Lipase DC* FS**

Diagnostic reagent for quantitative in vitro determination of lipase in serum or plasma on photometric systems

Order Information

Cat. No.	Kit size					
1 4321 99 10 021	R1	5 x	20 mL	+ R2	1 x	25 mL
1 4321 99 10 023	R1	1 x	800 mL	+ R2	1 x	200 mL
1 4321 99 10 930	R1	4 x	20 mL	+ R2	2 x	10 mL
1 4321 99 90 314	R1	10 x	20 mL	+ R2	2 x	30 mL

Summary [1,2]

Lipases are enzymes which hydrolyze glycerol esters of long fatty acids. The enzyme and its cofactor colipase are produced in the pancreas, lipase being also secreted in small amounts by the salivary glands as well as by gastric, pulmonary and intestinal mucosa. Bile acids and colipase form micellar complexes with the lipids and bind lipase on the substrate/water interface. Determination of lipase is used for investigation of pancreatic disorders. In acute pancreatitis the lipase concentrations rise to 2 - 50 fold the upper reference limit within 4 – 8 hours after the beginning of abdominal pain peaking at 24 hours and decrease within 8 to 14 days. Elevated lipase values can also be observed in chronic pancreatitis and obstruction of the pancreatic duct.

Method

Enzymatic color test
A synthetically produced lipase substrate (1,2-o-dilauryl-rac-glycero-3-glutaric acid-(6-methylresorufin) ester) is added to a micro-emulsion which is specifically split by lipase in the presence of colipase and bile acids. The combination of lipase and bile acids make this specific and reliable for pancreatic lipase without any reaction due to lipolytic enzymes or esterases. The reagent composition has been thoroughly optimized so there are no serum matrix effects.
The generated methylresorufin-ester is spontaneously degraded to methylresorufin. The absorbance by this red dye is directly proportional to the lipase activity in the sample.

Principle

Lipase catalyses the reaction

1,2-o-Dilauryl-rac-glycero-3-glutaric acid(6-methylresorufin) ester

$$\xrightarrow{\text{Lipase / Colipase}}$$

1,2-o-Dilauryl-rac-glycerin + Glutaric acid-(6-methylresorufin)-ester

Glutaric acid-(6-methylresorufin)-ester $\xrightarrow{\text{spontaneous degradation}}$

Glutaric acid + Methylresorufin

The increase in absorbance is determined photometrically.

Reagents

Components and Concentrations

Reagent 1:		
Good's Buffer	pH 8.0	50 mol/L
Taurodesoxycholate		4.3 mmol/L
Desoxycholate		8.0 mmol/L
Calcium chloride		15 mmol/L
Colipase		2.2 mg/L
Reagent 2:		
Tartrate Buffer	pH 4.0	7.5 mmol/L
Taurodesoxycholate		17.2 mmol/L
Color Substrate		0.65 mmol/L

Storage Instructions and Reagent Stability

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 – 8°C and contamination is a voided. Do not freeze the reagents and store them protected from light!

Waste Management

Please refer to local legal requirements.

Reagent Preparation

The reagents are ready to use. Do not shake!

Materials required but not provided

NaCl solution 9 g/L
General laboratory equipment

Warnings and Precautions

1. Reagent 2: Warning. H319 Causes serious eye irritation. P280 Wear protective gloves/protective clothing/ eye protection/face protection. P305+P351+P338 If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P337+P313 If eye irritation persists: Get medical advice/attention.
2. Reagent 1 contains sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
3. Many other clinical reagents contain lipase or high concentrations of detergents. Avoid contamination and carry over! Special care should be taken in combination with triglycerides, HDL and LDL reagents. Cuvettes and other glassware must be cleaned thoroughly after being used for other assays. In case of automated measurement refer to the instrument manual for special washing programs.
4. In very rare cases, samples of patients with gammopathy might give falsified results [11].
5. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
6. For professional use only!

Specimen

Serum or heparin plasma

Stability [8]:	7 days	at	20 – 25°C
	7 days	at	4 – 8°C
	1 year	at	–20°C

Discard contaminated specimens!
Only freeze once!

* direct color
** fluid stable

Assay Procedure

Application sheets for automated systems are available on request.

Wavelength	580 nm, Hg 578 nm
Optical path	1 cm
Temperature	37℃
Measurement	Against reagent blank

	Blank	Sample
Sample or calibrator	-	20 µL
Dist. water	20 µL	-
Reagent 1	1000 µL	1000 µL
Mix carefully (do not shake), incubate 1 to 5 min. Start reaction by adding reagent 2:		
Reagent 2	250 µL	250 µL
Mix, incubate 2 min at 37℃, read absorbance and st art stop watch. After exactly 1 and 2 min read absorbance again and then calculate ΔA/min.		

ΔA/min = [ΔA/min sample or calibrator] – [ΔA/min blank]

Calculation

With calibrator:

$$\text{Lipase [U/L]} = \frac{\Delta A/\text{min Sample}}{\Delta A/\text{min Calibrator}} \times \text{Conc. Calibrator [U/L]}$$

Conversion factor

Lipase [U/L] x 0.0167 = Lipase [µkat/L]

Calibrators and Controls

For the calibration of automated photometric systems, DiaSys TruCal U calibrator is recommended. The assigned values of the calibrator have been made traceable to the molar extinction coefficient of an available measuring method. DiaSys TruLab N and P controls should be assayed for internal quality control. Each laboratory should establish corrective action in case of deviations in control recovery.

	Cat. No.	Kit size
TruCal U	5 9100 99 10 063	20 x 3 mL
	5 9100 99 10 064	6 x 3 mL
TruLab N	5 9000 99 10 062	20 x 5 mL
	5 9000 99 10 061	6 x 5 mL
TruLab P	5 9050 99 10 062	20 x 5 mL
	5 9050 99 10 061	6 x 5 mL

Performance Characteristics

Measuring range

The test has been developed to determine lipase concentrations up to 300 U/L. When values exceed this range samples should be diluted 1 + 1 with NaCl solution (9 g/L) and the result multiplied by 2.

Specificity/Interferences

No interference was observed by ascorbic acid up to 30 mg/dL, free and conjugated bilirubin up to 60 mg/dL, hemoglobin up to 500 mg/dL and lipemia up to 1000 mg/dL triglycerides. For further information on interfering substances refer to Young DS [9].

Sensitivity/Limit of Detection

The lower limit of detection is 3 U/L.

Precision

According to protocol EP-5 of the NCCLS (National Committee of Clinical Laboratory Standards)

Within run precision n = 40	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	13.4	0.24	1.81
Sample 2	58.9	0.60	1.01
Sample 3	103	1.50	1.45

Between day precision n = 40	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	13.4	0.24	1.81
Sample 2	58.9	0.49	0.82
Sample 3	103	0.65	0.63

Method Comparison

A comparison of DiaSys Lipase DC FS (y) with a commercially available colorimetric test (x) using 67 samples gave following results:
Y = 0.96 x – 1.15 U/L; r = 0.999.

Reference Range [10]

≤ 60 U/L ≤ 1.00 µkat/L

Each laboratory should check if reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Literature

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Manufacturer

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