## Enzymatic colorimetric determination of pancreatic alpha-amylase according to the IFCC mod. recommendations in serum and plasma

### REAGENT 1: 2 x 40 mL

### REAGENT 2: 1 x 20 mL

**PRECAUTIONS IN USE**

In addition to the possible risks regarding the reactive components, product may contain non-reactive components such as preservatives (i.e. sodium azide or other) and detergents. The total concentration of these components is lower than the limits reported by the 67/548/EEC and 1999/45/EC directives and modifications and amendments regarding classification, labelling and packaging of dangerous preparations (reagents) have been made accordingly. However, it is recommended that this product be handled carefully, that ingestion and contact with eyes, skin and mucous membranes be avoided and that laboratory reagents are used according to good laboratory practice.

### SUMMARY

Alpha-amylases are hydrolitic enzymes which break down starch into maltose. In the human body, alpha-amylases originate from various organs that give the corresponding name to the enzyme. The pancreatic alpha-amylase is produced almost exclusively by the pancreas and released into the intestinal tract; the salivary alpha-amylase, mainly synthesized in the salivary glands, is secreted into saliva and is also present in tears, sweat and amniotic fluid. Pancreatic alpha-amylase assays are suitable for monitoring acute pancreatitis and acute attacks during chronic pancreatitis.

### PRINCIPLE

The enzymatic colorimetric assay for pancreatic alpha-amylase determination is carried out in two successive steps. In the first incubation step, the activity of the human salivary alpha-amylase is carried out in two successive steps. In the first reaction step the pancreatic alpha-amylase catalyzes the hydrolysis of the EPS substrate (Ethylidene Protected Substrate) p-nitrophenyl-maltotetraose 4,6-ethylidene-blocked (ethylidene-G7PNP) forming 2 ethylidene-G6 + 2 G2PNP + 2 ethylidene-G4+ 2 G3PNP + ethylidene-G5 + G4PNP. The α-glucosidase hydrolyses all fragments of G2PNP, G3PNP and G4PNP into p-nitrophenol (PNP) and glucose (G). The increase of absorbance, due to PNP formation, is proportional to the activity of pancreatic alpha-amylase in the examined sample.

### REAGENTS

Reagents, stored at 2-8 °C in unopened vials, are stable up to the expiry date indicated on the package.

**Reagents must be limpid; do not use if turbid.**

Components of the kit and initial concentration of reactive components:

- **REAGENT 1**
  - HEPES* buffer 52.5 mmol/L pH 7.15, 4,6-ethylidene-G3PNP ≥ 4 mmol/L, sodium azide < 0.1%  
  
  *HEPES: 2-[4-(2-hydroxyethyl)-1-piperazinyl]-ethane sulfonic acid

- **REAGENT 2**
  - HEPES* buffer 52.5 mmol/L pH 7.15, 4,6-ethylidene-G7PNP ≥ 4 mmol/L, sodium azide < 0.1%  

Barcode and bottle code number, if printed on reagent labels, are referred to the use of the product on Hitachi 911/912 analyzers. Please refer to the application and detailed information available upon request.

### NOTES AND LIMITATIONS

1. Do not pipette by mouth and avoid any contact with skin because both saliva and sweat contain alpha-amylase.
2. For diagnostic purposes, pancreatic alpha-amylase results should always be assessed in conjunction with the patient’s medical history, clinical examination and other findings.
3. A slight yellow color of REAGENT 2 does not influence the reagent performance.
4. In order to avoid wrong clinical interpretations, use only the calibrators that are commutable with the examined patient sera and control materials.

#### Preparation of reagent solutions

**REAGENT 1 and REAGENT 2**: ready to use. Reagents in unopened vials are stable up to the expiry date indicated on the package. Stability: 90 days at 2-8 °C after opening, if contamination is avoided and vials are recapped immediately after use.

#### CALIBRATION

For the calibration, it is recommended that the following materials be used:

**Clin Chem Cal** [REF 16550] 4x3 mL  
Lyophilized calibration serum. For use, follow the instructions contained in the kit.

#### QUALITY CONTROL

The use of following control materials at different levels of analyte is recommended to verify test accuracy:

**Clin Chem Control 1** [REF 16150] 6x5 mL  
Lyophilized control serum. For use, follow the instructions contained in the kit.

**Clin Chem Control 2** [REF 16250] 6x5 mL  
Lyophilized control serum. For use, follow the instructions contained in the kit.

#### SAMPLE

Serum or plasma (heparin, EDTA). Collect samples in accordance with the NCCLS procedure reported in the bibliography (1). Stability of the sample: 30 days at 2-8 °C.
Instrumentation and materials required but not provided
- Usual laboratory equipment
- Filters photometer or spectrophotometer

ANALYTICAL PROCEDURE
Wavelength: 405 nm
Pathlength: 1 cm
Temperature: 37 °C
Sample/REAGENT 1/REAGENT 2: 1/25/6.25
Reaction: kinetic (increase)

Allow reagents to reach working temperature before using. A proportional variation of the reaction volumes indicated in the analytical procedure does not change the result.

Put into cuvette:
- Sample 0.04 mL
- REAGENT 1 1.0 mL

Mix and incubate for about 5 minutes at working temperature. Add:
- REAGENT 2 0.25 mL

Mix carefully. Read the absorbance after 1 minute and repeat the absorbance readings after exactly 1, 2 and 3 minutes. Calculate the mean of ΔA/min.

CALCULATION

ΔA/min x F = pancreatic alpha-amylase activity (U/L)

F = 3730 at 405 nm

This F factor is valid only for the above-mentioned working conditions (wavelength, sample volume, final reaction volume and pathlength).

REFERENCE VALUES
Serum-plasma: 8 - 53 U/L

It is recommended that each laboratory establish its own expected range.

PERFORMANCES
(determined on Hitachi automatic analyzer)

Interferences: the test is not affected by the presence of bilirubin up to 40 mg/dL, hemoglobin up to 0.5 g/dL, and triglycerides up to 1500 mg/dL.
Measuring range: 2 - 1500 U/L. Samples with concentration higher than 1500 U/L must be diluted 1:10 with normal saline and result multiplied by 10.

Intra-Assay Precision: it was determined on 20 replicates of each control (3 levels - L1/L2/L3). Results were as follows:

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Within run SD</th>
<th>CV%</th>
<th>Run to run SD</th>
<th>CV%</th>
<th>Total SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>27.70</td>
<td>0.55</td>
<td>1.98</td>
<td>0.17</td>
<td>0.60</td>
<td>0.57</td>
<td>2.07</td>
</tr>
<tr>
<td>L2</td>
<td>113.50</td>
<td>1.18</td>
<td>1.04</td>
<td>1.38</td>
<td>1.22</td>
<td>1.82</td>
<td>1.60</td>
</tr>
<tr>
<td>L3</td>
<td>246.85</td>
<td>2.56</td>
<td>1.04</td>
<td>3.48</td>
<td>1.41</td>
<td>4.32</td>
<td>1.75</td>
</tr>
</tbody>
</table>

Sensitivity: 2 U/L. Sensitivity was calculated on 10 replicates of normal saline and reported as the "mean zero value + 3 SD".

Accuracy: this test (y) was compared with a commercially available method (x).

Results were as follows:
N = 60, r = 0.99871, y = 1.081 x -0.0543

WASTE MANAGEMENT
Reagents must be disposed of in accordance with local regulations.

BIBLIOGRAPHY

Note: changes in comparison to the previous version are indicated by a vertical bar in the text margin.