COD 11583 5 x 5 mL	COD 11550 6 x 25 mL
STORE AT 2-8ºC	
Reagents for measurement of α-amylase concentration Only for *in vitro* use in the clinical laboratory	

α-AMYLASE-DIRECT

BioSystems

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α-AMYLASE-DIRECT
DIRECT SUBSTRATE

PRINCIPLE OF THE METHOD

α-Amylase catalyzes the hydrolysis of 2-chloro-4-nitrophenyl-malto-trioside (CNP-G3) to 2-chloro-4-nitrophenol (CNP). The catalytic concentration is determined from the rate of 2-chloro-4-nitrophenol formation, measured at 405 nm[1,2,3].

$$\text{CNP – G3} \xrightarrow{\alpha\text{-amylase}} \text{CNP + maltotriose}$$

CONTENTS

	COD 11583	COD 11550
A. Reagent	5 x 5 mL	6 x 25 mL

COMPOSITION

A. Reagent: MES 50 mmol/L, calcium chloride 5 mmol/L, sodium chloride 300 mmol/L, sodium thiocyanate 450 mmol/L, CNP-G3 2.25 mmol/L, pH 6.1.

STORAGE

Store at 2-8ºC.

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

Indications of deterioration:

– Reagent: Presence of particulate material, turbidity, absorbance of the blank over 0.500 at 405 nm (1 cm cuvette).

WARNING AND PRECAUTIONS

Exercise the normal precautions required for handling all laboratory reagents. Safety data sheet available for professional user on request. Disposal of all waste material should be in accordance with local guidelines. Any serious incident that might occur in relation to the device shall be reported to BioSystems S.A.

REAGENT PREPARATION

Reagent (A) is 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.

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

α-Amylase in urine is stable for 1 month at 2-8ºC if pH is adjusted to approximately 7 before storage.

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)	1.0 mL	1.0 mL
Sample	20 µL	10 µL

3. Mix and insert the cuvette into the photometer. Start the stopwatch.
4. Record initial absorbance and at 1 minute intervals thereafter for 3 minutes.
5. Calculate the difference between consecutive absorbances, and the average absorbance difference per minute (ΔA/min).

CALCULATIONS

The α-Amylase concentration in the sample is calculated using the following general formula:

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

The molar absorbance (ε) of CNP at 405 nm is 15490 and the lightpath (l) is 1 cm. For serum and plasma samples, the total reaction volume (Vt) is 1.02 at 37ºC and the sample volume (Vs) is 0.02 at 37ºC. For urine samples, the total reaction volume (Vt) is 1.01 at 37ºC and the sample volume (Vs) is 0.01 at 37ºC. 1 U/L are 0.0166 µkat/L. The following formulas are deduced for the calculation of the catalytic concentration:

		37ºC
ΔA/min	Serum, plasma	x 3292 = U/L x 54.9 = µkat/L
	Urine	x 6520 = U/L x 108.7 = µkat/L

REFERENCE VALUES

Reaction temperature	Serum, plasma U/L	Serum, plasma µkat/L	Urine U/L	Urine µkat/L
37ºC[4,5]	22-80	0.37-1.33	< 321	< 5.35

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. 18005, cod. 18009 and cod. 18042), level II (cod. 18007, cod. 18010 and 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: 1.8 U/L = 0.03 µkat/L

– Linearity limit: 1317 U/L = 22 µkat/L (serum and plasma) and 2600 U/L = 43.5 µkat/L (urine). For higher values dilute sample 1/5 with distilled water and repeat measurement.

– Repeatibility (within run):

Mean Concentration	CV	n
64 U/L = 1.07 µkat/L	1.8%	20
338 U/L = 5.63 µkat/L	0.5 %	20

– Reproducibility (run to run):

Mean Concentration	CV	n
64 U/L = 1.07 µkat/L	3.5 %	25
338 U/L = 5.63 µkat/L	1.0 %	25

– Sensitivity:0.304 ΔmA·L/U·min = 18.2 ΔmA·L/µkat·min

– 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: Lipemia (triglycerides 10 g/L) and bilirubin (20 mg/dL) do not interfere. Hemoglobin (2.5 g/L) interfere. Other drugs and substances may interfere[5].

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 dextrans, maltose and some glucose molecules. α-Amylase is produced mainly by the exocrine pancreas (P-type) and the salivary glands (S-type) but it is also found in other tissues.

Assays of 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)[6,7].

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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