



Store at: +2+8°C.

Presentation:

Cod. EZ004 CONT: R 2 x 50 mL.

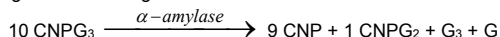
Procedure

Quantitative determination of α-amylase (AMS)

Only for in vitro use in clinical laboratory (IVD)

TEST SUMMARY

The direct alpha amylase hydrolyzes the 2-chloro-4-nitrophenyl-α-D-maltotriose (CNPG₃) to release 2-chloro-nitrophenol (CNP) and form 2-chloro-4-nitrophenyl-α-maltoside (CNPG₂), matotriose (G₃) and glucose (G) according to the following reaction:



The rate of 2-chloro-4-nitrophenol formation, measured photometrically, is proportional to the catalytic concentration of alpha amylase present in the sample¹.

REAGENTS COMPOSITION

R (notes)	MES pH 6.0	100 mmol/L
	CNPG ₃	2.25 mmol/L
	NaCl	350 mmol/L
	Calcium acetate	6 mmol/L
	Potassium thiocyanate	900 mmol/L
	Sodium azide	0.95 g/L

REAGENT PREPARATION AND STABILITY

Liquid reagent is ready to use.

Stability: Once opened is stable 60 days at 2-8°C when properly capped immediately after each opening.

All the components of the kit are stable until the expiration date on the label when stored at 2-8°C, protected from light and contamination prevented during their use.

Do not use reagents over the expiration date.

Signs of Reagent deterioration:

- Presence of particles and turbidity.
- Blank absorbance at 405 nm \geq 0.40

All the reagents of the kit are stable up to the end of the indicated month and year of expiry. Store tightly closed at 2-8°C, Do not use reagents over the expiration date.

SPECIMEN

Serum or plasma¹: remove from cells as soon as possible. It is recommended to use heparin as anticoagulant.

Urine: adjust pH to approximately 7.0 prior to storage.

Stability: 1 month at 2-8°C.

MATERIAL REQUIRED BUT NOT PROVIDED

- Spectrophotometer or colorimeter measuring at 405 nm.
- Thermostatic bath at 37°C. (note 1)
- Matched cuvettes 1.0 cm light path.

General laboratory equipment (note 2).

TEST PROCEDURE

- Assay Conditions
 - Wavelength: 405 nm.
 - Cuvette: 1 cm. light path.
 - Constant temperature 37°C.
- Adjust the instrument to zero with distilled water or air.
- Pipette into a Cuvette:

	Serum or plasma	Urine
R (mL)	1.0	1.0
Sample (μL)	20	10

- Mix and incubate for 30 seconds.
- Read the absorbance (A) of the sample, start the stopwatch and read absorbance at 1 min. interval thereafter for 3 min.
- Calculate the difference of absorbance and the average absorbance difference per minute ($\Delta A/\text{min}$).

CALCULATIONS

Serum, plasma $\Delta A/\text{min} \times 3954^* = \text{U/L of } \alpha\text{-amylase}$

Urine $\Delta A/\text{min} \times 7908^* = \text{U/L of } \alpha\text{-amylase}$

Units: One international unit (IU) is the amount of enzyme that transforms 1 μmol of substrate per minute, in standard conditions. The concentration is expressed in units per litre of sample (U/L).

Conversion factor: U/L x 0.01667 = μkat/L.

QUALITY CONTROL

Control sera are recommended to monitor the performance of the procedure, H Normal Control QC003 and H Pathological Control QC004. If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.

Serum controls are recommended for internal quality control. Each laboratory should establish its own Quality Control scheme and corrective actions.

REFERENCE VALUES³

Serum or plasma: < 90 U/L.

Urine: < 450 U/L.

(These values are for orientation purpose).

It is suggested that each laboratory establish its own reference range.

CLINICAL SIGNIFICANCE

Alpha-amylase is an enzyme that helps to digest the glycogen and the starch. It is produced mainly by exocrine pancreas and salivary glands. This determination is made mainly in diagnosis or to control diseases of the pancreas as acute or chronic pancreatitis. It can also reflect biliary or gastrointestinal disease and other upheavals^{2, 5, 6}.

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENT PERFORMANCE

- Measuring Range:

From detection limit of 0.2439 U/L. to linearity limit of 2200 U/L., under the described assay conditions.

If results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L. and multiply result by 2.

- Precision:

Mean (U/L)	Intra-assay n= 20		Inter-assay n= 20	
	77	194	77.2	197
SD	1.12	2.22	1.08	2.96
CV (%)	1.45	1.15	1.39	1.50

- Sensitivity: 1 U/L = 0.00025 ΔA/min

Accuracy: Results obtained GPL reagents did not show systematic differences when compared with other commercial reagents

The results obtained were the following:

Correlation coefficient (r): 0.98628.

Regression equation: $y=0.746x - 1.2697$

The results of the performance characteristics depend on the analyzer used.

INTERFERING SUBSTANCES

- Hemolysis interferes in the results¹. EDTA and citrate could inhibit alpha-amylase activity.
- A list of drugs and other interfering substances with α-amylase determination has been reported by Young et al.^{3,4}.

NOTES

- The activity of alpha-amylase is temperature dependent. Assays performed at temperatures lower than 37° C. or higher than 37° C. will show an apparent decrease or increase levels.
- Saliva and sweat contain α-amylase. Avoid mouth pipetting and skin contact with the reagent or material used.
- Contains potassium thiocyanate. Avoid inhalation, skin or eyes contact. If it happens, wash with plenty of water and consult a doctor.
- Formulation to reach constant:

$\Delta A/\text{min} \times 3954^*$ or 7908* = U/L α-amylase	$\frac{* \text{ Tv} \times 1000}{\epsilon \times \text{LP} \times \text{Sv}}$	Tv= Total volume in mL ε CNP = at 405 nm LP= Light path Sv= Sample volume in mL
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BIBLIOGRAPHY

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