LIPASE CE

REF 1143005	REF 1143010
1 x 60 mL	1 x 60 mL
CONTENTS	**CONTENTS**
R1. Reagent 1 x 50 mL	R1. Reagent 1 x 50 mL
R2. Reagent 1 x 10 mL	R2. Reagent 1 x 10 mL
CAL 1 x 1 mL	
Only for *in vitro* diagnostic use	

LIPASE

Enzymatic colorimetric method

KINETIC

PRINCIPLE

The method for the determination of lipase is based on the cleavage of specific chromogenic lipase substrate 1,2-O-dilaurylrac-glycero-3-glutaric acid-(6'methyl-resorufin)-ester emulsified in stabilized micro-particles. In the presence of specific activators of pancreatic lipase as colipase, calcium ions and bile acids, the substrate is con-verted to 1,2-O-dilauryl-rac-glycerol and glutaric acid-6'-methylresorufinester which decomposes spontaneously to glutaric acid and methylresorufin. The increase of absorbance at 580 nm, due to methylresorufin formation, is proportional to the activity of lipase in the sample.

REAGENT COMPOSITION

R1 **Buffer lipase** TRIS 40 mmol/L pH 8.3, colipase≥ 1 mg/L, desoxycholate ≥ 1.8 mmol/L, taurodesoxycholate ≥ 7.0 mmol/L.

R2 **Substrate lipase** Tartrate buffer 15 mmol/L pH 4.0, lipase substrate ≥ 0.7 mmol/L, Ca^{+2} ≥ 1 mmol/L.

CAL **Lipase Calibrator** Activity given in U/L methylresorufin at 37ºC on the label. Freeze-dried.

STORAGE AND STABILITY

Store at 2-8ºC.
All the kit compounds are stable until the expiry date stated on the label. Do not use reagents over the expiration date.
Store the vials tightly closed, protected from light and prevented contaminations during the use.
Stability: 90 days at 2-8 °C after opening,

Discard If appear signs of deterioration:
- Presence of particles and turbidity.
- Reagent **R2** is a turbid orange-colored stabilized micro-emulsion. Discard if it turns red.

REAGENT PREPARATION

- Reagents **R1** and **R2** are ready to use. **R2** Swirl the vial gently before performing the assay.

Calibrator. Reconstitute the contents of one vial with **1.0 mL** of distilled water, swirling gently until complete dissolution. Stable 7 days at 2-8ºC. Aliquoted into small volumes and frozen is stable for 3 months at –20ºC.

SAMPLES

Fresh serum and heparinized plasma. Stable 7 days at 2-8ºC or frozen at -20ºC for a longer period of time.

INTERFERENCES

- Hemoglobin up to 150 mg/dL and bilirubin up to 20 mg/dL do not interfere.
- Triglycerides (>300 mg/dL) affect negatively the assay.
- Morphine and certain cholinergic drugs may increase serum lipase levels.

MATERIALS REQUIRED

- Photometer or spectrophotometer with a thermostatted cell compartment set at 37ºC, capable to read at 580 ± 10 nm.
- Stopwatch, strip-chart recorder or printer.
- Cuvettes with 1-cm pathlength.
- Pipettes to measure reagents and samples.

PROCEDURE

1. Preincubate the reagents, samples and standard to reaction temperature.
2. Set the photometer to 0 absorbance with distilled water.
3. Pipette into a cuvette:

TUBES	Blank	Sample	Standard
Distilled water	10 µL	–	–
Sample	–	10 µL	–
CAL	–	–	10 µL
R1	1.0 mL	1.0 mL	1.0 mL
R2	0.2 mL	0.2 mL	0.2 mL

4. Mix gently by inversion. Insert cuvette into the cell holder and start stopwatch.
5. Incubate for 1 minute and record initial absorbance reading.
6. Repeat the absorbance readings exactly after 1 and 2 minutes.
7. Calculate the difference between absorbances.
8. Calculate the mean of the results found for the Blank, Sample and Standard to obtain the average change in absorbance per minute (ΔA/min).

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CALCULATIONS

Substract the ΔA/min of the Blank from the ΔA/min of Sample and Standard to obtain the respectives ΔA/min corrected. Apply:

$$\frac{\Delta A/min_{Sample}}{(\Delta A/min)_{Calibrator}} \times \text{Calibrator activity} = \text{U/L lipase}$$

Lipase unit. One (U) is defined as the amount of enzyme activity which liberates 1 µmol of methylresorufin from the substrate per minute at 37ºC.

REFERENCE VALUES[2]

Serum, plasma

Normal subjects ≤ 38 U/L

It is recommended that each laboratory establishes its own reference range.

QUALITY CONTROL

To ensure adequate quality control (QC), each run should include a set of controls (normal and abnormal) with assayed values handled as unknowns.

CLINICAL SIGNIFICANCE

Lipase values have approximately the same significance as amylase values in the diagnosis of *acute pancreatitis* being the greatest increases found in acute rather than chronic pancreatitis. Since serum lipase is not derived from as many tissues as amylase, it is more specific than amylase for acute pancratitis.
During *pancreatic disturbances*, the serum lipase activity may rise more slowly than the serum amylase, but it may remain elevated for a longer period of time and therefore more useful in the diagnosis and follow-up of the illness.

ANALYTICAL PERFORMANCE

- ***Linearity.*** Up to 250 U/L

- ***Precision***

U/L	Within-run			Between-run		
Mean	11.6	119.6	215	11.6	119.6	215
SD	2.6	4.1	6.0	1.2	5.4	10.8
CV%	22	3.4	2.8	10	4.5	5
N	20	20	20	20	20	20

Replicates: 20 for each level. Instrument: HITACHI 917

Replicates: 20 for each level for 8 days.

- ***Sensitivity.*** 5 U/L.

- ***Correlation.*** This assay (y) was compared with a similar commercial method (x). The results were:

N = 20 r = 0.997 y = 0.500x + 3.944

REFERENCES

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3. EU-Dir 1999/11 Commission Directive of 8 March 1999 adapting to technical progress the principles of Good Laboratory Practice as specified in Council Directive 87/18/EEC.
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