Reactivos GPL

Barcelona, España

CE

- AMYLASE LQ-

ALPHA AMYLASE

$CNPG_3$. Liquid. Kinetic.

Store at: +2+8ºC.

Presentation:
Cod. EZ004 CONT: R 2 x 50 mL .

Procedure

Quantitative determination of alpha amylase

Only for *in vitro use in clinical laboratory* (IVD)

TEST SUMMARY

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

$$10\ CNPG_3 \xrightarrow{\alpha-amylase} 9\ CNP + 1\ CNPG_2 + G_3 + G$$

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

REAGENTS COMPOSITION

R	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 %

PRECAUTIONS

Contains potassium thiocyanate. Avoid inhalation, skin or eye contact. If it happens, wash with plenty of water and consult a doctor.

REAGENT PREPARATION AND STABILITY

Liquid reagent is ready to use.
Stability: Once opened is stable 60 days at 2-8ºC 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 2-8ºC, 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 ≥ 0.50

SPECIMEN

Serum or plasma: 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 2-8ºC.

MATERIAL REQUIRED BUT NOT PROVIDED

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

General laboratory equipment.

TEST PROCEDURE

1. Assay Conditions
- Wavelength : ..... 405 nm.
- Cuvette: ..... 1 cm. light path.
- Constant temperature . . . . . 37ºC.

2. Adjust the instrument to zero with distilled water or air.
3. Pipette into a Cuvette(note1):

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

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

CALCULATIONS

Serum, plasma ΔA/min x 3594* = U/L of α-amylase

Urine ΔA/min x 7098* = U/L of α-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, Normal Control QC001 and Pathological Control QC002. 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 VALUES[1]

37ºC.
Serum or plasma < 90 U/L.
Women up to < 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 1 U/L. to linearity limit of 2000 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*:

	Intra-assay n= 20		Inter-assay n= 20	
Mean (U/L)	61.2	165	65.1	172
SD	1.00	2.44	2.84	4.57
CV (%)	1.64	1.47	4.36	2.65

- Sensitivity: 1 U/L = 0.0003 ΔA/min
- Accuracy: Results obtained GPL reagents did not show systematic differences when compared with other commercial reagents
  The results of the performance characteristics depend on the analyzer used.

INTERFERING SUBSTANCES

- Hemolysis interferes in the results [1]. 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

1. The activity of alpha-amylase is temperature dependent. Assays performed at temperatures lower than 37º C. or higher than 37º C. will show an apparent decrease or increase levels.
2. Use clean disposable pipette tips for its dispensation. Saliva and sweat contain α-amylase. To reduce the possibility of contamination do not pipette by mouth and avoid contact of the sample and reagent with the skin.
3. Formulation to reach constant:

ΔA/min x 3494* or 7098* = U/L α-amylase

$$* \frac{Tv \times 1000}{\varepsilon \times LP \times Sv}$$

Tv= Total volume in mL
ε CNP = at 405 nm
LP= Light path
Sv= Sample volume in mL

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