| COD 11799 1 x 25 mL |
|---|
| STORE AT 2-8ºC |
| Reagents for measurement of pancreatic α-amylase concentration<br>Only for *in vitro* use in the clinical laboratory |

**α-AMYLASE-PANCREATIC** BioSystems

CE

# α-AMYLASE-PANCREATIC
Immunoinhibition

## PRINCIPLE OF THE METHOD

Amylase catalyzes the hydrolysis of 4-nitrophenyl-maltoheptaoside-ethylidene to smaller oligosacharides which are hydrolyzed by α-glucosidase liberating 4-nitrophenol. The catalytic concentration is determined from the rate of 4-nitrophenol formation, measured at 405 nm[1,2]. Specific antibodies inhibits the salivary isoenzyme and thus allow the measurement of pancreatic α-amylase[3,4].

## CONTENTS AND COMPOSITION

A. Reagent: 1 x 20 mL. HEPES 50 mmol/L, calcium chloride 0.075 mmol/L, sodium chloride 90 mmol/L, magnesium chloride 13 mmol/L, α-glucosidase > 4 U/mL, pH 7.1, monoclonal antibodies (mouse) 50 mg/L.
B. Reagent: 1 x 5 mL. HEPES 50 mmol/L, 4-Nitrophenyl-maltoheptaoside-ethylidene 18 mmol/L, pH 7.1.

## STORAGE

Store at 2-8ºC.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

– Reagents: Presence of particulate material, turbidity, absorbance of the blank over 0.300 at 405 nm (1 cm cuvette).

## REAGENT PREPARATION

Reagents are provided ready to use.

## ADDITIONAL EQUIPMENT

– Analyzer, spectrophotometer or photometer with cell holder thermostatable at 37ºC and able to read at 405 nm.
– Cuvettes with 1 cm light path.

## SAMPLES

Serum, plasma or urine collected by standard procedures.

Pancreatic α-amylase in serum or plasma is stable for 1 month at 2-8ºC. Use heparin or EDTA as anticoagulant.

Pancreatic α-amylase in urine is stable for 1 month at 2-8ºC if pH is adjusted to approximately 7 before storage. Centrifuge or filter before testing.

## PROCEDURE

1. Bring the Reagent and the instrument to reaction temperature.
2. Pipette into a cuvette: (Notes 1,2)

| | Serum or plasma | Urine |
|---|---|---|
| Reagent (A) | 0.8 mL | 0.8 mL |
| Sample | 30 µL | 15 µL |

3. Mix and insert the cuvette into the instrument. Start the stopwatch. After 3-5 minutes, add:

| | | |
|---|---|---|
| Reagent (B) | 0.2 mL | 0.2 mL |

4. Mix.
5. After 2 minutes, record initial absorbance at 405 nm and at 1 minute intervals thereafter for 3 minutes.
6. Calculate the difference between consecutive absorbances, and the average absorbance difference per minute (ΔA/min).

## CALCULATIONS

Pancreatic α-amylase concentration in the sample is calculated using the following general formula:

$$\Delta A/min \times \frac{Vt \times 10^6}{\varepsilon \times l \times Vs} = U/L$$

The molar absorbance (ε) of 4-nitrophenol at 405 nm is 10600 and the lightpath (l) is 1 cm. For serum and plasma samples, the total reaction volume (Vt) is 1.030 and the sample volume (Vs) is 0.030. For urine samples, the total reaction volume (Vt) is 1.015 and the sample volume (Vs) is 0.015. 1 U/L are 0.0166 µkat/L. The following formulas are deduced for the calculation of the catalytic concentration:

| | | |
|---|---|---|
| ΔA/min | Serum, plasma | x 3239 = U/L<br>x 53.8 = µkat/L |
| | Urine | x 6384 = U/L<br>x 105.9 = µkat/L |

## REFERENCE VALUES

| Serum, plasma[5] | | Urine[5] | |
|---|---|---|---|
| U/L | µkat/L | U/L | µkat/L |
| 13-53 | 0.22-0.88 | 7-356 | 0.12-5.92 |

These ranges are given for orientation only; each laboratory should establish its own reference ranges.

## QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18042), level II (cod. 18043) and the Biochemistry Control Urine (cod. 18054 and cod. 18066) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

## METROLOGICAL CHARACTERISTICS

– Detection limit: 6.7 U/L = 0.11 µkat/L.
– Linearity limit: 1300 U/L = 21.6 µkat/L (serum and plasma) and 2600 U/L = 43.2 µkat/L (urine). For higher values dilute sample 1/5 with distilled water and repeat measurement.
– Repeatibility (within run):

| Serum. Mean Concentration | CV | n |
|---|---|---|
| 62.4 U/L = 1.04 µkat/L | 3.9 % | 20 |
| 138 U/L = 2.29 µkat/L | 1.1 % | 20 |

– Reproducibility (run to run):

| Serum. Mean Concentration | CV | n |
|---|---|---|
| 62.4 U/L = 1.04 µkat/L | 4.3 % | 25 |
| 138 U/L = 2.29 µkat/L | 2.8 % | 25 |

– Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.
– Interferences: Hemoglobin (10 g/L) and bilirubin (20 mg/dL) do not interfere. Lipemia (triglycerides 30 g/L) interfere. Other drugs and substances may interfere[6].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

## DIAGNOSTIC CHARACTERISTICS

α-Amylase catalyzes the hydrolysis of α-1,4-linkages of carbohydrates constituted of α-D-glucose units. The result is the formation of dextrins, maltose and some glucose molecules. α-Amylase is produced mainly by the exocrine pancreas (P-type; P-AMY) and the salivary glands (S-type; S-AMY) but it is also found in other tissues. The enzyme present in normal serum and urine is predominantly of pancreatic and salivary gland origin.

Assays of α-amylase activity in serum and urine are largely of use in the diagnosis of pancreatic diseases such as acute or chronic pancreatitis. Hyperamylasemia can also be due to renal insufficiency, acute pain of the abdomen, tumors of the lungs and the ovaries, salivary glands lesions, macroamylasemia, diabetic ketoacidosis, biliary tract disease, cerebral trauma, chronic alcoholism and drugs (opiates). The lack of specificity of total α-amylase measurements has led to the interest in the direct measurement of pancreatic α-amylase instead of total enzyme activity for the differential diagnosis of patients with acute abdominal pain[7,8].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

## NOTES

1. Saliva and skin do contain α-amylase, therefore never pipette by mouth and avoid skin contact with the reagents.
2. This reagent may be used in several automatic analysers. Instructions for many of them are available on request.

## BIBLIOGRAPHY


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