CHRONOLAB

CE
IVD

α- Amylase

CNPG$_3$. Kinetic. Liquid

Quantitative determination of α-amylase (AMS)

PACKAGING

Ref.: 101-0572	Cont.: 20 x 3 mL
Ref.: 101-0682	Cont.: 2 x 100 mL

Store at 2-8º C

CLINICAL SIGNIFICANCE

α-Amylase (AMS) 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.

PRINCIPLE OF THE METHOD

α-Amylase hydrolyzes the 2-chloro-4-nitrophenyl-α-D-maltotrioside (CNPG3) to release 2-chloro-4-nitrophenol (CNP) and form 2-chloro-4-nitrophenyl-α-D-maltoside (CNPG2), maltotriose (G3) y glucose (G) according to the following reaction:

$$10\ CNPG_3 \xrightarrow{Amilase} 9\ CNP + 1\ CNPG_2 + G_3 + G$$

The rate of 2-chloro-4-nitrophenol formation, measured photometrically, is proportional to the catalytic concentration of α-amylase present in the sample[1].

REAGENTS

R (Note 3)	MES pH 6,0	100 mmol/L
	CNPG3	2.25 mmol/L
	Sodium clorhidre	350 mmol/L
	Calcium acetate	6 mmol/L
	Potassium thiocyanate	900 mmol/L
	Sodium azide	0.95 gr/L

Optional (not included in the kit)

Contro-N	Ref.: 101-0252	4 x 5 mL	Lyophilized human control serum
	Ref.: 101-0083	20 x 5 mL	
Contro-P	Ref.: 101-0253	4 x 5 mL	Lyophilized human control serum
	Ref.: 101-0084	20 x 5 mL	

PREPARATION

The reagent is ready to use.

STORAGE AND STABILITY

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

Do not use reagents over the expiration date.

After opening, the reagent is stable for 60 days when properly capped immediately after each opening and stored at 2-8º C.

Signs of reagent deterioration:

- Presence of particles and turbidity.
- Blank absorbance (A) at 405 nm ≥ 0.40.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 405 nm.
- Thermostatic bath at 37º C(Note 1).
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment(Note 2).

SAMPLES

- Serum or plasma[1], 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 2-8º C.

PROCEDURE

1. Assay conditions:
   Wavelength: . . . . . 405 nm
   Cuvette: . . . . . 1 cm light path
   Constant temperature: . . . . . 37º C
2. Adjust the instrument to zero with distilled water.
3. Pipette into a cuvette:

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

4. Mix, incubate for 30 seconds.
5. Read initial absorbance (A) of the sample, start the stopwatch and read absorbances at 1 minute intervals thereafter for 3 minutes.
6. Calculate the difference between absorbances and the average absorbance differences per minute (ΔA/min).

CALCULATIONS

Serum or plasma	ΔA/min x 3954 = U/L AMS
Urine	ΔA/min x 7908 = U/L AMS

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 assay procedures. If control values are found outside the defined range, check the instrument, reagents and technique for problems.

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[1]

Serum or plasma	Up to 90 U/L of α-amylase
Urine	Up to 450 U/L of α-amylase

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range: From detection limit of 0.2439 U/L to linearity limit of 2200 U/L.

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

Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (U/L)	77.0	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 using CHRONOLAB reagents (y) did not show systematic differences when compared with other commercial reagents (x).

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.

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INTERFERENCES

Hemolysis interferes in the results[1].

α-Amylase activity can be inhibited by chelating agents like citrate and EDTA. A list of drugs and other interfering substances with α-amylase determination has been reported by Young et. al[3,4].

NOTES

1. α-Amylase enzyme activity is temperature dependent. Assays performed at temperatures <37º C or >37º C will show an apparent decrease or increase levels.
2. Saliva and sweat contain α-amylase. Avoid mouth pippeting and skin contact with the reagent or material used.
3. Contains potassium thiocyanate. Avoid inhalation, skin or eyes contact.
   If it happens, wash with plenty of water and consult a doctor.
4. **CHRONOLAB has instruction sheets for several automatic analyzers.**

BIBLIOGRAPHY

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5. Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.
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