α AMYLASE KIT

(Direct Substrate method)

For the determination of α Amylase activity in serum, plasma or urine
(For Invitro Diagnostic Use Only)

Summary

α Amylase is secreted by the pancreas into the duodenum where it aids the catabolism of carbohydrates to simple sugars. Damage to the pancreas or obstruction to the pancreatic duct causes the enzyme to enter the blood stream. Elevated levels are found in acute pancreatitis, perforated / penetrating peptic ulcers, paraotitis (mumps). Patients with chronic pancreatic disorders having pancreatic cell destruction do not have high levels as less amylase is produced by the pancreas.

Principle

α Amylase catalyses the hydrolysis of a 2 - chloro - 4 nitro phenol salt to chloro nitrophenol (CNP). The rate of hydrolysis is measured as an increase in absorbance due to the formation of chloro nitrophenol which is proportional to the α Amylase activity in the sample.

$$\text{CNP - Gal - G2} + H_2O \xrightarrow{\alpha\ \textit{Amylase}} \text{CNP} + \text{Gal - G2}$$

Normal reference values

Serum : upto 90 U/L at 37°C
Urine : upto 490 U/L at 37°C
It is recommended that each laboratory establish its own normal range representing its patient population.

Contents	**2 x 10 ml**	**2 x 30 ml**
L1 : Amylase Reagent	2 x 10 ml	2 x 30 ml

Storage / stability

Contents are stable at 2-8°C till the expiry mentioned on the label.

Reagent Preparation

Reagents are ready to use. Do not pipette with mouth.

Sample material

Serum,heparinised plasma, urine.
α Amylase is reported to be stable in the sample for 5 days at 2-8°C. Separate serum from clot as soon as possible.

Procedure

Wavelength / filter : 405 / (Hg405) / violet
Temperature : 37°C
Light path : 1 cm

For Serum as Sample :

Pipette into a clean dry test tube labelled as Test (T) :

Addition Sequence	**T** (37°C)
Amylase Reagent (L1)	1.0 ml
Sample	0.02 ml

Mix well and read the initial absorbance A_0 after 1 minute & repeat the absorbance reading after every 1, 2, & 3 minutes. Calculate the mean absorbance change per minute (ΔA / min.)

For Urine as Sample :

Pipette into a clean dry test tube labelled as Test (T) :

Addition Sequence	**T** (37°C)
Amylase Reagent (L1)	1.0 ml
Sample	0.01 ml

Mix well and read the initial absorbance A_0 after 1 minute & repeat the absorbance reading after every 1, 2, & 3 minutes. Calculate the mean absorbance change per minute (ΔA / min.)

Calculations

α Amylase Activity in U/L (Serum) = ΔA / min. x 3954
α Amylase Activity in U/L (Urine) = ΔA / min. x 7830

Linearity

The procedure is linear upto 1000 U/L at 37°C. If the absorbance change (ΔA / min.) exceeds 0.300, use only the value of the first two minutes to calculate the result, or dilute the sample 1 + 9 with normal saline (NaCl 0.9%) and repeat the assay (Results x 10).

Note

Anticoagulants like oxalate and EDTA bind Calcium which is needed for α Amylase activity and should not be used. Heparin may be used. Saliva and sweat contain α Amylase. Avoid contamination of reagent and sample during use.
For users to convert the values obtained by this method to the EPS substrate methods, multiply the results obtained by 2.45.

References

IFCC methods for the measurment of catalytic concentrations of enzymes, J. Clin. Chem. Acta. (1999) 281 : 5

System Parameters

Reaction	:	Kinetic
Wavelength	:	405 nm
Zero Setting	:	Distilled water
Incub. Temp.	:	37^{0}C
Incub. Time	:	---
Delay Time	:	60 sec.
Read Time	:	180 sec.
No. of read.	:	4
Interval	:	60 sec.
Sample Vol.	:	0.02 ml / 0.01 ml
Reagent Vol.	:	1.00 ml
Standard	:	---
Factor	:	3954 / 7830
React. Slope	:	Increasing
Linearity	:	1000 U/L
Units	:	U/L

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