



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 99891	R1 5 x 30 mL	R2 1 x 150 mL	R3 1 x 50 mL	R4 2 x 3 mL	R5 2 x 5 mL
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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IVD IN VITRO DIAGNOSTIC

CLINICAL SIGNIFICANCE (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., which reaction scheme is as follows:
Serum lipase acts on 1,2-diglyceride to form 2-monoglyceride which is then hydrolyzed 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 H₂O₂, 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

Vial R1 ENZYMES-SUBSTRATE (Lyophilisate)	
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	0.07 %
(N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine)	
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	
Vial R2 BUFFER	
Cholic acid (Ox or Sheep)	5.3 mmol/L
Buffer	pH 6.8
Sodium azide	< 0.1 %
Vial R3 START REAGENT	
Deoxycholate (Ox or Sheep)	36 mmol/L
4-Aminoantipyrine (4-AAP)	0.12 %
Sodium azide	< 0.1 %

Vial R4 LIPASE CALIBRATOR

Pancreatic lipase (human origin)
BIOLABO Lipase Calibrator contains human pancreatic lipase, serum albumin (bovine), and preservative.

Refer to the label of the vial to know the exact value of the Lipase Calibrator.

Vial R5 DILUENT FOR LIPASE CALIBRATOR

MATERIAL REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. Normal and pathological controls
3. Lipase Calibrator REF 95801(1 x 3 mL)

SAFETY CAUTION (7)

- BIOLABO reagents are designated for professional, in vitro diagnostic use.
- Verify the integrity of the contents before use.
- Caution: Human origin (vial R4). Each component of this product has been tested with FDA-approved methods and found non reactive for the presence of HBsAg, HCV and antibodies to HIV1/2. Because no known test method can offer complete assurance that infectious agents are absent, this material should be handled as potentially infectious.
- Use adequate protections (overall, gloves, glasses). Do not pipette with mouth.
- Avoid contact with skin and eyes. If spilt, thoroughly wash affected areas with plenty of water.
- Reagents contain sodium azide (concentration < 0.1%) which may react with copper and lead plumbing. Flush with plenty of water when disposing.
- Material Safety Data Sheet is available upon request.
- Waste disposal: Respect legislation in force in the country.

All specimens should be handled as potentially infectious, in accordance with good laboratory practices using appropriate precautions. Respect legislation in force in the country.

REAGENT PREPARATION

Once opened, add promptly to the contents of vial R1, the amount of buffer (vial R2) stated on the label.

Mix gently and wait for complete dissolution before using reagents (approximately 10 minutes).

The contents of vial R3 is ready for use.

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

- Using a volumetric classe 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

Store away from light, in the original vial, tightly closed at 2-8°C.

When free from contamination, used and stored as described in the insert, reagents are stable until expiry date stated on the label.

- Working reagent (vial R1 + vial R2) is stable for at least 28 days at 2-8°C.
- Once reconstituted, Calibrator (vial 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**)

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

INTERFERENCES (3)

Haemoglobin	no interference up to 2000 mg/dL
Free bilirubin	no interference up to 20 mg/dL (342 µmol/L)
Conjugated bilirubin	no interference up to 25 mg/dL (428 µmol/L)
Intralipids	no interference up to 1 %
Glycerol	no interference up to 250 mg/dL
Ascorbic acid	no interference up to 50 mg/dL
Triglycerides	no interference up to 1000 mg/dL.

Enzymes in triglycerides and cholesterol reagents may contaminate the Lipase reagents. To avoid contamination, ensure probes, cuvettes and tubes of the automatic analyzers 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.

CALIBRATION

- Use BIOLABO Lipase Calibrator enclosed in this kit or order **REF 95801 (1 x 3 mL)**

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

It is recommended to calibrate in the following cases:

1. When changing vial of reagent.
2. After maintenance operations on the instrument.
3. When control values obtained are out of range, even after using a new vial of fresh serum.

QUALITY CONTROL

- BIOLABO EXATROL-N Level I **REF 95010**
- BIOLABO EXATROL-P Level II **REF 95011**
- Assayed human control sera referring to the same method.
- 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. Repeat the test with the same control.
2. If control is still out of range, prepare a fresh control serum and repeat the test.
3. If control is still out of range, verify analysis parameters: wavelength, temperature, specimen/reagent ratio, time counting and calibration factor.
4. If control is still out of range, use a new vial of reagent and re-assay
5. If control is still out of range, please contact BIOLABO technical support or your local Agent.

EXPECTED VALUES (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 CHARACTERISTICS

Within run N = 20	Low level	Medium level	High level	Between run N = 20	Low level	Medium level	High level
Mean IU/L	33	118	269	Mean IU/L	34	120	275
S.D. IU/L	0.8	1.5	2.1	S.D. IU/L	1.5	2.7	6.3
C.V. %	2.4	1.2	0.8	C.V. %	4.4	2.3	2.3

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

Comparison studies with commercially available reagent:

$$y = 0.44x - 62.07 \quad r = 0.97$$

LINEARITY

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.

MANUAL PROCEDURE

Use BIOLABO Lipase Calibrator enclosed in this Kit or **REF 95801 (1 x 3 mL)**

Let stand reagents, calibrator, controls 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	Blank	Calibrator	Assay
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.			

Notes: Specific procedures are available upon request for automated instruments. Please contact BIOLABO technical support.

CALCULATION

Calculate the result as follows:

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

For information only:

$$\mu\text{kat/L} = \frac{\text{IU/L}}{60}$$

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, "Biosafety in Microbiological and Biomedical Laboratories", 1988



Manufacturer



Use by



In vitro diagnostic



Temperature limitation



Catalogue number



See insert



Batch number



Store away from light



sufficient for



dilute with