α - AMYLASE (Liquid)

ENZOPAK

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(KINETIC METHOD)
Reagent kit for quantitative estimation of α -Amylase in serum or plasma

- Colorimetric/Spectrophotometric (405 nm) Kinetic Reagent.
- Latest Technology with (GAL- G_2 - CNP) Substrate.
- Stable Reagent.

BACKGROUND AND SYNOPSIS:
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.

ENZOPAK α -Amylase uses Blocked *α* -(2-Chloro-4-Nitrophenyl) β -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.

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

PRINCIPLE:
ENZOPAK 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).

Gal-G_2- CNP+ H_2O+ Amylase $\longrightarrow$ CNP + Gal – G_2

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

PRECAUTION :
Amylase is for *IN-VITRO* diagnostic use only.
Reagent contains Sodium Azide. DO NOT INGEST.

DIAGNOSTIC SIGNIFICANCE:
Amylase is mostly measured for the diagnosis of acute pancreatitis wherein 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 and raptured ectopic pregnancy.

REAGENT COMPOSITION:

Active Ingredients	Concentration
Reagent-1	
* Buffer	100 mmol/L
* Calcium Acetate	3 mmol/L
* CNPG-3	2 mmol/L

pH 6.0 ± 0.1 at 25^{0}C
Also contains non-reactive fillers and Stabilizers.

PRESENTATION:

		No. of Bottles	
Store all reagents at 2-8^0 C	5ml	2 x 25 ml.	2x5 ml.
Amylase-L (Substrate)	1	2	2

REAGENT STORAGE AND STABILITY:
ENZOPAK α -Amylase reagents are stable until the expiry date stated on the label when stored at 2-8^0 C. Opened vial is stable for 4 weeks at 2-8^{0}C.

SPECIMEN COLLECTION:
Serum or heparinised plasma are suitable. EDTA, Oxalate or Citrate inhibit the amylase activity, hence cannot be used. Amylase activity in serum samples remain stable for 20 days at 2-8^0 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 (0.050 ml)
• Reagent Volume	:	1.0 ml.
• Factor	:	1628
• Light Path	:	1.0 cm.
• Zero setting with	:	Distilled Water

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. (Δ A/min) and calculate the test 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.

TEST RESULTS:
Serum Amylase Activity (IU/L) = Δ A/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	=	Milimolar absorbance of 2 – Chloro – 4 Nitrophenol.		
1000	=	to convert activity per ml. to per liter		

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

LIMITATIONS :
Avoid contaminants like Saliva, Cough, since these contain many units of Amylase which might effect the reagent for colour development.

LINEARITY :
This method is linear upto 1500 IU/L. For values above 1500 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.

REFERENCE:

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