

## Lipase Color

### For *in vitro* diagnostic use

#### INTENDED USE

For the quantitative determination of pancreatic lipase in serum or plasma.

#### SUMMARY

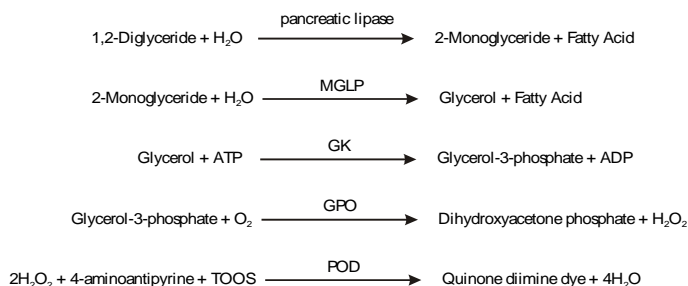
Pancreatic lipase in serum is closely associated with pancreatic diseases. The activity of this enzyme is measured as an important marker for diagnosing pancreatic diseases and the associated monitoring of therapeutic effects. Pancreatic lipase measurement has been reported using titrimetric, turbidimetric,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

The Pancreatic Lipase Color Assay is a colorimetric, kinetic assay that uses a clear substrate solution of 1,2-diglyceride, which is a “natural” substrate. The assay is highly sensitive and specific for pancreatic lipase, using colipase and deoxycholate as activators.<sup>5</sup>

The assay shows excellent reproducibility and stability. Furthermore, the simplicity of this procedure makes it readily adaptable for use on automatic 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.<sup>5</sup>



MGLP: monoglyceride lipase  
 GK: glycerol kinase  
 GPO: glycerol-3-phosphate oxidase  
 POD: peroxidase  
 TOOS: N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine sodium salt, dihydrate

#### REAGENTS

##### Composition of Materials

Lipase Color Reagent	Source	Reconstituted Concentration
1,2-Diglyceride (PCDG)	Egg	1.1 mM
Monoglyceride Lipase (MGLP)	Bacillus sp.	0.88 U/mL
Glycerol Kinase (GK)	S. canus	<1.34 U/mL
Glycerol-3-Phosphate Oxidase (GPO)	Streptococcus sp.	<40.0 U/mL
TOOS		0.07%
ATP	Bacterial	0.66 mM
Peroxidase (POD)	Horseradish	<1.34 U/mL
Colipase	Porcine	<40.0 U/mL
Buffer		pH 6.8
Human Serum Albumin	Human	0.27%
Ascorbate Oxidase	Cucumber, zucchini, <u>Acremonium</u> sp.	<2.66U/L
Stabilizers		
<b>Lipase Color Solvent</b>		
Cholic Acid	Ox or Sheep	5.3 mM
Buffer		PH 6.8
Sodium Azide		0.05%
<b>Lipase Color Activator</b>		
Deoxycholate	Ox or Sheep	36.0 mM
4-Aminoantipyrine		0.12%
Buffer		PH 8.7
Sodium Azide		0.05%

#### Precautions and Warnings:

- For In Vitro Diagnostic Use.
- Do not pipette by mouth.
- Warning:** Human source material. Treat as potentially infectious. Each serum/plasma donor unit used in the preparation of this product has been tested by an FDA approved method and found non-reactive for the presence of HBsAg, HCV and antibody to HIV 1/2. 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<sup>7</sup>.

- 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.
- Warning:** 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.

**Reagent Preparation:**

- Lipase Color Reagent: Reconstitute each vial with the appropriate amount of Solvent.  
**10 mL Reagent:** Reconstitute the **10 mL** Reagent, by pouring the contents of the solvent, directly into the Reagent vial. Allow to stand a minimum of ten minutes at room temperature. Mix gently by inversion before use.  
**30 mL Reagent:** Reconstitute the **30 mL** Reagent with 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.
- Lipase Color Solvent: Supplied ready to use.
- Lipase Color Activator: Supplied ready to use.

**Storage and Stability**

- The unopened Lipase Color Reagent, Solvent and Activator are stable until the expiration date printed on the label when stored at 2-8°C.
- Once reconstituted, the Lipase Color Reagent solution is stable for 28 days when stored 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.

**Indications of Deterioration**

Presence of extreme turbidity.

**SPECIMEN COLLECTION AND PREPARATION**

Serum, EDTA-treated plasma or lithium and sodium heparinized plasma are the recommended specimens.

Serum: Collect whole blood by venipuncture and allow to clot. Centrifuge and remove the serum as soon as possible after collection.<sup>8</sup> (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.<sup>8</sup> (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**

**Manual Assay**

- Into appropriately labeled tubes, pipette 25 µL of deionized water (blank), Lipase Color Calibrator and serum samples.
- Add 1.5 mL of reconstituted Lipase Color Reagent Solution to each tube, mix and incubate for 3-5 minutes at 37°C.
- Add 0.5 mL of the Lipase Color Activator Solution to each tube, mix well and incubate for 3-5 minutes at 37°C.
- Read and record the rate of increase in absorbance at 550 nm for the blank, Lipase Color Calibrator and samples in one minute intervals until the rate of change is constant.

**Automated Assay**

All analyzer applications should be validated in accordance with CLIA recommendations. For assistance with applications on automated analyzers, please contact Technical Service at Equal Diagnostics Inc. at 1-800-999-6578.

**Materials Provided**

All Equal Lipase Color Reagents are packaged in kits.

**Kit Part Number 905-C**

Lipase Color Reagent	100 x 30 mL
Lipase Color Solvent	3 x 1 L
Lipase Color Activator	1 x 1 L
Lipase Color Calibrator	6 x 3 mL

**Kit Part Number 905-D**

Lipase Color Reagent	100 x 30 mL
Lipase Color Solvent	3 x 1 L
Lipase Color Activator	2 x 1 L
Lipase Color Calibrator	20 x 3 mL

**Kit Part Number 905-E**

Lipase Color Reagent	33 x 30 mL
Lipase Color Solvent	1 x 1 L
Lipase Color Activator	1 x 1 L
Lipase Color Calibrator	6 x 3 mL

**Kit Part Number 905-B**

Lipase Color Reagent	5 x 30 mL
Lipase Color Solvent	1 x 200 mL
Lipase Color Activator	1 x 60 mL
Lipase Color Calibrator	2 x 3 mL

### 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. Lipase control sera or quality control material (see "Quality Control").

### Calibration

The Lipase Color Calibrator is required for calibration. Refer to the Lipase Color Calibrator package insert for information. 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 Equal Diagnostics, Inc. Technical Service at 1-800-999-6578. 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  $\mu$ 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  $1.65 \times 10^{-8}$  Kata/U<sup>5</sup>.

$$U/L \times (1.6 \times 10^{-8} \text{ Kata/U}) = \text{Kata/L Lipase}$$

3. Calculation:

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

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  
Rate of change per minute for the Lipase  
 $\Delta A/\text{min}_c$  = Color Calibrator  
U/L Calib. = Stated activity of the Lipase Color Calibrator

### Limitations/Interfering Substances

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

Interfering Substance	Concentration (mg/dL)
Unconj. Bilirubin	20
Conj. Bilirubin	25
Hemoglobin	2000
Triglyceride	1000
Liposyn	1%
Glycerol	250
Ascorbic Acid	50

Samples containing interfering substances greater than the levels listed exhibited bias >10% when diluted with saline as compared to the control sample. Dilutions are not recommended to reduce interferences.

1. Refer to the work of Young, *et. al.*<sup>9</sup> for a review of the effects of drugs on clinical laboratory tests.
2. 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 Diagnostics/Boehringer Mannheim Corporation Hitachi 911 system. Ranges were calculated as recommended by NCCLS guideline C28-A<sup>10</sup>. 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

The accuracy of the Lipase Color method, performed using the Roche Diagnostics/Boehringer Mannheim Corporation Hitachi 911 Analyzer was verified by comparison to the Dade Lipase method, performed on a Dade Dimension analyzer, producing the following results:

Method	Lipase Color Assay	Dade Lipase Assay
N	40	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 Diagnostics/Boehringer Mannheim Corporation 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
C.V. (%)	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 of 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
C.V. (%)	4.4	2.3	2.3

#### Limit of Detection

Limit of detection of the Lipase Color assay, quantified as 2 SD plus the mean of twenty replicate measurements of saline, is 2 U/L on a Roche Diagnostics/Boehringer Mannheim Corporation Hitachi 911 Analyzer.

#### Linearity

The Lipase Color method is linear to 750 U/L lipase on the Roche Diagnostics/Boehringer Mannheim Corporation 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

1. Imamura S, Misaki, H. "A sensitive method for assay of lipase activity by coupling with  $\beta$ -oxidation enzymes of fatty acid." Selected Topics in Clinical Enzymology; 2:73 (1984).
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5. Imamura S, et al., Chin. Chem., Abstract Issue in the 41<sup>st</sup> National Meeting; 1120 (1989).
6. Tietz NW, "Clinical Guide to Laboratory Tests", 2nd ed., Philadelphia, PA: WB Saunders Co.; 364 (1990).
7. Centers for Disease Control/National Institutes of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories", 1988.
8. National Committee for Clinical Laboratory Standards, "Procedures for the Collection of Diagnostic Blood Specimens by Skin Puncture", Approved Standard, Third Edition, NCCLS publication H4-A3, Villanova, PA (1991).
9. Young D.S. Effects of Drugs on Clinical Laboratory Tests, 4th Edition, AACC Press, Washington, DC; 3-398 to 3-400 (1995).
10. National Committee for Clinical Laboratory Standards, "How to Define and Determine Reference Intervals in the Clinical Laboratory: Approved Guideline", NCCLS Document C28-7. Vol. 15, No. 4, June 1995.

#### Manufactured by:

**Genzyme Diagnostics**  
**One Kendall Square**  
**Cambridge, Massachusetts 02139, USA**  
**Phone: (800)999-6578**