

LIPASE COLORIMETRIC LIQUID

KINETIC DETERMINATION IN THE SERUM
 METHOD WITH 1,2-O-DILAURYL-RAC-GLYCERO-3GLUTARIC ACID-(6'-METHYLRESORUFIN)-ESTER ((DLGGM))
 For in vitro diagnostic use only

Kit: 2 x 18 ml

Cod. LIP3542

SUMMARY

The determination of lipase is useful to investigate pancreatic disorders such as pancreatitis. In acute pancreatitis lipase activity increases along with amylase activity, however lipase levels are higher than those of amylase. So it's better to use tests for both of them for doing a correct diagnosis.

PRINCIPLE

The pancreatic lipase in the serum catalyzes the hydrolysis of substrate DLGGM, giving methyl resorufin (chromogen). The increase of absorbance in the unit time at 580 nm, is proportional to the activity of lipase in the sample.

REAGENTS

Components of the kit:

	Cod. LIP3542
*REAGENT 1 (liquid)	2 x 12 ml
*REAGENT 2 (microemulsion)	1 x 12 ml
Good buffer pH 8.0	50 mmol/L
Taurodesoxycolate	7.2 mmol/L
Desoxycolate	1.8 mmol/L
DLGGM	0.24 mmol/L
Colipase	1 mg/L
Ca Chloride	0.1 mmol/L
Tartrate	1.6 mmol/L

*REAGENT 3 (Iyo)	Calibrator	1 x 3 ml
*REAGENT 4 (liquid)	Calibrator Diluent	1 x 4 ml

Pancreatic Lipase (values in U/L methylresorufine at 37°C on the label)

STABILITY: the reagents, stored at 2-8°C, are stable up to the expiry date shown on the package if **not contaminated during handling**.

AUXILIARY REAGENTS QUALITY CONTROL (Not supplied with the kit)

To grant the correct test performances we suggest to use following kits:

- NORMAL CONTROL	Cod. CNU
- PATHOLOGICAL CONTROL	Cod. CPU

PREPARATION OF THE WORKING REAGENT

Ready to use.

Mix gently per inversion the *Reagent 2 before use.

STABILITY: 4 weeks at 2-8°C in dark place, after the first open.

Mix kindly and let the working reagent reach the working temperature before use. **Close immediately after handling. Incompetent handling will release us from any responsibility.**

PREPARATION OF THE STANDARD

Add 3 ml of the *Reagent 4 to the *Reagent 3 Calibrator (Iyo); mix gently until complete solution. Avoid foam.

STABILITY: 4 days at 2-8°C in dark place.
 2 months a -20°C, fractionated in small volumes.

CAUTION

The Calibrator is from human origin and has to be used as a potential transfer of infective pathologies. This product has been tested and found to be negative for HIV, HCV and HBsAg antibodies by an approved method. Because no test method can offer complete assurance that all infectious agents are absent, it is recommended that this product and all the samples be handled as though capable of trasmitting infectious disease.

SAMPLE

- No haemolyzed fresh serum.

PROCEDURE

• Wavelength:	570 or 580 nm
• Pathlength:	1 cm
• Reading:	against Reagent Blank
• Temperature:	37°C
• Method:	kinetic
• Reaction:	about 3 minutes
• Sample/Reagent:	1/100/50

Let reagent s reach the working temperature before using.

Pipette into a test tube or cuvette labelled:

R/B: Reagent Blank, S: Sample ST: Standard:

	R/B	ST	S
*Reagent 1	600 µl	600 µl	600 µl
Distilled water	6 µl	----	----
*Reagent 3 Calibrator	----	6 µl	----
Sample	----	----	6 µl

Mix and wait 5 min. at 37°C, then add:

*Reagent 2	300 µl	300 µl	300 µl
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Mix carefully and measure the absorbance after 1 minute at 37°C; read initial absorbance for Calibrator (Ast), Sample (As) and Reagent Blank (Arb).

Repeat the reading after 90 seconds.

Determine the average of the absorbance/minute readings ($\Delta A/\text{min}$).

$$\Delta A_{1st/\text{min.}} = \Delta A_{st/\text{min.}} - \Delta A_{rb/\text{min.}} \quad (\text{Calibrator})$$

$$\Delta A_{1s/\text{min.}} = \Delta A_{s/\text{min.}} - \Delta A_{rb/\text{min.}} \quad (\text{Sample})$$

CALCULATION

Insert the average found in the following formula:

$$\text{Pancreatic Lipase (U/L } 37^\circ\text{C methylresorufine)} = \frac{\Delta A_{1s/\text{min.}} \text{ Sample}}{\text{values of Calibrator} \times \frac{\Delta A_{1st/\text{min.}} \text{ Calibrator}}$$

REFERENCE VALUES

< 60 U/L (method methylresorufine, 37°C)

It is advisable that every laboratory determine its normal reference values.

PERFORMANCE CHARACTERISTICS

These performance characteristics was determined using a spectrophotometer or analyzers typically found in clinical laboratories, under the stated assay conditions..

Linearity: Lipase activity is determined between 3 – 400 U/L.
 For activity of Lipase \geq 400 U/L dilute the sample 1:4 with saline solution, repeat the determination and multiply the result \times 4.

Sensitivity: The minimum detectable is 3 U/L.

Within-run Precision:

Sample	Mean (U/L) ± 2s	CV %
Serum 1	38 ± 2	1,1
Serum 2	168 ± 4	0,9

Run-to-run (Day-to-day) Precision:

Sample	Mean (U/L) ± 2s	CV %
Serum 1	39 ± 3	1,3
Serum 2	167 ± 6	1,5

Accuracy: with commercially available Control(s)

	Waited	Found
Pathological	165 (141 - 188)	161-167-170
Normal	64,2 (52,5 – 75,9)	63 – 66 - 67

Interferences: See References point 2.

Correlation: A group of 20 samples was assayed by this procedure and using a similar commercially available Lipase Reagent. Comparison of the data gave following results:

Linear regression $Y = 1,0096X - 1,2$
 $r = 0,9996$ $n = 20$

NOTE

1. A proportional variation of the reaction volumes does not change the result.
 2. We suggest do not mix reagents from different production lots.
 3. For lipase activity higher than 400 U/l dilute the sample 1:4 with saline solution, repeat the determination and multiply the result x 4.
 4. This method do not gives interference from cholesterol reagent; the triglycerides reagent give a progressive interference. In automation avoid the determination of lipase after triglycerides; or wash very well the instrument to avoid contamination.
 5. It exists a correlation with turbidimetric method (Roche-BM, 37°C): U/L (turbidim. at 37°C, Roche-BM) = 3.4 x U/L (methylresorufine at 37°C).
 6. It exists a progressive interference from triglycerides for concentration higher than 200 mg/dL. .
 7. No interference from bilirubin up to 45 mg/dL, hemoglobin up to 150 mg/dL.
 8. PAY ATTENTION!
- Applications on routine Analyzers may be totally different from what we developed as manual determination, and also from themselves.
9. Very deep attention must be given to interfering substances: certain drugs and other substances are able to influence levels of Lipase (see References 2.).
 10. The reagent must be used only for the intended destinations, by expert people and in the due lab. conditions.
 11. The clinical diagnosis cannot be done using the result of only one test, but have to be done integrating different lab. and clinical data.

REFERENCES

1. Textbook of Clinical Chemistry, Ed. by N.W. Tietz, W.B. Saunders Co., Philadelphia (1999).
2. Young D.S. et al., Clin. Chem. 21, 302D (1975)
3. Junge W. et al., J.Clin. Chem. Clin. Biochem. 21, 445 (1983)

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