DIALAB Produktion und Vertrieb von chemisch-technischen Produkten und Laborinstrumenten Gesellschaft m.b.H.
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Lipase, Enzymatic, colorimetric

(en) English

REF	Content								
D01441	4 x	50	mL	R1	+	1 x	50	mL	R2
D01440	4 x	25	mL	R1	+	1 x	25	mL	R2
D01443	4 x	10	mL	R1	+	1 x	10	mL	R2
D44911	4 x	50	mL	R1	+	2 x	25	mL	R2
D0433917	4 x	50	mL	R1	+	1 x	50	mL	R2
DA0837	4 x	20	mL	R1	+	1 x	20	mL	R2
DT1037	4 x	20	mL	R1	+	1 x	20	mL	R2
DK0735	4 x	50	mL	R1	+	1 x	50	mL	R2
DE1837	2 x	50	mL	R1	+	2 x	12.5	mL	R2
DB20327	4 x	50	mL	R1	+	1 x	12.5	mL	R2

For professional in vitro diagnostic use only.

INTENDED USE

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

DIAGNOSTIC SIGNIFICANCE[1,2]

Lipases are enzymes which hydrolyze glycerol esters of long fatty acids. The enzyme and its cofactor colipase are produced in the pancreas. In small amounts, lipase is also secreted 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.

TEST PRINCIPLE

The colour substrate 1,2-o-dilauryl-rac-glycero-3-glutaric acid-(-6-methylresorufin) ester is cleaved by pancreatic lipase in the presence of colipase and bile acids, and the resulting dicarboxilic acid ester is hydrolysed under alkaline test conditions to yield the chromophore methylresorufin.
The kinetic of colour formation at 580 nm is monitored and it is proportional to lipase activity in the sample.

1,2-o-Dilauryl-rac-glycero-3-glutaric acid (6-methylresorufin) ester $\xrightarrow{\text{Lipase/Colipase}}$ 1,2-o-Dilauryl-rac-glycerol + Glutaric acid-(6-methylresorufin)-ester

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

REAGENT COMPOSITION

COMPONENTS		CONCENTRATION	
Reagent 1			
Good´s Buffer	pH 8.0		
Colipase		≥ 1	mg/L
Desoxycholate		≥ 1.0	mmol/L
Taurodesoxycholate		≥ 1.0	mmol/L
Calcium ions		≥ 1.0	mmol/L
Detergent			
Preservative			
Reagent 2			
Tartrate Buffer	pH 4.0		
Lipase Substrate		≥ 0.1	mmol/L
Stabilizer			
Preservative			

MATERIAL REQUIRED BUT NOT PROVIDED

Standard or Calibrator eg:

REF	Name	Content
D98485	Diacal Auto	5 x 3 mL
D98485SV	Diacal Auto	1 x 3 mL

Controls, eg:

REF	Name	Content	Description
D98481	Diacon N	12 x 5 mL	Control normal
D14481	Diacon N	5 x 5 mL	Control normal
D98481SV	Diacon N	1 x 5 mL	Control normal
D98482	Diacon P	12 x 5 mL	Control abnormal
D14482	Diacon P	5 x 5 mL	Control abnormal
D98482SV	Diacon P	1 x 5 mL	Control abnormal

- NaCl solution (9 g/L).
- Photometric device.
- General laboratory equipment.

REAGENT PREPARATION

Reagents are ready to use.
Avoid strong shaking!

STORAGE AND STABILITY

Conditions	Store at 2 – 8 °C. Protect from light. Close immediately after use. Avoid contamination. Do not freeze the reagents!
Stability	60 days after first opening of the primary container

Reagent R2 is a microemulsion. Therefore, a slight apparent precipitation could occur, showing a light red deposit on the bottom of vial. This is normal. It is recommended to resuspend solution before analysis, with a mild shaking.

WARNINGS AND PRECAUTIONS

1. Reagent 2: Danger.

H318: Causes serious eye damage.
P280: Wear protective gloves/protective clothing/eye 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.
P310: Immediately call a doctor.

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!
4. Special care should be taken in combination with triglycerides, HDL and LDL reagents containing microbial lipases that could stick on the surface of instrument cuvettes. 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 before lipase determination.
5. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.
6. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
7. In the event of an incident related to the device, report it to the manufacturer and your competent authority as required.
8. For professional use only!

SPECIMEN COLLECTION AND STORAGE

Serum, heparinized plasma.

Stability[9]:
In serum/plasma at 2 - 8 °C 7 days
Discard contaminated specimens.

TEST PROCEDURE

Method: Enzymatic colorimetric, kinetic, increasing reaction
Wavelength: 580 nm
Optical path: 1 cm
Temperature: 37 °C

Bring reagents and samples to room temperature.

Pipette into test tubes	Blank	Calibrator	Sample
Reagent 1	1000 µL	1000 µL	1000 µL
Sample	-		20 µL
Calibrator	-	20 µL	-
Dist. water	20 µL	-	-
Mix carefully (do not shake!), incubate 5 min. at 37 °C. Then add:			
Reagent 2	250 µL	250 µL	250 µL
Mix. Incubate 2 min. at 37°C, read absorbance against Reagent blank and start stop watch. Read absorbance again after exactly 1 and 2 minutes. Calculate: ΔA/min = [ΔA/min sample or calibrator] – [ΔA/min blank]			

Automation

Special adaptations for automated analysers can be made on request.

INTERPRETATION OF RESULTS

Calculation
Serum/Plasma:

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

Unit Conversion
Lipase [U/L] x 0.01667 = Lipase [µkat/L]

QUALITY CONTROL AND CALIBRATION

It is suggested to perform an internal quality control. We recommend the DIALAB serum controls **Diacon N** (control serum with values in the normal range) and **Diacon P** (control serum with values in the abnormal range).
Each laboratory should establish corrective action in case of deviations in control recovery.

Calibration
The assay requires the use of a Lipase Standard or Calibrator.
We recommend the DIALAB multi calibration serum **Diacal Auto**.

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PERFORMANCE CHARACTERISTICS

Tests were performed on the instrument Ilab650.

Exemplary data mentioned below may slightly differ in case of deviating measurement conditions.

Precision

Within run (n=10)	Sample 1	Sample 2
Mean [U/L]	49.9	110.5
CV [%]	1.30	1.53
Between day (n=20)	**Sample 1**	**Sample 2**
Mean [U/L]	50.0	110.9
CV [%]	2.87	3.53

Analytical Sensitivity

Limit of detection: 1 U/L.

Linearity and measuring range

The assay has been developed to determine lipase within a measuring range from 1 – 300 U/L. If this value is exceeded, samples should be diluted 1 + 1 with NaCl solution (9 g/L) and the result multiplied by 2.

Analytical specificity

Interfering substance	No interference up to:
Ascorbic acid	50 mg/dL
Bilirubin	50 mg/dL
Hemoglobin	400 mg/dL
Lipemia (Triglycerides)	1000 mg/dL

For further information on interfering substances refer to Young DS[10].

Clinical performance

Method comparison (n=76)	
Test x	DIALAB Lipase, Enzymatic, colorimetric Previous formulation
Test y	DIALAB Lipase, Enzymatic, colorimetric Current formulation
Slope	1.017
Intercept	-1.452 U/L
r^2	0.990

TRACEABILITY

The assigned values of Lipase in the calibrator Diacal Auto have been made traceable to the molar extinction coefficient ε according to an available measurement procedure.

EXPECTED VALUES

Normal subjects[8]	≤ 60 U/L (≤ 1.00 µkat/L)

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

LIMITATIONS

- Eventual Lipase (Enzymatic, colorimetric) carry-over to reagents Calcium (Arsenazo), Calcium (CPC), Magnesium (Xylidyl blue) and Triglycerides (GPO-PAP). The actual carry-over depends on the analyser.

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

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