantitative determination of Lipase concentration in human serum and plasma on Alinity c analyzer.

SUMMARY AND EXPLANATION OF THE TEST
Human pancreatic lipase (EC 3.1.1.3; triacylglycerol acylhydrolase) is a single-chain form having 48 kDa molecular weight and approximately 5.8 isoelectric point. The lipase gene is located on chromosome 10. To show full catalytic activity and highest specificity, it requires bile salts and the presence of a cofactor called colipase, a small molecular weight protein of 10 kDa secreted by pancreatic acinar cells.[1] Human lipase can be fully activated by colipases from other species (e.g. porcine colipase) in vitro; this property is used in analytical formulations of the lipase assay.[2]

Lipases (including lipoprotein lipase) are defined as enzymes that hydrolyze glycerol esters of long-chain fatty acids. Only ester bonds at carbons 1 and 3 (α-positions) are attacked and the reaction products contain 2 mol of fatty acids and 1 mol of 2-acylglycerol (β-monoglyceride) per mole of substrate. β-monoglyceride is resistant to hydrolysis, probably due to steric hindrance, but can spontaneously isomerize to the α-form (3-acylglycerol). This isomerization allows the third fatty acid to be cleaved at a much slower rate.[1] The control of lipase secretion and associated factors appears to be driven by the contents of the gastrointestinal lumen, particularly by the presence of acid or digested proteins and fats in the duodenal lumen. Secretion of cholipase, bile acids and lipase is driven by the release of cholecystokinin and secretin.[3] Most of the lipase activity found in serum originates from pancreatic acinar cells, but some is secreted by the gastric and intestinal mucosa.

The concentration of lipase in the pancreas is about 5000 times higher than in other tissues, and the concentration difference between pancreas and serum is about 20,000 times.[1,4]

Lipase is a small enough molecule to be filtered through the glomeruli, but is completely reabsorbed by the renal tubules and is not physiologically detectable in urine. Evidence suggests that pancreatic lipase may exist in at least two isoforms, but the exact nature of these forms is unknown. Complete absence of lipase has been reported in the literature. This congenital absence results in fat malabsorption and severe steatorrhea. Lipase has much less tissue distribution than P-type amylase and therefore its elevation in serum is less associated with non-pancreatic disease states.[1]

Serum lipase measurement is the recommended laboratory test for the diagnosis of acute pancreatitis. Clinical sensitivity ranges from 80 to 100% depending on the diagnostic predictive value chosen, while clinical specificity ranges from 85 to 100% depending on the patient population studied. After an episode of acute pancreatitis, serum lipase activity increases within 4-8 hours, peaks at approximately 24 hours and decreases within 7-14 days. In this case, increases between 2 and 50 times the upper reference limit (URL) have been reported. However, the increase in serum lipase activity is not always proportional to the severity of the attack. In addition, in pediatric acute pancreatitis, a serum lipase activity greater than seven times the URL within 24 hours of the attack was associated with a 7.1-fold risk ratio (95% confidence interval, 2.5 to 20.5) for developing severe pancreatitis.[1,5]

The diagnosis of acute pancreatitis is sometimes difficult because it must be differentiated from other acute intra-abdominal diseases with similar clinical manifestations, such as acute cholecystitis, perforated gastric or duodenal ulcer, intestinal obstruction or ruptured abdominal aortic aneurysm. Because treatment of other conditions mimicking pancreatitis typically involves surgery and surgical intervention is generally contraindicated in pancreatitis, accurate diagnosis of pancreatitis is vital.[1]

Also for the diagnosis, in the absence of renal failure, an increase in serum lipase activity more than three times the URL is a more specific diagnostic finding than an increase in serum α-amylase activity.[6] In fact, the mean peak increase in lipase activity after acute pancreatitis is about four times greater than that of amylase. Finally, lipase concentrations remain elevated longer than those of α-amylase, which is another advantage over α-amylase measurement in patients with delayed diagnosis.[1] Therefore, it is recommended that lipase replace α-amylase as the first diagnostic test for acute pancreatitis in the emergency department.[7] However, international practice differs in this respect.[8]

Serum lipase activity may be increased in patients with significantly reduced glomerular filtration rate (GFR). Thus, great caution is required in the interpretation of increased serum lipase values in the presence of chronic kidney disease. In a rare case, inaccuracies in serum lipase estimation have also been shown to be due to the presence of lipase macroforms consisting of IgG-bound enzyme, poorly filtered and excreted by the kidneys due to its large size, which would lead to elevated values if enzyme activity in the blood were measured. In addition, examination of the biliary tract by endoscopic retrograde pancreatography or opiate use (as it causes contraction of the sphincter of Oddi) may increase serum lipase activity.[1]

PRINCIPLES OF THE PROCEDURE
The test has Enzymatic colorimetric measurement method. Cleavage of the synthetic substrate 1,2-O-dilauryl-rac-glycerol-3-glutaric acid-(6'methylresorufin) ester in alkaline solution by the catalytic action of pancreatic lipase yields 1,2-O-dilauryl-rac-glycerol and an unstable intermediate, glutaric acid-(6'methylresorufin) ester. Decomposition of this intermediate product by self- hydrolyzing in alkaline medium leads to the formation of glutaric acid and methylresorufin with chromogenic properties.The absorbance value of this red colored product at 580 nm wavelength is directly proportional to the lipase activity in the sample.

1,2 – O – dilauryl – rak – glycero – 3 - glutaric acid – (6 – methylresorufin) esther $\xrightarrow{\text{Lipase}}$ 1,2 – O – dilauryl – rak – glycerol + glutaric acid – (6 – methylresorufin) esther

Glutaric acid – (6 – methylresorufin) esther $\xrightarrow{\text{spontaneous decay}}$ glutaric acid + methylresorufin

REAGENTS

Kit Contents

R1 Tris buffer (40 mmol/L). Colipase (≥ 1 mg/L). Deoxycholate (≥ 1.8 mmol/L). Taurodeoxycholate (≥ 7.0 mmol/L).

R2 Tartarate buffer (15 mmol/L). Lipase substrate (≥ 0.70 mmol/L). Calcium ions (≥ 1 mmol/L).

Warnings and Precautions
IVD: For in Vitro Diagnostic use only.
Do not use expired reagents.
Reagents with two different lot numbers should not be interchanged.
For professional use.
Follow Good Laboratory Practice (GLP) guidelines.

CAUTION: Human source samples are processed with this product. All human source samples must be treated as potentially infectious materials and must be handled in accordance with OSHA standards.

Danger
EUH032 : Releases a very toxic gas if contacts with acid.
H317 : May cause allergic skin reaction.

Precaution
P280 :Use protective gloves / clothes / glasses / mask.

P264 :Wash your hands properly after using.
P272 :Contaminated work clothes should not be allowed to be used outside of the workplace.

Intervention
P302+P352 :Wash with plenty of water and soap if it contacts with skin.
P333+P313 :Seek medical help if it irritates your skin or develops rash.
P362+P364 :Remove contaminated clothes and wash properly before using.

Disposal
P501 :Dispose the vials and contents according to the local regulations.

Reagent Handling
- Reagent cartridges should be kept in an upright position for at least 1 hours after receipt and before use to eliminate any air bubbles that may have formed inside.
- If the cartridge is dropped, it should be kept upright for 1 hours before use to allow the air bubbles to dissipate.
- Foam and air bubbles may form in the reagents; this may prevent accurate detection of the reagent level inside the cartridge, leading to insufficient reagent aspiration and potentially affecting the analysis results.

Reagent Storage [9]

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened	+2/+8°C	Until expiration date	Store in upright position.
Onboard	System Temperature	30 days	

Indications of Reagent Deterioration
When a calibration error occurs or the control value falls outside the accepted range, the reagents may be considered to have deteriorated. In this case, the test results are not valid and the samples should be reanalyzed. Recalibration may be required.

Alternative Result Units
Use the unit conversion table provided below for alternative result units.

Unit	Conversion Factor	Result Unit
U/L	0.0167	µkat/L

SAMPLE COLLECTION AND PREPARATION FOR ANALYSIS

Sample Types
The recommended sample types for this test are listed below.

Sample Type	Collection Tube
Serum	Serum tubes
Plasma	Li-heparine sample collection tubes

Follow the instructions provided with your sample collection tubes for use and handling.

Sample Conditions
- To obtain reliable results, serum should be free of fibrin, blood cells, or foreign particles. In samples obtained from individuals receiving anticoagulant or thrombolytic therapy, fibrin residues may be observed due to incomplete clotting.
- To reduce the risk of contamination between samples, a new pipette or pipette tip should be used for each procedure.

Preparation for Analysis
- When preparing sample tubes, the manufacturer's instructions should be followed; separation by standing alone is not sufficient.
- Samples should be free of air bubbles. Before analysis, bubbles should be removed using a stick, and a separate stick should be used for each sample.
- If the samples contain fibrin, red blood cells, or other particulate matter, centrifuge them again before testing to ensure reliable results.
- If samples do not appear homogeneous, they should be mixed by low-speed vortexing or by inverting the tube 10 times.
- The sample should be placed in a centrifuge tube and centrifuged; particularly in samples containing a lipid layer, the lipid-containing portion should not be transferred, and only the supernatant should be transferred to a separate container or secondary tube for testing.

Sample Storage [21]

Temperature	Maximum Storage Time
+2/+8°C	7 days
+20/+25°C	7 days
-20°C	1 year

The information provided applies to serum and plasma samples. Repeated freeze–thaw cycles of samples should be avoided.

PROCEDURE

Materials Provided
Lipase Reagent Kit
Materials Required but Not Provided
- Lipase Calibrator (Lyophilized) and commercially available controls
- Saline solution (0.85%–0.90% NaCl) for sample dilution

Assay Procedure
- If primary or aliquot tubes are used, ensure that a sufficient amount of sample is available.
- To prevent evaporation of the specimen, make sure that the tube volume is adequate before each test.
- Minimum Sample Volume Requirements:
  - Sample volume for a single test: 1,5 µL
  - This volume does not include dead space or the additional aspiration volume.

Sample Dilution Procedures
This method shows measurement linearity up to 600 U/L. For samples with results above this value, the automatic dilution procedure of the autoanalyzer should be applied or manual dilution may be performed.

Automatic Dilution Protocol
The system dilutes the sample at a 1:10 ratio and automatically calculates the concentration by multiplying the result by the dilution factor.

Manual Dilution Procedures
Dilute the sample with a 0.85%–0.90% NaCl solution. Enter the dilution factor on the relevant screen of the device. If the user does not enter the dilution factor, the result must be manually multiplied by the correct factor before it is reported. If the result of the diluted sample is below 5 U/L, which is the lower limit of the measurement range, the result is not reported, and the procedure is repeated with an appropriate dilution.

Calibration
The use of a Lipase Calibrator is required for this test.

Lipase Calibrator Set (Lyophilized)
Ref.No: 06R56-03

Calibration stability is 15 days. Changes in the reagent lot number require recalibration. Calibration is necessary if quality control results are not within acceptable limits.

Quality Control Procedures
Commercially available control materials with values assigned by this method may be used.

At least two levels of control should be run every 24 hours. Each laboratory should establish its own quality control procedures. If more stringent control monitoring is required, refer to the quality control procedures established for your laboratory.

EXPECTED VALUES

Each laboratory should investigate the transferability of expected values for its own patient population and, if necessary, establish its own reference range.

Reference Range [10,11]
Adults [22] : ≤ 60 U/L

SPECIFIC PERFORMANCE CHARACTERISTICS

Precision

			Within-Run (Repeatability)[15,16]		Within-Laboratory (Total)*[15,16]	
Sample	n	Mean (U/L)	SD	%CV	SD	%CV
Level 1	80	50.08	0.71	1.42	0.82	1.63
Level 2	80	154.90	1.74	1.23	1.82	1.17

*: Includes within-run, between-run, and between-day variability.

Lower Measurement Limits

The lower measurement limits refer to the range between the lower limit of quantitation (LLoQ) and the upper limit of quantitation (ULoQ), within which the analyte concentration can be measured with the required medical and laboratory accuracy without dilution, concentration, or any pre-treatment.[12] The LoD and LoQ values have been verified using the CLSI EP17-A2:2012 protocol.[13]

	U/L
LoD**	2.6
LoQ***	5.0

**** :** The LoD represents the lowest concentration at which the analyte can be detected with 95% probability, based on ≥ 60 replicates of samples with low analyte levels.

*****:** When determining the LoQ value, a coefficient of variation (%CV) of ≤20% is used as the basis.

Measuring Interval

This method shows measurement linearity up to 5- 600 U/L. For samples with results above this value, the automatic dilution procedure should be applied. For additional information, please refer to the instrument's user manual. Linearity studies data have been verified by using CLSI EP06-A:2003 protocol.[16]

Interference

Endogenous interferant and analyte concentrations that have been used in the Lipase scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.[18,19]

The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from Lipase interference scanning test is appropriate, is determined as ±10%.[20]

In Lipase test results, no significant interaction has been observed in the determined endogenous interferant and analyte concentrations or between interferants and analyte.

Interferant-Concentration	Lipase Target (U/L)	n*	Observed Recovery %
Hemoglobin 1260 mg/dL	31	3	97
Bilirubin 9.47 mg/dL	33	3	94
Lipemia 570 mg/dL	26	3	100

*In calculating how many times the control and test samples (containing interferent), prepared as a serum pool, would be run repetitively, the within-run SD values previously determined for the relevant method and the total allowable error rate defined as the interference limit were used. In the calculations, a type 1 error (α error) rate of 5% and a type 2 error (β error) rate of 10% (90% power) were accepted.[19]

It should be noted that, in addition to endogenous interferents, various drugs and metabolites; anticoagulants (e.g., heparin, EDTA, citrate, oxalate) and preservatives (e.g., sodium fluoride, iodoacetate, hydrochloric acid); substances that may come into contact with samples during collection and handling (such as serum separation devices, sample collection containers and their contents, catheters, catheter flushing solutions, skin disinfectants, hand sanitizers and lotions, glassware detergents, powdered gloves, etc.); dietary substances known to affect certain tests (e.g., caffeine, beta-carotene, poppy seeds); or certain substances present in a sample that may cause interference due to foreign proteins (e.g., heterophilic antibodies), autoimmune responses (e.g., autoantibodies), or malignancy (e.g., paraproteins causing interference in phosphate tests and indirect ion-selective electrode methods) may lead to various interferences and erroneous evaluations for many different reasons.[19]

These performance characteristics were obtained using an autoanalyzer. Results may vary when different equipment or manual procedures are used.

Method Comparison

As a result of the statistical evaluation of method comparison data, the table was constructed according to the Passing–Bablok regression method as follows:

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	Units	n	Correlation Coefficient	Intercept	Slpoe	Concentration Range
Serum	U/L	100	0.9987	-0.828	1.002	5,2-573

REFERENCES

1. Rifai, N., Chiu, R. W., & Young, I., et al., (2023) Tietz Textbook of Laboratory Medicine (7th ed.), Chapter 32: Serum Enzymes, p.350-e36, Elsevier, St. Louis, Missouri 63043
2. Tietz NW, Shuey DF. Lipase in serum—the elusive enzyme: an overview. Clin Chem 1993;38:1000–10.
3. Brownlee IA, Forster DJ, Wilcox MD, Dettmar PW, Seal CJ, Pearson JP. Physiological parameters governing the action of pancreatic lipase. Nutr Res Rev 2010;23:146–54.
4. Panteghini M. Electrophoretic fractionation of pancreatic lipase. Clin Chem 1992;38:1712–6.
5. Coffey MJ, Nightingale S, Ooi CY. Serum lipase as an early predictor of severity in pediatric acute pancreatitis. J Pediatr Gastroenterol Nutr 2013;56:602–8.
6. Tenner S, Baillie J, DeWitt J, Vege SS; American College of Gastroenterology. American College of Gastroenterology guideline: management of acute pancreatitis. Am J Gastroenterol 2013;108:1400–15.
7. Lippi G, Panteghini M, Bernardini S, Bonfanti L, Carraro P, Casagranda I, et al. Laboratory testing in the emergency department: an Italian Society of Clinical Biochemistry and Clinical Molecular Biology (SIBioC) and Academy of Emergency Medicine and Care (AcEMC) consensus report. Clin Chem Lab Med 2018;56:1655–9.
8. Gomez D, Addison A, De Rosa A, Brooks A, Cameron IC. Retrospective study of patients with acute pancreatitis: is serum amylase still required? BMJ Open 2012;2:e001471.
9. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline. CLSI Document EP25-A. Wayne, PA: CLSI; 2009.
10. Clinical and Laboratory Standards Institute (CLSI). Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – Third Edition. CLSI Document EP28-A3c. Wayne, PA: CLSI; 2010.
11. Clinical and Laboratory Standards Institute (CLSI). Verification of Reference Intervals in the Medical Laboratory Implementation Guide – Third Edition. CLSI Document EP28 ED3IG. Wayne, PA: CLSI; 2022.
12. Clinical and Laboratory Standards Institute (CLSI). Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking – 1st Edition. CLSI Document EP34. Wayne, PA: CLSI; 2018
13. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition. CLSI Document EP17-A2. Wayne, PA: CLSI; 2012.
14. Clinical and Laboratory Standards Institute (CLSI). Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach - 1st Edition. CLSI Document EP06-A. Wayne, PA: CLSI; 2003.
15. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition. CLSI Document EP05-A3. Wayne, PA: CLSI; 2014.
16. Clinical and Laboratory Standards Institute (CLSI). Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Second Edition. CLSI Document EP05-A2. Wayne, PA: CLSI; 2004.
17. Bablok W et al. A General Regression Procedure for Method Transformation. J Clin Chem Clin Biochem 1988; 26:783-790.
18. Clinical and Laboratory Standards Institute (CLSI). Supplemental Tables for Interference Testing in Clinical Chemistry - First Edition. CLSI Document EP37. Wayne, PA: CLSI; 2018.
19. Beurskens DMH, Huckriede JP, Schrijver R, et al. The anticoagulant and nonanticoagulant properties of heparin. Thromb Haemost 2020;120(10):1371-1383. doi:10.1055/s-0040-1715460
20. Connor WE, Armstrong ML. Plasma lipoprotein lipase after subcutaneous heparin. Circulation 1961;24:87-93. doi: 10.1161/01.cir.24.1.87

21. Guder W, Fonseca-Wollheim W, Heil O, et al. Maximum permissible transport and storage times for analysis of blood (serum, plasma),
22. urine and cerebrospinal fluid. DG Klinische Chemische Mitteilungen 1995; 26:207-224.
23. Junge W, Abicht K, Goldmann J, et al. Evaluation of the Colorimetric Liquid Assay for Pancreatic Lipase on Hitachi Analyzers in 7 Clinical Centers in Europe, Japan and USA. Clin Chem Lab Med 1999;37(Special Suppl):469.

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SYMBOLS

IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
R1	Reagent 1
R2	Reagent 2
GTIN	Global Trade Item Number
REF	Reference Number
GLP	Good Laboratory Practice
FOR USE WITH	Identifies Products to Be Used Together
PRODUCT OF TURKEY	Product of Turkey
	Manufacturer
	Expiration Date
	Temperature Limits
	Consult Instructions for Use
	Caution
	Number of Tests