Lipase
Color Liquid

CE

Kinetic colorimetric direct determination of lipase activity in serum and plasma.
Store at 2-8°C .

REF: LIP-027B

Summary:
Lipase enzymes are produced in the pancreas and also secreted in small amounts by the salivary glands as well as by gastric, pulmonary and intestinal mucosa. Determination of lipase is used for diagnosis and treatment of diseases of pancreas such as acute and chronic pancreatitis and obstruction of the pancreatic duct.

Principle:
The method for the determination of lipase is based on the cleavage of specific chromogenic lipase substrate 1,2-O-dilauryl-rac-glycero-3-glutaric acid-(6'-methylresorufin)-ester emulsified in stabilized microparticles. In the presence of specific activators of pancreatic lipase as colipase, calcium ions and bile acids, the substrate is converted in 1,2-O-dilauryl-rac-glycerol and glutaric acid-6'-methylresorufin-ester which decomposes spontaneously in glutaric acid and methylresorufin. The increase of absorbance, due to methylresorufin formation, is proportional to the activity of lipase in the sample.

Composition:
The components of the kit, stored at 2-8 °C in unopened vials, are stable up to the expiry date indicated on the package.
R1: tris buffer - 40 mmol/L pH 8.3, colipase - ≥ 1 mg/L, desoxycholate - ≥ 1.8 mmol/L, taurodesoxycholate - ≥ 7.0 mmol/L
R2: tartrate buffer - 15 mmol/L pH 4.0, lipase substrate - ≥ 0.7 mmol/L, calcium ions - ≥ 1 mmol/L

Precautions:
- For in vitro diagnostic use.
- Do not use components beyond the expiration date.
- Do not mix materials from different kit lot numbers.
- Safety Data Sheets are available

Instruments:
Instrument applications are available upon request.

Preparation:
R1 and R2 are ready to use.
Reagent 1 is in a clear liquid form, discard if turbid.
Reagent 2 is a turbid orange-colored micro-emulsion, discard if turning to 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.

Stability:
On Board: 30 days, if contamination is avoided.
Calibration: 30 days. Repeat the calibration at any variation in the reagent lot.

Samples:
Serum or plasma (EDTA or heparin).
Stability: 7 days at 2-8 °C or 12 months at -20 °C.

Equipment:
General Laboratory Equipment
Calibrator
Control Serum (normal and pathologic)

Procedure:
1. Assay conditions:
Wavelength: . . . . 570 nm (main)
. . . . 800 nm (reference)
Cuvette: . . . . . .1 cm. light path
Temperature: . . . . . .37°C
Sample/R1/R2: 1/100/200
Reaction: . . . . . .Fixed time (increase)

Allow reagents to reach working temperature before using.
A proportional variation of the indicated reaction volumes does not change the results.
Example of analytical procedure on automated intruments
Time 0
Calibrator/Controls/Sample = 3 µL
R1 = 300 µL
After 300 sec
Addition of R2 = 60 µL
After 150 sec
Reading 1
After 150 sec
Reading 2

Calculations:
1. Plot a calibration curve on a graph paper by tracing absorbance (y axis) according to corresponding U/L activity (x axis) for each Calibrator.
2. Indicate on the calibration curve the absorbance value obtained for Samples and Controls.
3. Extrapolate the U/L value for Samples and Controls from the calibration curve.

Conversion factor: LPS [U/L] x 0. 01667 = LPS [µkat/L]

Lipase activity (reported on the CALIBRATOR TcA) in U/L methylresorufin method at 37 °C, can be converted in turbidimetric U/L at 37 °C with triolein substrate (Roche turbidimetric) multiplying the LPS value in TcA by the calculated factor of 6.2.

Quality control:
All clinical laboratories should establish an Internal Quality Control program. Check instrument and reagent performance with recommended controls or similar. The values obtained for QC should fall within manufacturer's acceptable ranges or should be established according to the Laboratory's QC program.
Controls should be assayed:
- Prior reporting patient results.
- Following any maintenance procedure on the photometer used.
- At pre-established intervals following the Q.C. Laboratory recommandations.

Reference values:
Lipase in normal subjects
(U/L methylresorufin at 37 °C): ≤ 38 U/L
It is recommended that each laboratory establish its own expected range.

Performance characteristics:
Sensitivity:
5.0 U/L. Sensitivity was calculated on 20 replicates of normal saline and reported as the «mean zero value + 3 SD»
y = 1.015x + 7.690
r = 0.991
Sample range: 12 to 124 U/l

Precision:

Determined from 1x3x20 tests (day x run x rep) on 2 commercial controls (L1/L2) and a human sera pool (L3).

	Intra-assay		
U/L	L1	L2	L3
Mean	36.8	64.8	12.8
SD	0.37	0.40	0.24
CV%	1.0	0.6	1.9

Determined from 10x2x2 tests (day x run x rep) on 3 commercial controls (L1/L2/L3).

	Inter-assay						
	Mean	Total Imprecision		Between Days		Repeatability	
	U/L	SD	CV%	SD	CV%	SD	CV%
L1	18.0	0.88	4.9	0.49	2.7	0.74	4.1
L2	33.6	0.85	2.5	0.47	1.4	0.68	2.0
L3	95.1	1.55	1.6	1.18	1.2	0.73	0.8

Method comparison:
Accuracy:
this test (y) was compared with a commercially methylresorufin available method (x).
The results were as follows:
N= 101
r = 0.997
y = 0.50 x + 3.94
this test (y) was compared with a commercially 1,2-digliceride available method (x).
The results were as follows:
N = 52
r = 0.996
y = 0.63 x + 6.62

Interferences:
The test is not affected by the presence of haemoglobin up to 500 mg/dL, bilirubin up to 60 mg/dL and lipids (intralipid) up to 300 mg/dL.

Analytical Range:
5.0 - 250 U/L.
Samples with values higher than 250 U/L must be diluted 1:10 with normal saline and the result multiplied by 10.

Waste Management:
Reagents must be disposed of in accordance with local regulations.

References:

1. NCCLS Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard - Fifth Edition (H3-A5). Wayne, PA: The National Committee for Clinical Laboratory Standards, 2003.
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3. US Department of Health and Human Services. Biosafety in Microbiological and Biomedical Laboratories, 5th Ed. Washington,DC: US Government Printing Office, January 2007.
4. World Health Organization. Laboratory Biosafety Manual, 3rd ed.Geneva: World Health Organization, 2004.
5. Sewell DL, Bove KE, Callihan DR, et al. Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline — Third Edition (M29-A3). Wayne, PA: Clinical and Laboratory Standards Institute, 2005.
6. Pesce, A.J., Kaplan, L.A.: "Methods in Clinical Chemistry", Mosby Ed. (1987).
7. Burtis C.A., Ashwood E.R.: "Tietz Textbook of Clinical Chemistry", W.B. Saunders Company Ed. (3rd edition, 1999).

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