BIOLABO
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LIPASE Kinetic Method

Reagent for quantitative determination of Pancreatic Lipase [EC 3.1.1.3] in human serum or plasma.

REF 99881 **R1** 3 x 10 mL **R2** 3 x 10 mL **R3** 1 x 12 mL **R4** 1 x 3 mL **R5** 1 x 5 mL

TECHNICAL SUPPORT AND ORDERS
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Latest revision: www.biolabo.fr

CE

IVD

Made In France

I: corresponds to significant modifications

INTENDED USE

This reagent is designated for professional use in laboratory. (manual or automated procedure).
It allows quantitative determination of pancreatic lipase in human serum or plasmas to determinate its activity.

GENERALITIES (1)

Pancreatic lipase in blood is closely associated with pancreatic diseases. The measurement of Pancreatic lipase activity is an important marker for the diagnosis of pancreatic diseases and for the associated monitoring of therapeutics effects.

PRINCIPLE (4) (5)

Enzymatic method described by Imamura S., et al. Serum lipase acts on 1,2-diglyceride to form 2-monoglyceride which is then hydrolysed in glycerol and free fatty acid by monoglyceride lipase.
Glycerol Kinase acts on glycerol to liberate glycerol-3-phosphate, which in turn acted on by glycerol-3-phosphate oxidase to generate hydrogen peroxide. Peroxidase converts H_2O_2, 4-AAP and TOOS into quinoneimine dye.
The rate of formation of quinoneimine dye, directly proportional to the Lipase activity in the specimen, is measured at 550 nm.

REAGENT COMPOSITION

R1	**LIPASE**		Enzymes-Substrate	
1,2-Diglycerides (egg)			1.1	mmol/L
Monoglyceride lipase (Bacillus sp.)			880	IU/L
Glycerol Kinase (S. Canus)			1340	IU/L
Glycerol-3-phosphate oxidase (Streptococcus sp.)			40	KU /L
TOOS (N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine)			0.07	%
ATP			0.66	mmol/L
Peroxidase (Horseradish)		1340	IU/L	
Colipase (porcine)			40	IU/L
Buffer	pH 6.8			
Ascorbate oxidase (cucumber, zucchini)		2.6	IU/L	
Stabilizers				

DANGER : Acute tox.4: H312+H332-Nocif en cas de contact cutané ou inhalation. Eye dam.1 : H318 – Provoque des lésions oculaires graves.
P280 : porter des gants/vêtements/équipement de protection des yeux/du visage. P305+P351+P338 : EN CAS DE CONTACT AVEC LES YEUX: Rincer avec précaution à l'eau pendant plusieurs minutes. Enlever les lentilles de contact si la victime en porte et si elles peuvent être facilement enlevées.
Continuer à rincer, P501 : éliminer le contenu et le récipient conformément à la règlementation sur les déchets dangereux. Substance à l'origine de la classification : Trade secret. Pour plus de détails, consulter la Fiche de données de Sécurité (FDS)

R2	**LIPASE**		Buffer	
Cholic acid (Ox or Sheep)			5.3	mmol/L
Buffer	pH 6.8			
Sodium azide			< 0.1	%

R3	**LIPASE**		Start Reagent	
Deoxycholate (Ox or Sheep)			36	mmol/L
4-Aminoantipyrine (4–AAP)			0.12	%
Sodium azide			< 0.1	%

R4	**LIPASE**		Calibrator	
Pancreatic lipase			(human origin)	

Serum albumin (bovine), and preservative.
The exact value of lipase is indicated on the label of the vial.

R5	**LIPASE**		Calibrant (diluant)	

Reagent R2, R3, R4 are not classified as dangerous according to 1272/2008/EC regulation

SAFETY CAUTION (7)

- Refer to current Material Safety Data Sheet available on request or on www.biolabo.fr
- Verify the integrity of the contents before use.
- Waste disposal: Respect legislation in force in the country.
- All specimens or reagents of biological origin should be handled as potentially infectious. Respect legislation in force in the country.

Any serious incident that has occurred in connection with the device is notified to the manufacturer and the competent authority of the Member State in which the user and/or patient is based.

REAGENT PREPARATION

Add promptly to the contents of vial R1, the amount of buffer R2 stated on the label.

Mix gently and wait for complete dissolution.

The contents of vial R3 is ready for use.

Open the vial R4 carefully, avoiding any loss of lyophilised material.

- Using a volumetric class A pipette or equivalent, reconstitute with exactly 3 mL (3000 µL) of the content of vial R5.
- Close the vial and allow to stand for 10 minutes at room temperature.
- Dissolve completely the contents by swirling gently before use.
- Do not shake (to prevent foam formation).

STABILITY AND STORAGE

Stored away from light, well cap in the original vial at 2-8°C, reagent is stable when stored and used as described in the insert:

Unopened,
- Until the expiry date stated on the label of the Kit.

Once opened:
- Reconstitute immediately reagent R1

Once reconstituted:
- Transfer the requested quantity, recap and store at 2-8°C.
- Working reagent (R1 + R2) is stable for at least 28 days.
- Once reconstituted, Calibrator (R4) is stable for:
  ✓14 days at 2-8°C, 4 months at –20°C (freeze once only).
- Discard any reagent or calibrator if cloudy.
- Don't use working reagent or calibrator after expiry date stated on the label.

SPECIMEN COLLECTION AND HANDLING (1) (2) (6)

Serum: Collect whole blood by veinipuncture and allow clotting. Centrifuge and remove the serum as soon as possible after collection (within 3 hours)
EDTA plasma, or lithium and sodium heparinised plasmas: Collect specimens with recommended anticoagulant. Centrifuge and remove plasma as soon as possible after collection (within 3 hours)

Lipase activity is stable in serum/plasma for:
- 1 week at room temperature, 3 weeks at 2-8°C, 3 months at -20°C (freeze once only)

LIMITES (5)

Bacterial contamination of the specimen may result in an increase of lipase activity.

Enzymes in triglycerides and cholesterol reagents may contaminate the Lipase reagents. To avoid contamination, ensure probes, cuvettes and tubes of the automatic analysers are thoroughly washed between triglycerides and cholesterol assays and use of the lipase assay.

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

MATERIALS REQUIRED BUT NOT PROVIDED

1.Basic medical analysis laboratory equipment.
2.Spectrophotometer or Biochemistry Clinical Analyzer

QUALITY CONTROL

- REF 95010 EXATROL-N Level I.
- REF 95011 EXATROL-P Level II.
- External quality control program.

It is recommended to control in the following cases:

- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.
- After maintenance operations on the instrument.

If control is out of range, apply following actions:

1. Prepare a fresh control serum and repeat the test.
2. If control is still out of range, use a new vial of calibrator or a fresh calibrator and repeat the test.
3. If control is still out of range, repeat with a new vial of reagent.

If control is still out of range, please contact BIOLABO technical support or your local Agent.

REFERENCE INTERVAL (2)

Serum (37°C)	Lipase (IU/L)	Lipase (µKat/L))
	7-59	[0.12-1.00]

Each laboratory should establish its own normal ranges for the population that it serves.

PERFORMANCE

On Roche/Hitachi 911 analyser

Detection limit: approximately 2 IU/L (0.03 µKat /L)

The assay is linear up to 750 IU/L (12.5 µKat/L).

If result is greater than 750 IU/L, dilute specimen with saline solution and re-assay taking into account the dilution factor. Linearity depends on the specimen/reagent ratio.

Precision

Within run N = 20	*Low level*	*Medium level*	*High level*
Mean IU/L	33	118	269
S.D. IU/L	0.8	1.5	2.1
C.V. %	2.4	1.2	0.8

Beetwen run N = 20	*Low level*	*Medium level*	*High level*
Mean IU/L	34	120	275
S.D. IU/L	1.5	2.7	6.3
C.V. %	4.4	2.3	2.3

Comparison studies with commercially available reagent on 41 patients between 5 and 315 IU/L:

y = 0.44 x -62.07 r = 0.97

Interferences:

Haemoglobin	Negative interference from 1500 mg/dL
Free bilirubin	Negative interference from 20 mg/dL (342 µmol/L)
Conjugated bilirubin	no interference up to 25 mg/dL (428 µmol/L)
Glycerol	Negative interference from 250 mg/dL
Ascorbic acid	no interference up to 50 mg/dL
Triglycerides	Negative interference from 750 mg/dL.
Intralipids	no interference up to 1 %

CALIBRATION

- REF 95801 Lipase Calibrator enclosed in this kit

The calibration frequency depends on proper instrument functions and on the preservation of the reagent.

Make a new calibration when changing reagent batch, if quality control results are found out of the range and after maintenance operations.

PROCEDURE

Manual method:

Let stand reagents and specimens at room temperature.

Pipette into 1 cm path length cuvette:	Blank	Calibrator	Assay
Reagent (R1+ R2)	1000 µL	1000 µL	1000 µL
Calibrator (vial R4)		20 µL	
Specimen			20 µL
Mix vigorously, let stand for 4 minutes at 37°C. Add:			
Start-Reagent (vial R3)	350 µL	350 µL	350 µL
Mix vigorously, let stand for 3 minutes at 37°C. Start a timer and record absorbance every minute during 3 minutes at 550 (546-550) nm.			

1- Performances with manual procedure should be validated by user.

2- Kenza applications and other applications proposal are available on request.

CALCULATION

Manual Procedure:

$$\text{Lipase Activity} = \frac{(\Delta\text{Abs/min})_{\text{Assay}} - (\Delta\text{Abs/min})_{\text{Blank}}}{(\Delta\text{Abs/min})_{\text{Calibrator}} - (\Delta\text{Abs/min})_{\text{Blank}}} \times \text{Calibrator Concentration}$$

Automatic Biochemistry analyzer:

The analyzer provides directly final result.

For more details about calibration and calculation of results, refer to User's manual and specific application.

$$\text{µkat/L} = \frac{\text{IU/L}}{60}$$

REFERENCES

(1) TIETZ N.W. Text book of clinical chemistry, 3rd Ed. C.A. Curtis, E.R. Ashwood, W.B. Saunders (1999) p.699-700.

(2) Clinical Guide to Laboratory Test, 4th Ed., N.W. TIETZ (2006) p. 676-677

(3) YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p. 3-398 to 3-400

(4) Imamura S., Misaki H., "A sensitive method for assay of lipase activity by coupling with β-oxidation enzymes of fatty acids." Selected topics in Clinical Enzymology; 2: 73 (1984)

(5) Imamura S., et al., Clin. Chem., Abstract issue in the 41st National meeting; 1120 (1989)

(6) NCCLS, "Procedures for the collection of Diagnostic Blood Specimens by Skin Puncture", approved standard, Third Edition, NCCLS publication H4-A3, Villanova, PA (1991).

(7) Centers for Diseases control/National Institutes of Health Manual, "Biosafty

(8) in Microbiological and Biomedical Laboratories", 1988

Manufacturer	Expiry date	In vitro diagnostic	Storage temperature	Dematerialized water	Biological risk
Product Reference	See Insert	Batch number	Store away from light	Sufficient for	Dilute with