LIPASE (Kinetic Colorimetric.)

INTENDED USE

Bioline Lipase is used for the quantitative determination of Lipase in human serum.

CLINICAL SIGNIFICANCE

Lipases are enzymes which hydrolyze glycerol esters of long fatty acids. The enzyme and its cofactor colipase are produced in the pancreas, lipase being also secreted in small amounts by the salivary glands as well as by gastric, pulmonary and intestinal mucosa. Bile acids and colipase form micellar complexes with the lipids and bind lipase on the substrate / water interface. Determination of lipase is used for investigation of pancreatic disorders. In acute pancreatitis the lipase concentrations rise to 2-50 fold the upper reference limit within 4-8 hours after the beginning of abdominal pain peaking at 24 hours and decrease within 8 to 14 days. Elevated lipase values can also be observed in chronic pancreatitis and obstruction of the pancreatic duct.

METHOD AND PRINCIPLE

Enzymatic color test A synthetically produced lipase substrate (1,2-o-dilaurylrac-glycero-3-glutaric acid-(6-methylresorufin) ester) is added to a micro-emulsion which is specifically split by lipase in the presence of colipase and bile acids. The combination of lipase and bile acids make this specific and reliable for pancreatic lipase without any reaction due to lipolytic enzymes or esterases. The reagent composition has been thoroughly optimized so there are no serum matrix effects. The generated methylresorufin-ester is spontaneously degraded to methylresorufin. The absorbance by this red dye is directly proportional to the lipase activity in the sample.

REAGENT COMPOSITION

Reagent 1: Goods Buffer pH 8.0 50 mmol/L Taurodesoxycholate 4.3 mmol/L Desoxycholate 8.0 mmol/L Calcium chloride 15 mmol/L Colipase 2.2 mg/L Detergent Preservative.

Reagent 2: Tartrate Buffer pH 4.0 7.5 mmol/L Taurodesoxycholate 17.2 mmol/L Color Substrate 0.65 mmol/L Coemulgator Stabilizer Preservative.

Calibrator - Lyophilized . Store at 2 - 8 ºC.

Check the calibrator concentration on the bottle label. After reconstitution, the calibrator is stable for 7 days at 2 - 8 ºC and 30 days at -20 ºC.

The calibration is valid only for the reagents and calibrator from the same lot number. **Reagents and calibrators are not interchangeable between kits with different lot numbers**.

WARNINGS AND PRECAUTIONS

1. **Many other clinical reagents contain lipase or high concentrations of detergents. Avoid contamination and carry over! Special care should be taken in combination with triglycerides, HDL and LDL reagents. Cuvettes and other glassware must be cleaned thoroughly after being used for other assays. In case of automated measurement refer to the instrument manual for special washing programs.**
2. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.

REAGENT PREPARATION

Reagent R1 and R2 are ready to use as supplied. Unopened reagents, when stored at indicated temperature, are stable up to the expiration date shown on the label.

REAGENT DETERIORATION

The reagent should be discarded if:

1. R2 colour changed to purple colour.It is a sign of reagent contamination.
2. The reagent does not meet stated performance parameters.

SPECIMEN COLLECTION AND STORAGE

Serum or heparin plasma Stability :
7 days at 4 - 8 °C
1 year at -20 °C

ASSAY PROCEDURE FOR SEMIAUTO ANALYZER.

Wavelength : 578 nm
Temperature : 37°C

	Blank	Calibrator	Sample
Reagent 1	0.8 mL	0.8 mL	0.8 mL
Calibrator	-	20 µL	-
Sample	-	-	20 µL

Mix and incubate at 37ºC for 5 min .

Reagent 2	0.2 ml	0.2 ml	0.2 ml.

Mix and read Abs A1 after 60 seconds and A2 after 120 sec of reading A1. Calculate the change of absorbance by subtracting A2-A1.

Calculation:

$$\text{Lipase IU/L} = \frac{\Delta \text{ Abs of sample} \times \text{Cal concn}}{\Delta \text{ Abs of Calibrator}}$$

LIMITATIONS

No interference was observed by ascorbic acid up to 30 mg/dL, free and conjugated bilirubin up to 60 mg/dL, hemoglobin up to 500 mg/dL and lipemia up to 1000 mg/dL triglycerides.

QUALITY CONTROL

It is recommended that controls be included in each set of assays. Commercially available control material the established Lipase values may be used for quality control. The assigned value of the control material must be confirmed by the chosen application. Failure to obtain the proper range of values in the assay of control material may indicate reagent deterioration, instrument malfunction, or procedural errors.

EXPECTED VALUES

< 60 IU/L

PERFORMANCE CHARACTERISTICS.

Measuring range:The test has been developed to determine lipase concentrations up to 300 U/L. When values exceed this range samples should be diluted 1 + 1 with NaCl solution (9 g/L) and the result multiplied by 2.

Specificity/Interferences: No interference was observed by ascorbic acid up to 30 mg/dL, free and conjugated bilirubin up to 60 mg/dL, hemoglobin up to 500 mg/dL and lipemia up to 1000 mg/dL triglycerides.

Sensitivity/Limit of Detection :The lower limit of detection is 3 U/L.

Method Comparison: A comparison between Bioline Lipase and a commercially available colorimetric test (x) using 67 samples gave following results: y = 0.96 x – 1.15 U/L; r= 0.999

General Technical Parameters

Mode	**2-point kinetics**
Wavelength (Filter)	**578 nm**
Secondary Wavelength/Bichromatic	**None / No**
Reaction Direction / Type	**Increasing / Positive**
Sample Vol.	**20 µL**
Reagent Vol.	**1000 µL**
Delay Time / Lag Time	**60 sec**
Measuring Time	**120 Seconds**
Reagent Blank Abs (Max.)	**< 0.500 Abs**
Calibration Method	**1 - Point**
Factor	**NA (Calculated by System)**
Standard (Conc.)	**See the Calibrator Vial**
Linearity	**300 IU/L**
Decimal Places	**1**
Temp.	**37 °C**
Unit	**IU/L**
Ref. Low	**< 3 IU/L**
Ref. High	**> 60 IU/L**

REFERENCES

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