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# Lipase (Colorimetric) Reagent Set

## Intended Use
For the *in vitro* quantitative kinetic determination of Pancreatic Lipase in serum.

## Method History
Serum lipase is recognized as an important indicator for the diagnosis, and therapeutic monitoring, of pancreatic diseases. There have been many methods developed for the assay of lipase activity: alkaline titration of fatty acid liberated from triglyceride gum Arabic emulsion, measurement of the decrease in turbidity of a triglyceride (olive oil) emulsion, and a colorimetric method using a synthetic substrate containing thiol ester of a short chain acid. However, each of these methods has specific deficiencies, including difficulty of the procedure, lack of specificity, lack of precision near the normal level, poor reproducibility, susceptibility to interferences, and poor adaptability to automated instrumentation.
This new colorimetric Lipase reagent uses a clear substrate solution of 1,2-diglyceride, a natural lipase substrate derived from egg lecithin. It is a highly specific method for pancreatic lipase, using co-lipase and deoxycholate as activators. [1,5] The colorimetric measurement of the rate of formation of the quinone dye from TOOS provides a highly sensitive reaction with excellent reproducibility and stability.
Finally, because of the simple two-part reagent system, the procedure is adaptable to many automated chemistry analyzers.

## Principle
Pancreatic Lipase
1,2-diglyceride + $H_2O$ ----------➢ 2-monoglyceride + fatty acid
MGLP
2-monoglyceride + $H_2O$ ----------➢ glycerol + fatty acid
GK
Glycerol + ATP ----------➢ glycerol-3-phosphate + ADP
GPO
Glycerol-3-phosphate + $O_2$ ----------➢ dihydroxyacetone phosphate + $H_2O_2$
POD
$2H_2O_2$ + 4-aminoantipyrine + TOOS ----------➢ quinone dye + $4H_2O_2$

Serum pancreatic lipase acts on a natural type of substrate, 1,2-diglyceride to liberate 2-monoglyceride. The 2-monoglyceride is hydrolyzed by monoglyceride lipase (MGLP) to produce glycerol and fatty acid. Glycerol kinase (GK) then acts on the glycerol to produce glycerol-3-phosphate which is converted to dihydroxyacetone phosphate and hydrogen peroxide in a reaction catayzed by glycerol-3-phosphate oxidase (GPO). The hydrogen peroxide then reacts with 4-aminoantipyrine and N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine sodium salt (TOOS) in a reaction catalyzed by peroxidase (POD) to yield a quinone dye. The rate of increase in absorbance at 550nm is directly proportional to the Lipase activity of the sample.

## Clinical Significance
Serum Lipase activity is found to be elevated in acute pancreatitis or obstruction of the pancreatic duct. The half-life of pancreatic lipase is longer than that of pancreatic amylase, making it a very reliable marker of pancreatic disease. Since this colorimetric Lipase is specific for pancreatic Lipase, it is ideally suited to run in conjunction with a total amylase reagent, which measures both pancreatic and salivary type amylase.

## Reagents
1. Lipase Substrate – 1,2-Diglyceride 63%, MGLP 87u/100ml, GK 133u/100ml, GPO 4,000u/100ml, Co-Lipase 4,000u/100ml, Buffer.
2. Lipase Substrate Buffer – Buffer, Cholic Acid 217mg%, pH = 6.8±0.1.
3. Lipase Activator-deoxycholate 1414mg%, 4-aminoantipyrine 120mg%, Buffer pH = 8.7±0.1.

## Precautions
1. Reagent is for *in vitro* diagnostic use only.
2. Avoid ingestion.

## Reagent Preparation
Reconstitute the Lipase Substrate vial with the amount of Lipase Substrate Buffer indicated on the vial label. Swirl to dissolve. The Lipase Activator solution is ready to use.

## Reagent Storage and Stability
Store reagent at 2-8°C.
Before reconstitution, all reagents are stable until the expiration date if stored at 2-8°C. Upon reconstitution, the Lipase Substrate solution is stable for four days at 25°C, and 21 days at 2-8°C. The Lipase standard is stable 30 days after reconstitution when stored at 2-8°C.

## Reagent Deterioration
Do not use if reagents show turbidity or other evidence of contamination or deterioration.

## Specimen Collection and Storage
1. Fasting, non-hemolyzed serum is the preferred specimen.
2. Separate the serum from the clot immediately after collection and measure the lipase activity promptly. If the assay is not performed immediately, the serum must be refrigerated or frozen until use. Never repeat freeze and thaw as the lipase can be inactivated.
3. Lipase is reported stable in serum for at least three days at 2-8°C.

## Interferences
1. Free glycerol will not interfere with the assay as long as concentrations are 100mg/dl or below. If the serum contains free glycerol above 100mg/dl, dilute with saline to make level of free glycerol below 100mg/dl before the assay.
2. Microbial lipase and cholesterol esterase can affect the assay.
3. For a listing of substances which may affect serum lipase levels, see Young et al.[6]

## Materials Provided
Lipase substrate and buffer reagents.
Lipase activator solution.
Lipase standard

## Materials Required but not Provided
1. Accurate pipetting devices.
2. Test tubes/rack
3. Timer
4. Heating bath or block (37°C).
5. Spectrophotometer with a temperature controlled cuvette.

## Test Procedure (Automated)
See appropriate instrument application instructions.

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## Test Procedure (Manual)

1. Label test tubes “Blank”, “Standard”, “Control”, “Patient”, etc.
2. Pipette 300 ul of reconstituted Lipase Substrate reagent to all tubes.
3. Pipette 5ul of distilled water to the blank tube and 5 ul of the appropriate sample to the tubes labeled “Standard”, “Control’, etc.
4. Mix each tube well and incubate for 3-5 minutes at 37°C.
5. After the pre-incubation, add 100 ul of Lipase activator to the blank tube. Mix well and incubate for 3 minutes at 37°C. Then measure the rate of increase in absorbance per minute at 550nm (540-560nm).
6. Repeat step 5 for all tubes.
7. See “Calculations” to obtain results.

## Procedure Notes

The above volumes may be multiplied by an appropriate factor if a larger total volume of reaction mixture is necessary for reading.

## Calibration

Use a pancreatic lipase standard.

## Quality Control

The integrity of the reaction should be monitored by use of normal and abnormal control sera with known lipase activity.

## Limitations

Samples with Lipase activity exceeding 600 U/L should be diluted with an appropriate amount of saline, re-assayed, and the final result multiplied by the appropriate dilution factor.

## Calculations

$$\frac{\Delta A \text{ Sample} - \Delta A \text{ Blank}}{\Delta A \text{ Standard} - \Delta A \text{ Blank}} \times \text{Conc. of Std. (u/l)} = \text{Lipase activity (U/L)}$$

## Expected Values

0-62 U/L

It is strongly recommended that each laboratory establish its own normal range.

## Performance

1. Linearity: 600 U/L
2. Correlation: A study performed comparing the Lipase (Colorimetric) method to turbidimetric Lipase procedure yielded a correlation coefficient of 0.956 with a regression equation of y=0.48x + 9.1.
3. Precision: The lipase activity of three samples was measured ten times each with the following results:

| Mean | S.D. | C.V.% |
|---|---|---|
| 46.7 | 1.70 | 3.64 |
| 254.0 | 1.70 | 1.47 |
| 516.5 | 4.65 | 0.90 |

## References


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5. Imamura, S., et al, Clin. Chem., Abstract Issue in the 41st National Meeting, 120 (1989).
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Rev. 5/03 P803-L7503-01