

LIPASE COLOR

INTENDED USE

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

SUMMARY

Pancreatic lipase in serum or plasma is closely associated with pancreatic diseases. The activity of this enzyme is measured as an important marker for diagnosing pancreatic diseases and the associated monitoring of therapeutic effects. Pancreatic lipase measurement has been reported using titrimetric, turbidimetric, fluorometric, and colorimetric methodologies.¹⁻⁶

The Pancreatic Lipase Color test is a colorimetric, kinetic assay that uses a clear substrate solution of 1,2-diglyceride that is a "natural" substrate. The assay is highly sensitive and specific for pancreatic lipase, using colipase and deoxycholate as activators.⁵

The assay shows excellent reproducibility and stability. Furthermore, the simplicity of this procedure makes it readily adaptable for use on automated analyzers.

PRINCIPLE

Serum lipase acts on a natural substrate, 1,2-diglyceride, to liberate 2-monoglyceride. This is hydrolyzed by monoglyceride lipase (a highly specific enzyme for monoglyceride) into glycerol and free fatty acid. Glycerol kinase acts on glycerol to form glycerol-3-phosphate, which is in turn acted on by glycerol-3-phosphate oxidase to generate hydrogen peroxide. Peroxidase converts the hydrogen peroxide, 4-Aminoantipyrine and TOOS (N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine) into a quinone dye. The rate of formation of the dye, measured as an increase in absorbance at 550 nm, is proportional to the lipase concentration in the sample.⁵

REAGENTS

Composition

Component	Ingredients	Concentration
Lipase Color Reagent	1,2-Diglyceride (Egg)	1.1 mM
	Monoglyceride Lipase (Bacillus sp.)	0.88 U/mL
	Glycerol Kinase (S. canus)	<1.34 U/mL
	Glycerol-3-Phosphate Oxidase (Streptococcus sp.)	<40 U/mL
	TOOS	0.07%
	ATP (Bacterial)	0.66 mM
	Peroxidase (Horseradish)	<1.34 U/mL
	Colipase (Porcine)	<40 U/mL
	Buffer	pH 6.8
	Human Serum Albumin (Human)	0.27%
	Ascorbate Oxidase (Cucumber, Zucchini)	<2.66 U/L
	Stabilizers	

Composition (continued)

Component	Ingredients	Concentration
Lipase Color Solvent	Cholic Acid (Ox or Sheep)	5.3 mM
	Buffer	pH 6.8
	Sodium Azide	0.05%
Lipase Color Activator	Deoxycholate (Ox or Sheep)	36 mM
	4-Aminoantipyrine	0.12%
	Buffer	pH 8.7
	Sodium Azide	0.05%

The Lipase Color Calibrator contains human pancreatic lipase, serum albumin (bovine), and preservative.

Precautions and Warnings

1. For *In Vitro* Diagnostic Use.
2. Do not pipette by mouth.
3. Do not use the calibrator after the expiration date printed on the label.
4. **Warning:** Human source material. Treat as potentially infectious. Each donor unit used in the preparation of this product has been tested by an FDA approved method and found non-reactive for HBsAg, HCV, HIV 1/2 and HIV-1 antigen. Because no known test method can offer complete assurance that Hepatitis B virus, Human Immunodeficiency Virus (HIV) or other infectious agents are absent, all human-based products should be handled in accordance with good laboratory practices using appropriate precautions.⁷
5. The enzymes in triglyceride and cholesterol reagents may contaminate the Lipase Color Assay. To avoid contamination, ensure probes, cuvettes or tubes of automated analyzers are thoroughly washed between triglyceride or cholesterol assays and use of the Lipase Color Assay.
6. **Caution:** Contains sodium azide, which may react with lead and copper plumbing to form potentially explosive metal azides. On disposal, flush drain with a large volume of water to prevent buildup. Dispose of in accordance with local, state, and federal regulations.

Preparation

1. Lipase Color Reagent: Reconstitute the Reagent vial with the 30 mL Solvent, using a Class A volumetric pipette. Allow to stand a minimum of ten minutes at room temperature. Mix gently by inversion before use.
2. Lipase Color Solvent: Supplied ready to use.
3. Lipase Color Activator: Supplied ready to use.
4. Lipase Calibrator: Reconstitute the calibrator vial with 3.0 mL of distilled, deionized, Type II water or equivalent using a Class A volumetric pipette⁸. Replace the cap/stopper and mix gently by inversion. Allow to stand for a minimum of 10 minutes at room temperature. Mix gently by inversion before use.

DO NOT SHAKE

Storage and Stability

Unopened Lipase Color Reagent, Solvent, Activator and Calibrator are stable until the expiration date shown on the label when stored at 2-8°C.

DO NOT FREEZE

Once reconstituted, the Lipase Color Reagent is stable for 28 days at 2-8°C.

DO NOT FREEZE

Once opened the Lipase Color Solvent and Activator are stable until the expiration date on the label when stored capped at 2-8°C.

DO NOT FREEZE

Once reconstituted, the Lipase Calibrator is stable up to 28 days at 2-8°C. Reconstituted stability of the calibrator may be extended by aliquotting and freezing the reconstituted calibrator at -20°C for at least 4 months.

FREEZE ONLY ONCE

Indications of Deterioration

Presence of extreme turbidity.

SPECIMEN COLLECTION AND PREPARATION

Serum, EDTA-treated plasma or lithium and sodium heparinized plasma are the recommended sample types. Specimens should be collected as per the National Committee for Clinical Laboratory Standards Guideline H4-A3.⁸

Serum: Collect whole blood by venipuncture and allow to clot. Centrifuge and remove the serum as soon as possible after collection.⁹ (Within 3 hours)

Plasma: Specimens may be collected in EDTA or lithium and sodium heparin. Centrifuge and remove the plasma as soon as possible after collection.⁹ (Within 3 hours)

If the assay is not performed immediately, the serum and plasma must be refrigerated or frozen until use. If not analyzed promptly, specimens may be stored at 2-8°C for up to 3 weeks. If specimens need to be stored for more than 3 weeks, they may be preserved at -20°C or below for up to 3 months. Samples may be frozen once. Refer to NCCLS Document H18-A for further instructions on specimen collection, handling and storage.

PROCEDURE

Assay

All analyzer applications should be validated in accordance with CLIA recommendations. For assistance with applications on automated analyzers, please contact Genzyme Technical Service in the U.S. at (800) 332-1042.

Materials Provided

The Lipase Color Reagent, Solvent, Activator and Calibrator are required for the measurement of Lipase. The Lipase Color reagents are packaged and sold together. A configuration of each of the following items will be included in the package you receive.

Description	Configuration	Catalog Number
Lipase Color	Reagent 5 x 30mL Solvent 1 x 200mL Activator 1 x 60mL Calibrator 2 x 3mL	905-B
Lipase Color	Reagent 100 x 30mL Solvent 3 x 1 L Activator 1 x 1 L Calibrator 6 x 3 mL	905-C
Lipase Color	Reagent 100 x 30mL Solvent 3 x 1 L Activator 2 x 1 L Calibrator 20 x 3 mL	905-D
Lipase Color	Reagent 33 x 30mL Solvent 1 x 1 L Activator 1 x 1 L Calibrator 6 x 3 mL	905-E

Materials Required but not Provided

1. Spectrophotometer or other instrument capable of reading at 550 nm.
2. Temperature controller or water bath.
3. Class A volumetric pipettes, tubes and timer.
4. Distilled or deionized water meeting specifications equivalent to USP purified water.
5. Lipase control sera or quality control material (See "Quality Control").

Calibration

The Lipase Color Calibrator is required for calibration. Refer to the instrument manual for analyzer specific calibrator procedures and for guidance in determining calibration frequency. The calibrator value can be found on the calibrator vial label.

Quality Control

Reliability of test results should be routinely monitored with quality control materials or serum pools that reasonably represent performance on patient specimens. Controls or serum pools should be run with each assay to ensure that the reagents are functioning properly and that correct procedures have been followed. Quality control materials are intended for use only as monitors of accuracy and precision. An acceptable range for each lot of control material should be established by the laboratory. If control values are not within the expected range confirm procedures were performed correctly and follow normal troubleshooting measures. If the problem persists call Genzyme Technical Marketing in the U.S. at (800) 332-1042.

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

RESULTS

1. Unit Definition: One unit (U) is defined as the amount of enzyme activity which liberates 1 μ mole of 2-monoglyceride from 1,2-diglyceride per minute at 37°C. Lipase activity is expressed in U/L.
2. Patient results may be reported in U/L. To convert from conventional units to S.I. units, multiply the conventional units by 1.65×10^{-8} Katal/U.⁵ U/L x (1.6×10^{-8} Katal/U) = Katal/L Lipase.

3. Calculation

$$\text{Lipase Activity (U/L)} = \frac{\Delta A/\text{min}_c - \Delta A/\text{min}_b}{\Delta A/\text{min}_a - \Delta A/\text{min}_b} \times \text{U/L Calib.}$$

Where:

- $\Delta A/\text{min}_a$ = Rate of change per minute for the sample
- $\Delta A/\text{min}_b$ = Rate of change per minute for the blank
- $\Delta A/\text{min}_c$ = Rate of change per minute for Lipase Color Calibrator
- U/L Calib. = Stated activity of the Lipase Color Calibrator (on vial label)

Limitations/Interfering Substances

No significant interference was detected in the Lipase Color assay up to and including the concentrations stated below:

Substance Tested	Concentration with no significant ($\pm 10\%$) interference
Unconj. Bilirubin	20 mg/dL
Conj. Bilirubin	25 mg/dL
Hemoglobin	2000 mg/dL
Triglyceride	1000 mg/dL
Liposyn	1%
Glycerol	250 mg/dL
Ascorbic Acid	50 mg/dL

Samples containing interfering substances greater than the levels listed exhibited bias $>10\%$. Dilutions are not recommended to reduce interferences.

- Refer to the work of Young et al¹⁰ for a review of the effects of drugs on clinical laboratory tests.
- The enzymes in triglyceride and cholesterol reagents may contaminate the Lipase Color Assay. To avoid contamination, ensure probes, cuvettes or tubes of automated analyzers are thoroughly washed between triglyceride or cholesterol assays and use of the Lipase Color Assay.

Expected Values

A normal range study was performed using the Lipase Color assay. A serum range of 21-67 U/L (138 healthy donors) was obtained on the Roche/Hitachi 911 system. Ranges were calculated as recommended by NCCLS guideline C28-A.¹¹ These results were obtained using a specific lot of reagent. It is recommended that each laboratory establish the normal range for its patient population.

SPECIFIC PERFORMANCE CHARACTERISTICS

Accuracy

Accuracy of the Lipase Color method, performed using the Roche/Hitachi 911 Analyzer was verified by comparison to the Dade Lipase method, performed on a Dade Dimension analyzer, producing the following results:

Method	Genzyme	Dade Lipase Assay
Lipase Assay	N	40
Mean (U/L)	54	260
Regression Analysis	$y=0.44(x)-62.07$	
Correlation Coefficient	$r=0.97$	
Reference Range	21-67 U/L	114-286 U/L

Precision

Within-run precision of the Lipase Color assay was determined using three frozen spiked human lipase pools. Each run consisted of 20 replicate samples. Within-run precision studies produced the following results on the Roche/Hitachi 911 Analyzer.

Serum Pool	Low	Mid	High
N	20	20	20
Mean (U/L)	33	118	269
S.D. (U/L)	0.8	1.5	2.1
CV (%)	2.4	1.2	0.8

Between run precision of the Lipase Color assay was determined using three frozen spiked human lipase pools tested in duplicate, once or twice per day, for 15 days.

Serum Pool	Low	Mid	High
N	20	20	20
Mean (U/L)	34	120	275
S.D. (U/L)	1.5	2.7	6.3
CV (%)	4.4	2.3	2.3

Limit of Detection

Limit of detection of the Lipase Color assay, quantified as 2 S.D. plus the mean of twenty replicate measurements of saline, is 2 U/L on a Roche/Hitachi 911 analyzer.

Linearity

The Lipase Color method is linear to 750 U/L lipase on the Roche/Hitachi 911 analyzer. If the result is greater than 750 U/L, dilute with saline, multiply the result by the dilution factor to obtain the lipase activity of the sample.

REFERENCES

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10. Young DS, Effects of Drugs on Clinical Laboratory Tests, 4th Edition, AACC Press, Washington, DC; 3-398 to 3-400 (1995).
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Definitions for Symbols



Catalog number



For *in vitro* diagnostic use



Temperature limitation



Manufactured by



Use by



Batch code



Consult instructions for use



Caution, consult accompanying document

Manufactured by:

genzyme
Diagnostics

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