**α-Amylase direct Fluid monoreagent**

**ENZYMATIC IN VITRO TEST FOR THE QUANTITATIVE DETERMINATION OF α-AMYLASE IN HUMAN SERUM, PLASMA AND URINE**

**QUALITY CONTROL**

Control Serum N 6x5ml 88 41 48B
Control Serum P 6x5ml 88 46 85B
The control intervals and limits must be adjusted to the individual laboratory and country-specific requirements. Values obtained should fall within established limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

**SUMMARY**

The α-amylases (1,4-α-D-glucanohydrolases, EC 3.2.1.1) catalyze the hydrolytic degradation of polymeric carbohydrates such as amylose, amylopectin and glycogen by cleaving 1,4-α-glucosidic bonds. In polysaccharides and oligosaccharides, several glycosidic bonds are hydrolyzed simultaneously. Maltotriose, the smallest such unit, is converted into maltose and glucose, albeit very slowly.

Two types of α-amylases can be distinguished, the pancreatic type (P-type) and the salivary type (S-type). Whereas the P-type can be attributed almost exclusively to the pancreas and is therefore organ-specific, the S-type can originate from a number of sites. As well as appearing in the salivary glands it can also be found in tears, sweat, human milk, amniotic fluid, the lungs, testes and the epithelium of the fallopian tube.

Because of the sparsity of specific clinical symptoms of pancreatic diseases, α-amylase determinations are of considerable importance in pancreatic diagnostics. They are mainly used in the diagnosis and monitoring of acute pancreatitis. Hyperamylasemia does not, however, only occur with acute pancreatitis or in the inflammatory phase of chronic pancreatitis, but also in renal failure (reduced glomerular filtration), tumors of the lungs or ovaries, pulmonary inflammation, diseases of the salivary gland, diabetic ketosis, cerebral trauma, surgical interventions or in the case of macroamylasemia. To confirm pancreatic specificity, it is recommended that an additional pancreas-specific enzyme - lipase or pancreatic-α-amylase- also be determined.

Numerous methods have been described for the determination of α-amylase. These either determine the decrease in the amount of substrate viscometrically, turbidimetrically, nephelometrically or amylolactically or measure the formation of degradation products saccharogenically or kinetically with the aid of enzyme-catalyzed subsequent reactions. The kinetic method described here is based on the cleavage of 2-chloro-4-nitrophenyl-α-D-maltotrioside (CNP-G3) by α-amylase.

**INTENDED USE**


**TEST PRINCIPLE**

Colorimetric test with 2-chloro-4-nitrophenyl-α-D-maltotrioside (CNP-G3) as direct substrate. Color is released directly as a result of a cleavage at the aglycone:

\[ \text{CNP-G3} \rightarrow \text{α-D-maltotrioside} \rightarrow \text{CNP+G} \]

(CNP = chloro-nitrophenol; G = glucose)

The increase of absorption of chloro-nitrophenol is directly proportional to the α-amylase concentration. The hydrolysis pattern in the formulation of the reagent show about less than 10 % CNP-G2 and less than 1 % CNP-G4 as by products.

**NOTES**

For intra vitam diagnostic use. Exercise the normal precautions required for handling all laboratory reagents.

**LIMITATIONS - INTERFERENCE**

Do not pipette by mouth, and ensure that the reagent does not come into contact with the skin. (Saliva and sweat contain α-amylase!)

- Criterion: Recovery within &10% of initial value.
- Impurities: No significant interference up to 70 mg/dl bilirubin.
- Hemolysis: No significant interference up to 170 mg/dl hemoglobin.
- Lipemia (Intralipid): No significant interference up to 2500 mg/dl triglycerides.

An increase of the initial absorbance of the reagent to A > 0.3 (405 nm) indicates a contamination of the reagent.

**MEASURING/REPORTABLE RANGE**

Measuring range: 7U/I (0.12 kkat/I) to 1500 U/I (25.8 kkat/I)
Dilute samples having higher activities with 0.9% NaCl (e.g. 1 + 9). Multiply the result by the appropriate dilution factor (e.g. factor 10).

**REFERENCE VALUE**

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Meas. U/I</th>
<th>SD U/I</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum/plasma</td>
<td>&lt; 220 U/I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spontaneously voided urine</td>
<td>&lt; 1000 U/I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 h urine</td>
<td>&lt; 900 U/I</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range. For diagnostic purposes the α-amylase results should always be assessed in conjunction with the patient’s medical history, clinical examinations and other findings.

**IMPRECISION**

Reproducibility within run was determined using human samples and controls (n = 20). The following results were obtained:

**METHOD COMPARISON**

A comparison of the AXIOM Amylase (y) with a commercial obtainable assay (x) gave with 36 samples the following result:

\[ y = 0.972x + 1.282; r = 0.999 \]

**REAGENT CONCENTRATION**

R1:
- MES buffer, pH 6.0 100 mmol/l
- NaCl 350 mmol/l
- Ca-Acetate 6 mmol/l
- Potassium thiocyanate 900 mmol/l
- CNP-G3 2.27 mmol/l
- Stabilizers and detergents > 0.1 %

**PREPARATION AND STABILITY**

R1: Ready for use
Stability opened: up to expiry date
- at +2°C to +8°C
- at +20°C to +25°C
- at +2°C to +8°C
- at +2°C to +8°C
Please note the legal regulations.

Testing Procedure

Materials provided
- Working solutions as described above
- Calibrators and controls as indicated below
- 0.9% NaCl

Manual procedure:
- Wavelength: Hg 405 nm (400 – 420 nm)
- Temperature: +37°C
- Cuvette: 1cm light path
- Zero adjustment: against air

Mix and incubate 1 min at +37°C. Then read initial absorbance and start stopwatch simultaneously. Read again after exactly 1, 2 and 3 minutes. Determine the mean change of absorbance per minute (ΔA/min) and use this for the calculation.

Calculation:
Use absorption differences to calculate ΔA/min. Multiply with the following factors:

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Ions</th>
<th>Other Metabolites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid Phosphatase</td>
<td>Ammonium fluid</td>
<td>Bilirubin T/D</td>
</tr>
<tr>
<td>Alkaline Phosphatase</td>
<td>Copper fluid</td>
<td>Creatinine Fluid</td>
</tr>
<tr>
<td>α-Amylase direct</td>
<td>Calcium fluid</td>
<td>Glucose GOD-PAP fluid</td>
</tr>
<tr>
<td>CK-NAC activated</td>
<td>Chloride fluid</td>
<td>Glucose Hexokinase fluid</td>
</tr>
<tr>
<td>CK-MB (NAC- activated)</td>
<td>Inorganic Phosphorus UV fluid</td>
<td>Urea Enzymatic fluid</td>
</tr>
<tr>
<td>γ-GT fluid</td>
<td>Iron fluid</td>
<td>Urea UV fluid</td>
</tr>
<tr>
<td>LDH fluid</td>
<td>TIBC</td>
<td>Uric Acid PAP fluid</td>
</tr>
<tr>
<td>Cholinesterase</td>
<td>Magnesium fluid</td>
<td></td>
</tr>
<tr>
<td>GOT/ASAT fluid</td>
<td>Potassium fluid</td>
<td></td>
</tr>
<tr>
<td>GPT/ALAT fluid</td>
<td>Sodium fluid</td>
<td></td>
</tr>
<tr>
<td>Lipase UV fluid</td>
<td>Urea Control Serum N</td>
<td></td>
</tr>
<tr>
<td>Lactate PAP</td>
<td>Control Serum P</td>
<td></td>
</tr>
<tr>
<td>α-HBDH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipids</td>
<td>Alcumin</td>
<td></td>
</tr>
<tr>
<td>Cholesterol fluid</td>
<td>CSF-Protein fluid</td>
<td></td>
</tr>
<tr>
<td>HDL Cholesterol</td>
<td>Microprotein fluid</td>
<td></td>
</tr>
<tr>
<td>LDL Cholesterol</td>
<td>Hemoglobin</td>
<td></td>
</tr>
<tr>
<td>Triglycerides fluid</td>
<td>Protein Total fluid</td>
<td></td>
</tr>
</tbody>
</table>

Disposal

Please note the legal regulations.

Literature

AXIOM Product range Clinical Chemistry