Pancreatic amylase CC FS **

Diagnostic reagent for quantitative in vitro determination of pancreatic amylase in serum, plasma or urine on photometric systems

Order Information

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Kit size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 0551 99 10 021</td>
<td>R1 5 x 20 mL + R2 1 x 25 mL</td>
</tr>
<tr>
<td>1 0551 99 10 023</td>
<td>R1 1 x 800 mL + R2 1 x 200 mL</td>
</tr>
<tr>
<td>1 0551 99 10 930</td>
<td>R1 4 x 20 mL + R2 2 x 10 mL</td>
</tr>
</tbody>
</table>

Summary [1,2]

α-Amylases are hydrolytic enzymes which break down starch into maltose. In the human body α-amylases originate from various organs: the pancreatic amylase is produced by the pancreas and released into the intestinal tract, the salivary amylase is synthesized in the salivary glands and secreted into saliva. As the pancreatic and the salivary amylase show a structural homology of 97%, the only method to distinguish both sufficiently is to use an assay based on monoclonal antibodies to inhibit the salivary enzyme. The amylase present in the blood is eliminated through the kidney and excreted into the urine. Therefore, an elevation of serum activity is reflected in the rise of urinary amylase activity.

Method

Enzymatic photometric test, in which the substrate 4,6-ethylidene-(G7)-p-nitrophenyl-(G1)-A-D-maltoheptaoside (EPS-G7) is cleaved by α-amylases into various fragments. These are further hydrolyzed in a second step by α-glucosidase producing glucose and p-nitrophenol [1,2]. As the salivary isoenzyme is inhibited selectively by a combination of two monoclonal antibodies during the preincubation phase, the increase in absorbance represents the pancreatic amylase activity in the sample [3-5].

Principle

\[
\text{5 EPS-G7} + 5 \text{H}_2\text{O} \quad \alpha\text{-Amylase} \quad 5 \text{PNP} \\
2 \text{G2PNP} + 2 \text{G3PNP} + \text{G4PNP} + 14 \text{G} \quad \text{PNP} \quad \alpha\text{-Glucosidase} \\
\text{5 PNP} + \text{PNP} \\
\text{PNP} = \text{p-Nitrophenol, G = Glucose}
\]

Reagents

Components and Concentrations

R1: Good’s buffer pH 7.15 0.1 mol/L
NaCl 62.5 mmol/L
MgCl₂ 12.5 mmol/L
α-Glucosidase ≥ 2.5 kU/L
Monoclonal antibodies against salivary amylase (mouse)

R2: Good’s buffer pH 7.15 0.1 mol/L
EPS-G7 8.5 mmol/L

Reagent Preparation

1. Mix, incubate for approx. 3 min., then add:
   - 20 µL Sample/Calibrator
   - 1000 µL Reagent 1
   - 250 µL Reagent 2

2. Mix, read absorbance after 2 min. and start stopwatch.

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, protected from light and contamination is avoided. Do not freeze the reagents!

Warnings and Precautions

1. The remaining activity of salivary α-amylase is up to 3%. Very rarely extremely high activities of salivary α-amylase may lead to increased readings of pancreatic α-amylase. However saliva and skin do contain α-amylase, therefore never pipette by mouth and avoid skin contact with the reagents.
2. The reagents contain 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 potentially infectious according to universal precautions and good laboratory practice.
4. In very rare cases, samples of patients with gammopathy might give falsified results [10].
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!

Waste Management

Please refer to local legal requirements.

Reagent Preparation

The reagents are ready to use.

Materials required but not provided

- NaCl solution 9 g/L
- General laboratory equipment

Specimen

Serum, heparin plasma or EDTA plasma, urine

Stability [8]:

- in serum/plasma: 7 days at 20 – 25°C
- 7 days at 4 – 8°C
- 1 year at -20°C
- in urine: 2 days at 20 – 25°C
- 10 days at 4 – 8°C
- 3 weeks at -20°C

Freeze only once! Discard contaminated specimens.

Assay Procedure

Application sheets for automated systems are available on request.

Wavelength

Hg 405 nm

Optical path

1 cm

Temperature

37°C

Measurement

Against reagent blank

<table>
<thead>
<tr>
<th>Sample/Calibrator</th>
<th>Serum / Plasma</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 µL</td>
<td>10 µL</td>
<td></td>
</tr>
</tbody>
</table>

Reagent 1

- 1000 µL
- 1000 µL

Reagent 2

- 250 µL
- 250 µL

Mix, incubate for approx. 3 min., then add:

Read absorbance after 2 min. and start stopwatch.

Read absorbance again 1, 2 and 3 min thereafter.
Calculation

With factor

Calculate ∆A/min from absorbance readings and multiply by the corresponding factor:

\[ \Delta A/\text{min} \times 5670 = \text{Pancreatic amylase activity} \ [\text{U/L}] \]

With calibrator

\[ P - \text{Amyl}. \ [\text{U/L}] = \frac{\Delta A/\text{min Sample}}{\Delta A/\text{min Calibrator}} \times \text{Conc. Calibrator} \ [\text{U/L}] \]

Conversion factor

Pancreatic amylase [U/L] x 0.0167 = Pancreatic amylase [µkat/L]

Calibrators and Controls

For the calibration of automated photometric systems, DiaSys TruCal U calibrator is recommended. This method is traceable to the molar extinction coefficient. For internal quality control DiaSys TruLab N and P controls should be assayed. Each laboratory should establish corrective action in case of deviations in control recovery.

<table>
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<th>Cat. No.</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 20 x 5 mL</td>
</tr>
</tbody>
</table>

Performance Characteristics

Measuring range

On automated systems the test is suitable for the determination of pancreatic amylase activities up to 2000 U/L.

In case of a manual procedure, the test is suitable for pancreatic amylase activities which correspond to a maximum of ∆A/min of 0.350.

If such value is exceeded the sample should be diluted 1 + 10 with NaCl solution (9 g/L) and results multiplied by 11.

Specificity/Interferences

No interference was observed by ascorbic acid up to 30 mg/dL, bilirubin up to 40 mg/dL, lipemia up to 2,000 mg/dL triglycerides and Hemoglobin up to 150 mg/dL. For further information on interfering substances refer to Young DS [9].

Sensitivity/Limit of Detection

The lower limit of detection is 5 U/L.

Precision

<table>
<thead>
<tr>
<th>Intra-assay precision</th>
<th>Mean [U/L]</th>
<th>SD [U/L]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>69.7</td>
<td>2.18</td>
<td>3.13</td>
</tr>
<tr>
<td>Sample 2</td>
<td>207</td>
<td>2.61</td>
<td>1.26</td>
</tr>
<tr>
<td>Sample 3</td>
<td>370</td>
<td>3.36</td>
<td>0.91</td>
</tr>
</tbody>
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<th>SD [U/L]</th>
<th>CV [%]</th>
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<tbody>
<tr>
<td>Sample 1</td>
<td>68.3</td>
<td>1.48</td>
<td>2.17</td>
</tr>
<tr>
<td>Sample 2</td>
<td>204</td>
<td>1.61</td>
<td>0.79</td>
</tr>
<tr>
<td>Sample 3</td>
<td>371</td>
<td>3.14</td>
<td>0.85</td>
</tr>
</tbody>
</table>

Method Comparison

A comparison of DiaSys Pancreatic amylase CC FS (y) with a commercially available test (x) using 58 samples gave following results:

\[ y = 0.97 \times x - 1.66 \ \text{U/L}; \ r^2 = 0.994. \]

Reference Range [7]

<table>
<thead>
<tr>
<th></th>
<th>Women</th>
<th>Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum/plasma</td>
<td>U/L</td>
<td>µkat/L</td>
</tr>
<tr>
<td></td>
<td>&lt; 53</td>
<td>&lt; 0.88</td>
</tr>
<tr>
<td>Urine</td>
<td>&lt; 319</td>
<td>&lt; 5.32</td>
</tr>
</tbody>
</table>

Each laboratory should check if the 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