**Lipase**

Enzymatic, colorimetric

2 Reagents

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

<table>
<thead>
<tr>
<th>Ref.No.</th>
<th>Kit Size</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>D01441</td>
<td>5 x 50 mL</td>
<td>4 x 50 mL R1 + 1 x 50 mL R2</td>
</tr>
<tr>
<td>D01440</td>
<td>5 x 25 mL</td>
<td>4 x 25 mL R1 + 1 x 25 mL R2</td>
</tr>
<tr>
<td>D01443</td>
<td>5 x 10 mL</td>
<td>4 x 10 mL R1 + 1 x 10 mL R2</td>
</tr>
<tr>
<td>D44911</td>
<td>5 x 50 mL</td>
<td>4 x 50 mL R1 + 2 x 25 mL R2</td>
</tr>
<tr>
<td>D433917</td>
<td>5 x 50 mL</td>
<td>4 x 50 mL R1 + 1 x 50 mL R2</td>
</tr>
<tr>
<td>DA0837</td>
<td>5 x 20 mL</td>
<td>4 x 20 mL R1 + 1 x 20 mL R2</td>
</tr>
<tr>
<td>DT1037</td>
<td>5 x 20 mL</td>
<td>4 x 20 mL R1 + 1 x 20 mL R2</td>
</tr>
<tr>
<td>DK0735</td>
<td>5 x 50 mL</td>
<td>4 x 50 mL R1 + 1 x 50 mL R2</td>
</tr>
<tr>
<td>DE1837</td>
<td>2 x 62.5 mL</td>
<td>2 x 50 mL R1 + 2 x 12.5 mL R2</td>
</tr>
</tbody>
</table>

Additionally available:
- D98485 5 x 3 mL Calibrator Diacal Auto
- D98485SV 1 x 3 mL Calibrator Diacal Auto
- D98481 12 x 5 mL Control normal Diacal N
- D14481 5 x 5 mL Control normal Diacal N
- D98481SV1 5 x 1 mL Control normal Diacal N
- D98482 12 x 5 mL Control abnormal Diacal P
- D14482 5 x 5 mL Control abnormal Diacal P
- D98482SV1 5 x 1 mL Control abnormal Diacal P

**TEST PARAMETERS**

- Method: Enzymatic colorimetric, kinetic, increasing reaction
- Temperature: 37°C
- Wavelength: 580 nm
- Sample: Serum, heparinized plasma
- Linearity: up to 300 U/L
- Sensitivity: The lower limit of detection is 1 U/L

**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. 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 decreasing within 8 to 14 days. Elevated lipase values can also occur, showing a light red deposit on the bottom of the vial. This is normal. It is recommended to resuspend the solution before analysis, with a mild shaking.

**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 \(<\text{Lipase} / \text{Colipase}\>)
1,2-o-Dilauryl-rac-glycero + Glutaric acid-(6-methylresorufin)-ester \(\text{spontaneous degradation}\) \(\rightarrow\) Glutaric acid + Methylresorufin

**REAGENT COMPOSITION**

**COMPONENTS**

**CONCENTRATION**

<table>
<thead>
<tr>
<th>Reagent 1:</th>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goods Buffer</td>
<td>pH 8.0</td>
<td></td>
</tr>
<tr>
<td>Colipase</td>
<td>≥ 2 mg/L</td>
<td></td>
</tr>
<tr>
<td>Desoxycholate</td>
<td>≥ 1.0 mmol/L</td>
<td></td>
</tr>
<tr>
<td>Taurodesoxycholate</td>
<td>≥ 1.0 mmol/L</td>
<td></td>
</tr>
<tr>
<td>Calcium ions</td>
<td>≥ 1.0 mmol/L</td>
<td></td>
</tr>
<tr>
<td>Detergent</td>
<td>Preservative</td>
<td></td>
</tr>
</tbody>
</table>

**REAGENT 2:**

- Tartrate Buffer pH 4.0
- Colour Substrate ≥ 0.1 mmol/L
- Stabiliser
- Preservative

**REAGENT PREPARATION**

Reagents are ready to use. Avoid strong shaking!

**REAGENT STABILITY AND STORAGE**

- Stability: at 2 – 8 °C
- Storage: up to the expiration date
- Stability: after first opening use preferably within 60 days when stored at 2 – 8 °C
- Reagent 2 is a microemulsion. Therefore, a slight precipitation can occur, showing a light red deposit on the bottom of the vial. It is recommended to resuspend the solution before analysis, with a mild shaking.

**SAMPLE STABILITY AND STORAGE**

- Stability: at 2 – 8 °C
- Stability: 7 days
- Discard contaminated specimens.

**MATERIALS REQUIRED BUT NOT PROVIDED**

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

**MANUAL TEST PROCEDURE**

Bring reagents and samples to room temperature.

<table>
<thead>
<tr>
<th>Pipette into test tubes</th>
<th>Blank</th>
<th>Calibrator</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 1</td>
<td>1000 µL</td>
<td>1000 µL</td>
<td>1000 µL</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>20 µL</td>
<td>-</td>
</tr>
<tr>
<td>Calibrator</td>
<td>-</td>
<td>20 µL</td>
<td>-</td>
</tr>
<tr>
<td>Dist. water</td>
<td>20 µL</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Mix carefully (do not shake!), incubate 5 min. at 37 °C. Then:

- Reagent 2: 250 µL, 250 µL, 250 µL
- Mix carefully (do not shake!), incubate 2 min. (37 °C), read absorbance against Reagent blank and start stop watch.
- Mix carefully again after exactly 1 and 2 minutes. Calculate:

\[ \Delta A/\text{min} = [\Delta A/\text{min sample or calibrator}] - [\Delta A/\text{min blank}] \]

**CALCULATION**

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

**REFERENCE RANGE [8]**

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

* Each laboratory should check if reference ranges are transferable to its own patient population and determine own reference ranges if necessary.
PERFORMANCE CHARACTERISTICS
LINEARITY, MEASURING RANGE
The assay is linear up to 300 U/L.
If this value is exceeded, samples should be diluted 1 + 1 with saline solution (9 g/L NaCl in dist. water) and the results multiplied by 2.

SENSITIVITY/LIMIT OF DETECTION
The limit of detection is 1 U/L.

PRECISION (at 37°C)
The limit of detection is 1 U/L.

<table>
<thead>
<tr>
<th></th>
<th>Intra assay</th>
<th>Inter assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean [U/L]</td>
<td>SD [U/L]</td>
</tr>
<tr>
<td>Sample 1</td>
<td>60.6</td>
<td>0.54</td>
</tr>
<tr>
<td>Sample 2</td>
<td>90.4</td>
<td>0.70</td>
</tr>
<tr>
<td>n = 20</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean [U/L]</td>
<td>SD [U/L]</td>
</tr>
<tr>
<td>Sample 1</td>
<td>59.9</td>
<td>1.76</td>
</tr>
<tr>
<td>Sample 2</td>
<td>90.3</td>
<td>1.80</td>
</tr>
</tbody>
</table>

SPECIFICITY/INTERFERENCES
No interference was observed in the presence of:
- Ascorbic acid ≤ 50 mg/dL
- Bilirubin ≤ 50 mg/dL
- Hemoglobin ≤ 400 mg/dL
- Triglycerides ≤ 1000 mg/dL

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

METHOD COMPARISON
A comparison between Dialab Lipase (y) and a commercially available colorimetric test (x) using 89 samples gave following results:
y = 0.93 x + 0.50 U/L; r² = 0.99.

QUALITY CONTROL
All control sera with Lipase values determined by this method can be used.
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.

AUTOMATION
Special adaptations for automated analyzers can be made on request.

WASTE MANAGEMENT
Please refer to local legal requirements.

WARNINGS AND PRECAUTIONS
1. Reagent 2: Danger.
   H318: Causes serious eye damage.
   P280: Wear protective gloves/ 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. For professional use only!

REFERENCES