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

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 makes 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 < Lipase / Colipase ->

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

Glutaric acid-(6-methylresorufin)-ester < 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 mmol/L
Taurodesoxycholate 4.3 mmol/L
Desoxycholate 8.0 mmol/L
Calcium chloride 15 mmol/L
Colipase (porcine) 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 avoided. Do not freeze the reagents and store them protected from light!

Note: A slight red precipitate may occur in reagent 2 which does not affect the performance of the test. Please do not resuspend before use!

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
2. Reagent 1 contains sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
3. Reagent 1: contains animal material. Handle the product as potential infectious according to universal precautions and good clinical laboratory practices.
4. 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.
5. In very rare cases, samples of patients with gamopathy might give falsified results [11].
6. 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.
7. 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!

Assay Procedure
Application sheets for automated systems are available on request.

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

Sample or calibrator
Blank Sample
Dist. water 20 µL 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°C, read absorbance and start 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

DiaSys TruCal U calibrator is recommended for the calibration of automated photometric systems. 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.

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Kit size</th>
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<tbody>
<tr>
<td>TruCal U</td>
<td>5 9100 99 10 063 20 x 3 mL</td>
</tr>
<tr>
<td>TruLab N</td>
<td>5 9000 99 10 062 20 x 5 mL</td>
</tr>
<tr>
<td>TruLab P</td>
<td>5 9050 99 10 061 6 x 5 mL</td>
</tr>
</tbody>
</table>

Performance Characteristics

Data evaluated on BioMajesty® JCA-BM6010/C

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

- Measuring range 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.
- Limit of detection: 5 U/L

<table>
<thead>
<tr>
<th>Interfering substance</th>
<th>Interferences &lt; 10% up to</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid</td>
<td>60 mg/dL</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>70 mg/dL</td>
</tr>
<tr>
<td>Ditaurobilirubin</td>
<td>60 mg/dL</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>600 mg/dL</td>
</tr>
<tr>
<td>Lipemia (triglycerides)</td>
<td>2000 mg/dL</td>
</tr>
<tr>
<td>N-acetylcysteine (NAC)</td>
<td>2000 mg/L</td>
</tr>
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</table>

For further information on interfering substances refer to Young DS [9].

Precision

<table>
<thead>
<tr>
<th></th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean [U/L]</td>
<td>30.9</td>
<td>60.9</td>
<td>286</td>
</tr>
<tr>
<td>CV [%]</td>
<td>1.26</td>
<td>0.611</td>
<td>0.263</td>
</tr>
</tbody>
</table>

Total Precision (n=80)

<table>
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<th>Sample 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean [U/L]</td>
<td>30.2</td>
<td>59.9</td>
<td>284</td>
</tr>
<tr>
<td>CV [%]</td>
<td>2.01</td>
<td>1.20</td>
<td>1.10</td>
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</table>

Method comparison (n=107)

<table>
<thead>
<tr>
<th></th>
<th>Competitor Lipase (Cobas c311)</th>
<th>DiaSys Lipase DC FS (BioMajesty® JCA-BM6010/C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope</td>
<td>0.982</td>
<td>0.982</td>
</tr>
<tr>
<td>Intercep</td>
<td>–0.168 U/L</td>
<td>–0.168 U/L</td>
</tr>
<tr>
<td>Coefficient of correlation</td>
<td>0.999</td>
<td>0.999</td>
</tr>
</tbody>
</table>

Reference Range [10]

\( \leq 60 \text{ U/L} \) \( \leq 1.00 \text{ µkat/L} \)

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

Literature


Manufacturer

DiaSys Diagnostic Systems GmbH
Alte Strasse 9 65558 Holzheim Germany

**IVD**

*Manufacturer*

DiaSys Diagnostic Systems GmbH
Alte Strasse 9 65558 Holzheim Germany

*** according to CLSI document EP17-A2, Vol. 32, No. 8