α – AMYLASE

(KINETIC METHOD)

ENZOPAK

Last update 09-2020

Ref. CC1-AMY.003, 20x1.1 ml

INTENDED USE

Reagent kit for quantitative estimation of α-Amylase in serum.

PRODUCT HIGHLIGHTS

- Colorimeter/Spectrophotometer (405 nm) Kinetic Reagent.
- Latest Technology with (GAL- G_2 - CNP) Substrate.
- Stable Reagent and Long Reconstituted Stability.
- Substrate in tablet forms in various sizes of 1.1, 2.2 and 5.5 ml. reconstitution.
- Internationally Standardized readings comparable to other methods with different substrates like pNPG7, pNPG6, pNPG5 etc.

INTRODUCTION

α-Amylase catalyses the hydrolysis of 1-4 glucosidic linkages of starch and other related polysaccharides to produce maltose and other oligosaccharides. The enzyme is a relatively small molecule which is rapidly cleared by the kidneys and excreted in the urine.

The old methods using starch digestion and subsequent testing with iodine are almost outdated and are replaced by chromogenic substrates in developed countries.

α-Amylase uses Blocked α-(2-Chloro-4-Nitrophenyl) ß-1, 4-Galactopyranosyl maltoside (GAL-G_2- CNP) as a substrate and does not require coupling of other enzymes and therefore gives a very stable substrate reagent.

α-Amylase gives an excellent correlation to other blocked substrates technology and a linear response to both pancreatic and salivary amylase.

DIAGNOSTIC SIGNIFICANCE

Amylase is mostly measured for the diagnosis of acute pancreatitis where in serum levels are found to be elevated. In acute pancreatitis α-Amylase starts rising approximately four hours after the onset of pain, reaches peak at 24 hours and remains elevated for 3 to 7 days. High levels of amylase are also associated with other disorders, like biliary tract diseases, severe glomerular dysfunction and salivary gland disorders.

PRINCIPLE

α-Amylase uses a chromogenic substrate Gal-G_2- CNP which, by the reaction of α-Amylase breaks down to release 2-Chloro-4-Nitrophenol (CNP).

$$\text{Gal-}G_2\text{-CNP} + H_2O + \text{Amylase} \longrightarrow \text{CNP} + \text{Gal-}G_2$$

The release of 2-Chloro-4-Nitrophenol (CNP) is measured at 405 nm and is proportional to α-Amylase activity.

PRESENTATION

Store all reagents at 2-8^0 C	No. of Bottles 20 x 1.1 ml.
• 1 AMYLASE (Substrate)	2 (10 Tablets)
• 2 AMYLASE (Buffer)	2
• Bottle for reconstitution	1

FINAL REAGENT COMPOSITION

Active Ingredients	Concentration
• CNPG-2	1mmol/L
• Sodium Chloride	1000 mmol/L
• Buffer	50 mmol/L

Ph 6.0 ± 0.1 at 25^{0}C

Also contains non-reactive fillers and Stabilizers.

PRECAUTION

Amylase is for *in-vitro* diagnostic use.

Reagent contains Sodium Azide, DO NOT INGEST.

PREPARATION OF WORKING REAGENT

For 20 x 1.1 ml.

Reconstitute one tablet of 1 AMYLASE with 1.1 ml. of 2 AMYLASE. Mix gently till complete dissolution. Use after 5 minutes.

REAGENT STORAGE AND STABILITY

α-Amylase reagents are stable until the expiry date stated on the label when stored at 2-8^0 C. Reconstituted reagent is stable for 4 weeks at 2-8^0 C.

SPECIMEN COLLECTION

Serum is essential. Amylase activity in serum samples remain stable for 20 days at 2-4^0 C.

REACTION PARAMETERS

• Type of Reaction	:	Kinetic/Increasing OD
• Wavelength	:	405 nm
• Flowcell Temperature	:	37^{0}C
• Delay Time	:	60 Seconds
• Interval Time	:	30 Seconds
• No. of readings	:	4
• Sample Volume	:	50 µl (0.05 ml)
• Reagent Volume	:	1.0 ml
• Factor	:	1628
• Light Path	:	1.0 cm
• Zero setting with	:	Distilled Water

TEST PROCEDURE

Pipette into Test Tubes	TEST
Working Reagent (ml)	1.0
Sample (ml)	0.05

Mix immediately and read first absorbance of test exactly at 60 seconds and then, second, third and fourth at an interval of 30 seconds at 405 nm. Determine the mean change in absorbance per minute. (ΔA/min) and calculate the test results.

TESTS RESULTS

Serum Amylase Activity (IU/L) = ΔA/min X F

$$F = \frac{1}{12.9} \times \frac{T.V}{S.V} \times 1000 = 1628$$

Where

T.V.	=	Total Volume	= 1.05
S.V.	=	Sample Volume	= 0.05
12.9	=	Millimolar absorbance of 2-Chloro-4-Nitrophenol.	
1000	=	to convert activity per ml. to per liter	

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NORMAL VALUES

35 - 140 IU / L at 37^0C.

LINEARITY

This method is linear upto 1200 IU/L. For values above 1200 IU/L, dilute the sample suitably with 0.9 % saline and repeat the assay. Apply correction due to dilution to arrive at a final results.

NOTE

For laboratories using instruments with cuvette capacity more / less than 1.0 ml, sample volume and reagent volume may be increased / decreased proportionately.

REFERENCES

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