α - AMYLASE

CNPG3 Method

Intended Use:
Amylase test reagent/kit is a medical device intended for the estimation of Amylase in serum or saliva

Clinical Significance
α-Amylase is most frequently measured in the diagnosis of acute pancreatitis with serum levels may be grossly elevated and therefore is useful diagnostic tool in critical care management. In acute pancreatitis α-Amylase levels reach a peak at 24 hours and remain elevated from 3-7 days. Hyperamylasemia is also is also associated with other acute abdominal disorders, biliary tract diseases, diabetic ketoacidosis, severe glomerular dysfunction, salivary gland disorders and ruptured ectopic pregnancy.

Principle
Substrate CNP G_3 is directly hydrolyzed by α-Amylase to produce CNP monitored at 405 nm during incubation of the reagent with a sample.

$$CNP\ G_3 \xrightarrow{\text{α-Amylase}} CNP + CNP\ G_2 + G_3 + G$$

CNP G_3 = 2-Chloro-4-nitrophenyl- α-Maltotrioside
CNP G_2 = 2-Chloro-4-nitrophenyl- α-Maltoside
CNP = 2-Chloro-4-nitrophenol

Reagent Composition

R1 Amylase Reagent	Tris buffer - 50 mmol/L CNP G3 - 5 mmol/L KSCN - 100 mmol/L Activators & Stabilizer

Working Reagent Preparation
Reagent is ready to use. Do not pipette with mouth.

Stability and Storage
Store at 2-8ºC
All the kit contents are stable until the expiry date stated on the label. Do not use reagents beyond the expiration date.
Store the vials tightly closed protected from light and prevent contaminations during the use.

Discard if signs of deterioration appear:
- Presence of particles and turbidity.

Materials required
- Photometer or spectrophotometer with a thermostat cell compartment set at 25/30/37ºC, capable of reading at 405nm.
- Stopwatch, strip-chart recorder or printer.
- Cuvettes with 1-cm path length
- Pipettes to measure reagent and samples.

Sample and Stability
Serum or plasma
Serum or heparinized plasma free of hemolysis.
α-Amylase is reported to be stable in serum or plasma for 5 days at 2-8ºC.

Known Interfering Substances
- Lipemia (intralipid 20 g/L) do not interfere.
- Bilirubin (20 mg/dL) does not interfere.
- Hemoglobin (>2 g/L) may affect the results.
- Other drugs and substances may interfere

Assay Procedure

Pipette into clean dry test tubes labeled as Test (T):

Addition Sequence	Test (T)
R1 Amylase Reagent	1000µl
Sample	25µl

Mix well and read the initial absorbance A0 after 1 minute and subsequently 2 more readings with 60 seconds interval at 405 nm. Calculate the mean absorbance change per minute (Δ Abs/30 seconds x 2)

Calculations:

α-Amylase activity in IU/L = ΔA/min. x 4640

Quality Control
To ensure adequate quality control (QC), each run should include a set of controls (normal and abnormal) with assayed values handled as unknowns.
If the values are found outside of the defined range, check the instrument, reagents and procedure.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable results.

Linearity
The procedure is linear up to 2000 IU/L at 37^{0}C. If the amylase activity is above 2000 IU/L, dilute the specimen suitably with normal saline. In such case the results obtained should be multiplied by dilution factor to obtain correct amylase activity.

Reference Values
Serum : Up to 140 IU/L
It is recommended that each laboratory establishes its own normal range representing its patient population.

General System Parameters

Mode	Kinetic
Reaction	Increasing
Wavelength	405nm
Blank with	Reagent
Sample Volume	25µl
Reagent Volume	1000µl
Factor	4640
Incubation Temp.	37^{0}C
Delay Time	60 sec
Read Time	120 sec
No. of Reading	03
Normal Range	Up to 140 IU/L
Linearity	Up to 2000 IU/L
Unit	IU/L

Notes
1. 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. Reagent should not be used if its absorbance exceeds 0.8 at 405 nm against distilled water.
2. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

Reference

1. Jungi W.et.al, Biochem 22,109 (1089)
2. Winn-Deen E.S. David H.Signett (1988)

For *in vitro* Diagnostic use only.