COD 11793 1 x 60 mL
STORE AT 2-8ºC
Reagents for measurement of lipase concentration Only for *in vitro* use in the clinical laboratory

LIPASE

BioSystems
REAGENTS & INSTRUMENTS

CE

LIPASE
COLOR

PRINCIPLE OF THE METHOD

Lipase catalyzes the hydrolysis of the chromogenic substrate 1,2-O-dilauryl-rac-glycerol-3-glutaric acid-(6'-methylresorufin)-ester to 1,2-O-dilauryl-rac-glycerol and an unstable intermediate, glutaric acid-(6'-methylresorufin)-ester. This descomposes spontaneously in alkaline solution to form glutaric acid and methylresorufin. The catalytic concentration is determined from the rate of the red dye formation measured at 580 nm[1,2].

1,2-O-dilauryl-rac-glycerol-3-glutaric acid-(6'-methylresorufin)-ester $\xrightarrow{\text{lipase}}$ 1,2-O-dilauryl-rac-glycerol + glutaric acid-(6'-methylresorufin)-ester

glutaric acid-(6'-methylresorufin)-ester $\xrightarrow{H_2O}$ glutaric acid + methylresorufin

CONTENTS AND COMPOSITION

A. Reagent: 1 x 50 mL. Tris buffer 40 mmol/L, colipase ≥ 1 mg/L, deoxycholate ≥ 1.8 mmol/L, taurodesoxycholate ≥ 7.0 mmol/L, pH 8.3.

B. Reagent: 1 x 10 mL. Tartrate buffer 15 mmol/L, 1,2-O-dilauryl-rac-glycerol-3-glutaric acid-(6'-methylresorufin)-ester ≥ 0.7 mmol/L, calcium ions ≥ 1 mmol/L, pH 4.0.

S. Lipase Standard: 1 for 1 mL. Human lipase in human serum matrix. Concentration is given on the label.

Components from human origin have been tested and found to be negative for the presence of antibodies anti-HIV and anti-HCV, as well as for Hbs antigen. However, they should be handled cautiously as potentially infectious.

STORAGE

Store at 2-8ºC.

Reagents and Standard are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

– Reagents: RA, presence of particulate material, turbidity. RB, is a turbid orange-colored microemulsion, discard if turning red. In some storage conditions (i.e. storage at a temperature lower than the one indicated) a precipitate may appear in the vial that will not influence the reagent performance, however, it is recommended to resuspend the product with a slight rotation of the vial before carrying out the analysis.

– Standard: Presence of moisture.

REAGENT PREPARATION

Reagents are provided ready to use.

Lipase Standard: Reconstitute with 1.00 mL of distilled water. Stable for 7 days at 2-8ºC or for 3 months at –18ºC when frozen in aliquots.

ADDITIONAL EQUIPMENT

– Thermostatic water bath at 37ºC.

– Analyzer, spectrophotometer or photometer with cell holder thermostatable at 37ºC and able to read at 580 ± 20 nm.

SAMPLES

Serum or sodium, lithium or ammonium heparin plasma collected by standard procedures.

Lipase in the sample is stable for 7 days at 2-8ºC.

PROCEDURE

1. Bring the Reagents and the instrument to 37ºC.
2. Pipette into a cuvette: (Note 1)

Reagent A	1000 µL
Serum / Standard (S)	10 µL

3. Mix and insert the cuvette into the instrument. Start the stopwatch. After 1-3 minute, add:

Reagent B	200 µL

4. Mix.
5. After 1 minute, record initial absorbance and at 1 minute intervals thereafter for 3 minutes.
6. Calculate the difference between consecutive absorbances, and the average absorbance difference per minute (ΔA/min).

CALCULATIONS

The lipase concentration in the sample is calculated using the following general formula:

$$\frac{\Delta A/min_{\ Sample}}{\Delta A/min_{\ Standard}} \times C_{\ Standard} = U/L$$

REFERENCE VALUES

Serum: ≤ 38 U/L = ≤ 0.633 µkat/L
These ranges are given for orientation only; each laboratory should establish its own reference ranges.

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042) and II (cod. 18007, 18010 and 18043) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

– Detection limit 5.0 U/L lipase = 0.083 µkat/L lipase

– Linearity limit: 250 U/L = 4.17 µkat/L lipase. For higher values dilute sample 1/2 with distilled water and repeat measurement.

– Repeatibility (within run):

Mean concentration	CV	n
119 U/L = 1.98 µkat/L	3.4 %	20
215 U/L = 3.58 µkat/L	2.8 %	20

– Reproducibility (run to run):

Mean concentration	CV	n
119 U/L = 1.98 µkat/L	4.5 %	25
215 U/L = 3.58 µkat/L	5.0 %	25

– Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.

– Interferences: Lipemia (triglycerides < 30 g/L) and bilirubin (< 20 mg/dL) do not interfere. Hemoglobin (> 5.0 g/L) interfere. Other drugs and substances may interfere[3].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

Lipases hydrolyzes glycerol esters of long-chain fatty acids. Although lipase can be secreted by other glands and mucosa, only pancreatic lipase is of interest in medical diagnosis. Therefore, lipase measurements on serum are used exclusively to investigate pancreatic disorders.

Serum lipase concentration increases after an attack of acute pancreatitis. In general, increases in amylase and lipase run in parallel course, but the elevation of lipase persists for a longer time. Elevations in serum lipase concentration may be also due to obstruction of the pancreatic duct by a calculus or by carcinoma, in acute and chronic renal disease as well as in treatments with opiates[4,5].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. These reagents may be used in several automatic analysers. Instructions for many of them are available on request.

BIBLIOGRAPHY

1. Neumann U, Kaspar P, Ziegerhorn J, Bergmeyer HU. Methods of enzymatic analysis, 3rd ed. Vol. 4:26-34,1984.
2. Panteghini M, Bonora R, Pagani F. Measurement of pancreatic lipase activity in serum by a kinetic colorimetric assay using a new chromogenic substrate. *Ann Clin Biochem* 2001;38:365-370.
3. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.
4. Junge W, Abicht K, Goldman J et al. Evaluation of the colorimetric liquid assay for pancreatic lipase on Hitachi analyzers in clinical centers in Europe, Japan, and USA. *Clin Chem Lab Med* 1999;37, special suppl:469.
5. Friedman and Young. Effects of disease on clinical laboratory tests, 4th ed. AACC Press, 2001.