

EN

Liquid N-geneous™ Lipase Reagent

INTENDED USE

For the quantitative measurement of lipase activity in serum or plasma.

SUMMARY

Lipase is a glycoprotein of pancreatic origin which plays a pivotal role in the digestion of lipids.¹ Elevated serum lipase levels are closely associated with pancreatic diseases. Acute pancreatitis is one disease state that must be considered in the differential diagnosis of acute abdominal pain. Laboratory tests can support the clinical impression of pancreatitis, including serum lipase, which can reach profoundly elevated levels in acute disease presentations.²

The Liquid N-geneous™ Lipase test uses 1, 2-O-dilauryl-rac-glycero-3-glutaric acid- (6'-methylresorufin)-ester as a substrate and is an adaptation of the colorimetric method developed by Neumann.³

The fully liquid nature of this reagent makes it both convenient and compatible for use on a wide variety of automated clinical chemistry analyzers.

PRINCIPLE

Serum lipase hydrolyzes the substrate 1,2-O-dilauryl-rac-glycero-3-glutaric acid- (6'-methylresorufin)-ester to liberate glutaric acid-6'-methylresorufin, which in turn is reduced to glutaric acid and methylresorufin. The rate of formation of methylresorufin is measured spectrophotometrically at an absorbance of 570 nm, and at 37°C. The rate of color change is proportional to the lipase activity in the sample.

Reaction Sequence

1,2-O-dilauryl-rac-glycero-3-glutaric acid (6'-methylresorufin)-ester

Pancreatic
Lipase →

1,2-O-dilauryl-rac-glycerol + glutaric acid-6'-methylresorufin)-ester (not stable)

H₂O →

glutaric acid + methylresorufin

REAGENTS

Composition

Component	Ingredients	Concentration
Reagent 1	Buffer	1.41%
	Sodium deoxycholate	0.09%
	Sodium azide	0.00081%
	Detergent	
Reagent 2	Calcium acetate	
	Buffer	23.2 U/mL
	Colipase	0.03%
	Methylresorufin-ester	
	Detergent	10%
	Propanol	1.00%
Stabilizer		

Precautions and Warnings

- For In Vitro Diagnostic Use.
- Do not use the reagents beyond the expiration date printed on the label.
- Warning:** All specimens used in the test should be considered potentially infectious. Universal precautions as they apply to your facility should be used for handling and disposal of materials during and after testing.⁴
- Liquid N-geneous™ Lipase Reagents must be used with the Liquid N-geneous™ Lipase Calibrator.
- Caution:** Do not store below 2°C.
- Caution:** Protect reagents from light.
- Caution:** Reagent 1 contains 0.09% sodium azide as an antimicrobial agent. Sodium azide may react with lead and copper

plumbing to form potentially explosive metal azide buildup. Flush with copious amounts of water when discarding material.

- Reagent 2 contains propanol. In the European Union this is classified as Irritant with the following Risk and Safety Phrases.

R41	Risk of serious damage to eyes.
S23	Do not breathe vapor.
S24/25	Avoid contact with skin and eyes.
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36/37/39	Wear suitable protective clothing, gloves and eye/face protection.

Preparation

Reagent 1: Liquid, ready to use.

Reagent 2: Liquid, ready to use.

Storage and Stability

Unopened reagent is stable until the expiration date shown on the label when stored at 2 - 8°C.

Once opened, the reagent is stable up to 60 days at 2 - 8°C or 7 days at 25°C.

DO NOT FREEZE.

PROTECT FROM LIGHT.

Onboard stability

Reagents are stable open on the Roche/Hitachi 912 analyzer for 60 days at 10 to 15°C.

Indications of Deterioration

Presence of any turbidity in R1 or excessive turbidity in R2 may indicate reagent deterioration or microbial growth.

Inability to recover control values.

SPECIMEN COLLECTION AND PREPARATION

Serum and lithium or sodium heparinized plasma are the recommended collection media. Use standard sample collection and preparation methods.⁵

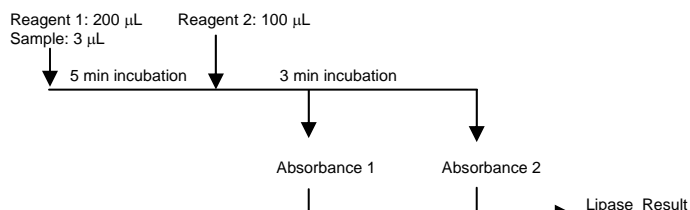
If not analyzed promptly, serum or plasma specimens may be stored at 2-8°C for 28 days, or at 20 - 25°C for 7 days. If specimens need to be stored for more than 7 days, they may be preserved at -20°C or below for up to 3 months.

Samples may be frozen and thawed twice.

PROCEDURE

Assay

Below is a general example of the Liquid N-geneous™ Lipase assay procedure for an automated analyzer. All analyzer applications should be validated.



For assistance with applications on automated analyzers inside the U.S., please contact Genzyme Diagnostics Technical Marketing at (800) 332-1042. Outside the U.S., please contact your local distributor.

Materials Provided

Liquid N-geneous™ Lipase Reagents 1 and 2 are required for the measurement of lipase. The Liquid N-geneous™ Lipase reagents are packaged and sold separately. Either of the following items may be included in the package you receive.

Description	Configuration	Catalog Number
Reagent 1	5 x 26 mL	80-6687-00
Reagent 2	5 x 13 mL	80-6688-00

Materials Required but not Provided

Description	Configuration	Catalog Number
Liquid N-geneous™ Lipase Calibrator	3 x 3 mL	80-6691-00

- Quality Control materials.
- Analyzer capable of running two-reagent chemistries.

Calibration

Only the Liquid N-geneous™ Lipase Calibrator should be used to calibrate the Liquid N-geneous™ Lipase assay.

Refer to the instrument operator's manual for analyzer specific calibration procedures and for guidance in determining calibration frequency.

Quality Control values should be within the expected ranges.

Quality Control

Reliability of test results should be monitored routinely with quality control materials or serum pools that reasonably represent performance with patient specimens. Controls or serum pools should be used to monitor that the reagents are functioning properly and that correct procedures are being followed. An acceptable range for each lot of control material should be established by the laboratory. If control values are not within the expected range, follow normal troubleshooting procedures. If assistance in the U.S. is required, please call Genzyme Technical Marketing (800) 332-1042. Outside the U.S., please contact your local distributor.

Quality control requirements should be established in accordance with local, state and/or federal regulations, or accreditation requirements.

RESULTS

Results are reported in U/L. One unit is defined as the amount of enzyme that liberates 1 μmole of methylresorufin per minute at 37°C.

Limitations/Interfering Substances

All interference studies were conducted according to NCCLS guideline EP7.⁶

Hemoglobin concentration of up to 500 mg/dL did not interfere (bias < 10%) in samples with lipase activity of 47 U/L. Hemoglobin concentration greater than 200 mg/dL showed a negative bias of up to 31% at lipase activity of 120 U/L.

Intralipid® concentrations of greater than 1.2% (3,600 mg/dL triglyceride equivalent) showed a positive bias of greater than 10% at a lipase activity of 47 U/L.

Refer to the work of Young et al.,⁷ for a review of the effects of drugs on clinical laboratory tests.

If triglyceride or cholesterol assays were run previously, ensure probes and cuvettes or tubes are thoroughly washed to avoid contamination by lipase or cholesterol esterase.

Expected Values

Samples from 72 male and 78 female apparently healthy adults, ranging in age from 20 to 70 were tested with the Liquid N-geneous™ Lipase assay.⁸ The reference interval defined at the 2.5 and 97.5 percentiles was observed to be 11.7 to 48.5 U/L.

Each laboratory should confirm the reference interval for the patient population it serves.

SPECIFIC PERFORMANCE CHARACTERISTICS

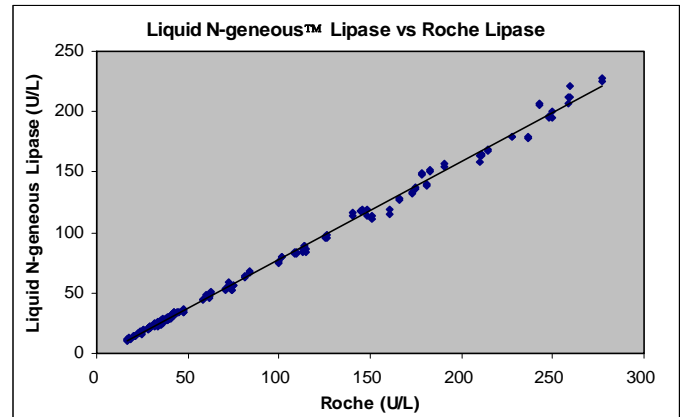
Accuracy

Comparative performance studies were conducted using the Liquid N-geneous™ Lipase Reagent on the Roche/Hitachi 912 clinical analyzer and both the Roche Diagnostics Lipase assay and Genzyme Diagnostics Lipase Color (LCK) assay (Enzymatic/Colorimetric) methods.

For Liquid N-geneous™ Lipase vs. Roche, 91 serum samples, with lipase concentrations between 16.6 and 276.8 U/L were tested over 2 days. The protocol followed the recommendations of NCCLS EP9.⁹

The regression analysis is provided below:

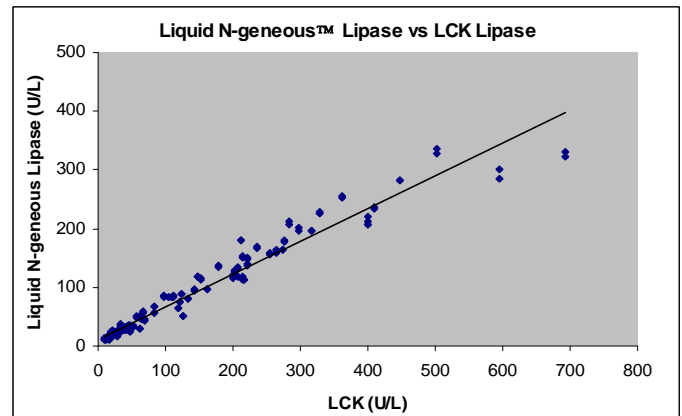
Liquid N-geneous™ Lipase vs. Roche (n = 91)	
Slope	0.808
Intercept (U/L)	-2.69
Correlation Coefficient (r)	0.998



For Liquid N-geneous™ Lipase vs. LCK, 97 serum samples, with lipase concentrations between 9.2 and 691.9 U/L were tested over 2 days. The protocol followed the recommendations of NCCLS EP9.⁹

The regression analysis is provided below:

Liquid N-geneous™ Lipase vs. LCK (n = 97)	
Slope	0.558
Intercept (U/L)	11.48
Correlation Coefficient (r)	0.978



Precision

Precision of the Liquid N-geneous™ Lipase Reagent was determined by running samples in duplicate, twice per day for 20 days on the Roche/Hitachi 912 analyzer across 20 calibrations and 4 reagent lots, using 3 levels of fresh pooled human serum supplemented with human pancreatic lipase following NCCLS EP5¹⁰. The following data are from a representative lot.

Within-Run Precision

Serum pool	Mean Recovery (U/L)	Standard Deviation (U/L)	CV
Level 1	24	0.29	1.2%
Level 2	56	0.59	1.0%
Level 3	129	0.82	0.6%

Total Precision

Serum pool	Mean Recovery (U/L)	Standard Deviation (U/L)	CV
Level 1	24	0.56	2.3%
Level 2	56	1.16	2.1%
Level 3	129	2.47	1.9%

Limit of Blank

The limit of blank is the concentration that is not statistically distinguishable from zero. Saline was run twenty times with Liquid N-geneous™ Lipase Reagent on the Roche/Hitachi 912 clinical analyzer and the mean plus two standard deviations of a sample that contained 0 U/L of lipase was used to define the detection limit: 0.3 U/L.

Specificity

The following substances, at the concentrations shown, did not affect the performance (bias <10%) of the Liquid N-geneous™ Lipase assay in a serum pool with approximately 47 U/L lipase.⁷

<u>Substance</u>	<u>Concentration Tested</u>
Bilirubin, Conj. & Unconj.	60 mg/dL
Hemoglobin	500 mg/dL
Ascorbic acid	125 mg/dL
Intralipid®	1.2% (3,600 mg/dL trig)
Glycerol	600 mg/dL
Acetaminophen (paracetamol)	20 mg/dL
Acetylsalicylic acid	50 mg/dL
Ampicillin	5 mg/dL
Caffeine	10 mg/dL
Captopril	6 mg/dL
Chlorpheniramine maleate	0.8 mg/dL
Cimetidine	10 mg/dL
Cyclosporin U	0.8 mg/dL
Doxycycline hyclate	6 mg/dL
Furosemide	2 mg/dL
Ibuprofen	40 mg/dL
Indomethacin	1 mg/dL
Levodopa	160 mg/dL
Lovastatin	1.6 mg/dL
Methotrexate	450 mg/dL
Methylidopa	2.5 mg/dL
Metoprolol tartrate	0.3 mg/dL
Metronidazole	1 mg/dL
Nicotinic acid	2 mg/dL
Omeprazole	7.2 mg/dL
Prednisone	1.2 mg/dL
Promethazine hydrochloride	1 mg/dL
Propranolol hydrochloride	0.5 mg/dL
Quinidine sulphate	5 mg/dL
Simvastatin	0.8 mg/dL
Theophylline	25 mg/dL
Tolbutamide	100 mg/dL

Linearity

Using NCCLS protocol EP6¹¹, the Liquid N-geneous™ Lipase method is linear from 0.3 U/L to 400 U/L. The samples were admixtures of low and high serum pools.

Specimens above 400 U/L may be diluted with physiological saline. Samples may be diluted twofold. Multiply the result by the dilution factor to obtain the lipase concentration for the sample.


References











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6. National Committee for Clinical Laboratory Standards. Interference Testing in Clinical Chemistry: Approved Guideline. NCCLS document EP7-A. Villanova, PA: 2002.
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9. National Committee for Clinical Laboratory Standards. Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline. NCCLS document EP9-A. Villanova, PA: 2002.
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Definitions for Symbols

 This product fulfills the requirements of the European Directive for In Vitro Diagnostic Medical Devices.

 Catalog number	 For in vitro diagnostic use
 Temperature limitation	 Manufactured by
 Use by	 Batch code
 Consult instructions for use	 Caution, consult accompanying document
 Irritant	 Authorized Representative in the European Community

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