LIPASE (colorimetric)
Quantitative determination of lipase
Only for *in vitro* use in the clinical laboratory **LIP-027**
Store at 2-8°C

LIPASE

SORACHIM

PRINCIPLE OF THE METHOD
The sequence of reactions involved in the enzymatic direct lipase determination is the following:

1-2-O-dilauryl-rac-glycerol-3-glutaric -(6' -methylresorufin)-ester
Lipase →
1-2-O-dilauryl-rac-glycerol + Glutaric-6'-methylresorufin-ester

Glutaric-6'-methylresorufin-ester $\xrightarrow{OH^-}$ Glutaric acid + Methylresorufin

The rate of methylresorufin formation, measured photometrically, is proportional to the catalytic concentration of lipase present in the sample.

CLINICAL SIGNIFICANCE.
Only pancreatic lipase is of interest in medical diagnosis. Lipase hydrolyzes glycerol esters of long chain fatty acids. Lipase measurement is used for diagnosis of diseases of pancreas such as acute and chronic pancreatitis and obstruction of the pancreatic duct[1,7,8]. Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

REAGENTS
R1 : TRIS 40 mmol/L pH 8.3, Colipase ≥ 1mg/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.
S: Calibrator. Lyophilized human serum.
Precautions: Components from human origin have been tested and found to be negative for the presence of HBsAg, HCV, and antibody to HIV (1/2). However handle cautiously as potentially infectious.

PREPARATION
R1 and R2 (Note 1) are provided ready to use.
Reagent 1 is a clear liquid: discard if turbid.
Reagent 2 is a turbid orange-colored micro-emulsion: discard if turned to red.
Calibrator: dissolve the contents with 1 mL of distilled water. Cap the vial and mix gently to dissolve the contents.
Some lipases of microbial origin, used in the manufacturing reagents for enzymatic determination of triglycerides, may result in a strong adhesion to the plastic cuvettes of the instruments. Therefore it is recommended to verify on random access analyzers any possible lipase contamination by performing a test with a reagent for lipase determination before its routine use and to adopt suitable actions (i.e. washing with acid solution) to avoid this problem.

STORAGE AND STABILITY
All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use.
R1 and R2: once opened are stable 3 months at 2-8°C.
S: once reconstituted is stable 1 week at 2-8°C or 3 months at -20°C.
Signs of component deterioration: Presence of particles and turbidity

ADDITIONAL EQUIPMENT
- General laboratory equipment.
- Spectrophotometer or photometer measuring at 570nm.

SAMPLES
Serum or plasma (EDTA or heparin). Avoid repeated frozen and unfrozen. Stability of the sample: 2 days at 2-8°C.

PROCEDURE (Note 2)
1. Assay conditions:
   Wavelength. . . . . 570nm.
   Cuvette: . . . . . 1cm light path
   Temperature. . . . . 37°C
2. Bring the reagents and the instrument to 37°C
3. Pipette into a cuvette:

	Blank	Cal./ Sample
R1 (mL)	1,0	1,0
R2 (µL)	200	200
Distilled water (µL)	10	--
Cal. / Sample (µL)	--	10

4. Mix thoroughly and incubate for 1 minute at 37°C.
5. Read the initial absorbance (A1) of the sample and standard, start the stopwatch and read the absorbances at 1 minute intervals thereafter for 2 minutes.
6. Calculate the average absorbance differences per minute (ΔA/min) for the sample, calibrator and blank.

CALCULATIONS
$$\frac{\Delta A \text{ Sample} - \Delta A \text{ blank}}{\Delta A \text{ calibrator} - \Delta A \text{ blank}} \times \text{calibrator activity} = U/L$$

Units: One international unit (IU) is the amount of enzyme that transforms 1 µmol of substrate per minute, in standard conditions. Concentration is expressed in units per litre of sample (U/L). Conversion factor: LPS [U/L] * 0.01667 = LPS [µkat/L]

QUALITY CONTROL
Control sera are recommended to monitor the performance of assay procedures. If control values are found outside the defined range, check the instrument, reagents and technique for problems.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[1]
≤ 38 U/L (methylresorufin at 37°C)
These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS
Measuring range: From detection limit of 5 U/L to linearity limit of 250 U/L. If the results obtained were greater than linearity limit, dilute the sample 1/10 with NaCl 9 g/L (normal saline) and multiply the result by 10.

	Intra-assay (n=20)			Inter-assay (n=20)		
Mean (U/L)	36.8	64.8	12.8	18.0	33.6	95.1
SD	0.37	0.40	0.24	0.88	0.85	1.55
CV%	1.0	0.6	1.9	4.9	2.5	1.6

Accuracy: This test (y) was compared with a commercially methylresorufin available method (x). The results were as follows: N=101, r=0.997, y=0.50x+3.94.
This test (y) was also compared with a commercially 1,2-digliceride available method (x). The results were as follows: N=52, r=0.996, y=0.63x+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 300mg/dl. Other drugs and substances may interfere[5].
The results of the performance characteristics depend on the analyzer used.

NOTES
1. In some storage conditions (i.e. storage at a temperature lower that the one indicate) a precipitate may appear in the vial that will not influence on the reagent performance; however, it is recommended to resuspend the reagent with a slight rotation of the vial before carrying out the analysis.
2. We have instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

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