Reagent kit for determination of the α-amylase activity in serum or urine based upon the IFCC EPS method.

Measurements of amylase are used primarily in the diagnosis and treatment of the diseases of the pancreas. Amylase is found in the pancreas and salivary glands. When released in the digestive tract, the enzyme hydrolyzes starch. Amylase determinations are useful in the diagnosis of diseases of the pancreas and parotids. Elevated serum levels are associated with acute pancreatitis and other pancreatic disorders as well as mumps and bacterial parotitis.

Principle

The procedure utilizes a different auxiliary enzyme α-glucosidase, which cleaves all primary degradation products and leads to a 100% chromophore release from the substrate.

\[ \text{5-Ethylidene-G7-pNP+5H}_2\text{O} \rightarrow \alpha-\text{amylase} \rightarrow \text{2Ethylidene-G5} + \text{2G2-pNP+2 Ethylidene-G4+2 G3-pNP+Ethylidene-G3+G4-pNP} \]

\[ +2 \text{G2-pNP+2G3-pNP+G4-pNP+14H}_2\text{O} \rightarrow \alpha-\text{glucosidase} \rightarrow \text{5-pNP+14G} + \text{G-glucose, pNP+nitrophenol} \]

Reference values

Serum: ≤100 U/l (1.67 µkat/l)
Urine: ≤400 U/l (6.67 µkat/l)

It is recommended that each laboratory should assign its own normal range.

Reagents

Reagent (R1)
HEPES buffer, pH=7.15
NaCl
MgCl₂
α-glucosidase

Reagent (R2)
4,6-Ethylidene-G7-pNP (EPS) 3 mmol/l
HEPES buffer, pH=7.15

Precaution

Discard cloudy reagent. Avoid contamination by using clean laboratory materials (pipettes, plastic vials,...) for analyzers. These reagents contain 0.1 % sodium azide. To avoid the possible build-up of azide compounds, flush waste-pipes with water after the disposal of unneeded reagent. Do not use citrate, oxalate or EDTA anti-coagulant. Do not pipette by mouth and avoid contamination with skin! (Sweat and saliva contain amylase activity at 405 nm).

Sample

Serum free of haemolysis and urine.

**PROCEDURE**

Preparation and stability of working reagent

One-reagent procedure:
Mix 5 volumes of R1 with 1 volume of R2.
Stability:
- at 20-25 °C: 5 days
- at 2-8 °C: 4 weeks

Two-reagent procedure:
The reagents are ready for use. If the absorbance of working reagent is higher than 0.5 at 405 nm the reagent can not be used.

Assay conditions

Wavelength: 405 (400-420) nm
Temperature: 37°C
Cuvette: 1-cm light path
Method: kinetic (increasing)
Read against: distilled water

One-reagent procedure

<table>
<thead>
<tr>
<th></th>
<th>Sample</th>
<th>Working reagent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working reagent</td>
<td>600 μl</td>
<td></td>
</tr>
<tr>
<td>Sample</td>
<td>8 μl</td>
<td></td>
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</tbody>
</table>

Mix and after a 1-minute incubation, measure the change of absorbance per minute (ΔA/min) for 3 minutes.

Two-reagent procedure

<table>
<thead>
<tr>
<th></th>
<th>Sample</th>
<th>R1</th>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>500 μl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample</td>
<td>8 μl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mix and wait 3 minutes:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R2</td>
<td>100 μl</td>
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</table>

Mix and after a 3-minute incubation, measure the change of absorbance per minute (ΔA/min) for 3 minutes.

**Calculation using calibration**

\[ \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{standard}}} \times C_{\text{standard}} = C_{\text{sample}} \]

A = Absorbance
C = Concentration

Quality control

A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

**PERFORMANCES DATA**

The following data were obtained using the Olympus 600 analyser (37°C).

**Linearity**

The test is linear up to 1800 U/l (30.0 µkat/l).

**Sensitivity**

It is recommended that each laboratory establishes its own range of sensitivity. As this is limited by the sensitivity of the spectrophotometer used. Under manual conditions however, a change of 0.001 Abs units/min is equivalent to 7.28 U/l (0.12 µkat/l) alpha-amylase activity at 405 nm.

**Correlation**

Comparative studies were done to compare our reagent with another commercial alpha-amylase (EPS) assay. The results from these studies are detailed below.

**Specificity**

Bilirubin 1026 μmol/l (60 mg/dl), lipid 1000 mg/dl, glucose 111 mmol/l (2000 mg/dl) and ascorbic acid 5.68 mmol/l (100 mg/dl) don’t interfere with the assay up to the given levels.

**Note**

Do not use reagents after the expiry date stated on each reagent container label. Do not use products, test solutions and reagents described above for any purpose other than described herein.

For in vitro diagnostic use only,

The following symbols are used on labels

**Bibliography**