Lipase Colorimetric Reagent

INTENDED USE
FOR IN VITRO DIAGNOSTIC USE
Lipase Reagent is intended for the quantitative determination of Pancreatic Lipase in serum.

SUMMARY
Serum in lipase is an indicator for the diagnosis, and therapeutic monitoring, of pancreatic diseases. To interferences, and poor adaptability to automated instrumentation.

PRINCIPLE OF PROCEDURE
Pancreatic Lipase
1,2-diglyceride + H2O -------------> 2-monoglyceride + fatty acid
MGLP
2-monoglyceride + H2O -------------> glycerol + fatty acid
Glycerol Kinase (GK) then acts on the glycerol to produce glycerol-3-phosphatase which is converted to dihydroxyacetone phosphate and hydrogen peroxide in a reaction catalyzed by glycerol-3-phosphatase 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.

INTERFERING SUBSTANCES
Free glycerol at concentrations at less than 100mg/dl will not interfere with the assay. Microbial lipase and cholesterol esterase can affect the assay. For a listing of substrates which may affect serum lipase levels, see Young.

PROCEDURE
Materials provided
85580
| Lipase Substrate | 4 x 10 mL
| Lipase Buffer   | 2 x 21 mL
| Lipase Activator| 1 x 14 mL
| Lipase Standard | 1 x 3 mL

Materials required but not provided
1. Automated chemistry analyzer

TEST PROCEDURE
1. Pipette 300μl of reconstituted Lipase Substrate reagent to all tubes.
2. Pipette 5μl of distilled water to the blank tube and 5μl of the appropriate sample to the tubes labeled “Standard”, “Control”, etc.
3. Mix each tube well and incubate for 3-5 minutes at 37 °C.
4. After the pre-incubation, add 100μl of Lipase activator to the blank tube. Mix well and incubate for 3 minutes at 37 °C.
5. 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.

CALIBRATION
Use the lipase standard provided in the kit. This assay should be calibrated in accordance with the instrument manufacturer’s specifications. Calibration stability is dependent upon the instrument performance and the proper storage of the reagents. Re-calibration is recommended at anytime, should one of the following occur.
1. Change in the reagent lot number.
2. Preventative maintenance is performed on the analyzer.
3. A critical element of the analyzer is replaced.
4. Control material results have shifted or are out of range and the use of a new bottle of reagents. Re-calibration is recommended at anytime, should one of the following occur.
5. The assay has been calibrated.
6. Preventative maintenance is performed on the analyzer.

QUALITY CONTROL
Controls are recommended to monitor the performance of the assay, providing a constant screening of the instrument, reagents and techniques. It is recommended that each laboratory establish its own procedures for corrective action if calibration is not acceptable.

REAGENT PREPARATION
Reconstitute the Lipase Substrate with Lipase Substrate Buffer as indicated on the vial label. Swirl to dissolve.
Reconstitute the Lipase Standard with 3 ml of distilled water.
4. A critical element of the analyzer is replaced.

5. The individual laboratory requirements specify that quality control material is to be run.

It is recommended that each laboratory establish its own control schedule and procedures for corrective action if controls do not recover within the specified tolerances.

PROCEDURAL 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.

PERFORMANCE CHARACTERISTICS

Linearity
This assay is linear to 600 U/L.

Accuracy
A study performed comparing the Lipase (Colorimetric) methods to a turbidimetric Lipase procedure yielded a correlation coefficient of 0.956 with a regression equation of y = 0.48x + 9.1.

Precision
The lipase activity of three samples was measured ten times each with the following results:

<table>
<thead>
<tr>
<th>Mean</th>
<th>S.D.</th>
<th>C.V.%</th>
</tr>
</thead>
<tbody>
<tr>
<td>46.7</td>
<td>1.70</td>
<td>3.64</td>
</tr>
<tr>
<td>254.0</td>
<td>1.70</td>
<td>1.47</td>
</tr>
<tr>
<td>516.5</td>
<td>4.65</td>
<td>0.90</td>
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</tbody>
</table>

EXPECTED VALUE
0-62 U/L
It is strongly recommended that each laboratory establish its own normal range.

REFERENCES