Pariksha Biotech
A game changer in IVD

α - Amylase Estimation Kit
High-Q α - Amylase- ML
(CNPG3 Method)

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Intended Use:

Kit for the quantitative determination of α-Amylase in human serum and heparinized plasma.

Clinical Significance:

The determination of amylase activity in serum is most commonly performed for the diagnosis and treatment of diseases of the pancreas.

Method History:

Amylase was first measured quantitatively by an iodometric method introduced by Wohlegemuth in 1908. Somogyi introduced a procedure in 1938 that standardized the amounts of starch and iodine. His work became the basis for the widely-used Amyloclastic and Saccharogenic methods introduced in 1956 and 1960 respectively. Disadvantages of these methods included long incubation times, endogenous glucose interference, and unstable reaction colors resulting in poor reproducibility and reliability. Rinderknecht et al introduced a dye-coupled starch method in 1967 that was relatively simple to perform. However, the procedure used an insoluble substrate, lacked linearity, and still required centrifugation or filtration.Turbidimetric procedures have been introduced that are relatively fast but they require special instrumentation and have difficulty producing stable and reproducible starch solutions.Several enzymatic procedures have been suggested including one that used the defined substrate maltotetraose. These methods represented significant improvement in amylase measurement, but were still subject to relatively long pre-incubation times, possible endogenous glucose interference, and a series of other potential interferences with the formation of NADH.Wallenfels et al introduced p-nitrophenylglycosides as defined substrates for α-amylase determination in a procedure that eliminated interference from endogenous glucose and pyruvate. A variety of coupling enzymes have been used to hydrolyze the short chain oligosaccharides resulting from the amylase activity in the specimen. Unfortunately, these coupling enzymes contained residual amylase activity that adversely affected the stability of these reagents.

Hyperamylasemia does not, however, only occur with acute pancreatitis or in the inflammatory phase of chronic pancreatitis, but also in renal failure (reduced glomerular filtration), tumors of the lungs or ovaries, pulmonary inflammation, diseases of the salivary gland, diabetic ketoacidosis, cerebral trauma, surgical interventions or in the case of macroamylasemia. To confirm pancreatic specificity, it is recommended that an additional pancreas-specific enzyme - lipase or pancreatic-á-amylase- also be determined.

Numerous methods have been described for the determination of α-amylase. These either determine the decrease in the amount of substrate viscometrically, turbidimetrically, nephelometrically and amyloclastically or measure the formation of degradation products saccharogenically or kinetically with the aid of enzyme-catalyzed subsequent reactions.

The present method is based on the use of a chromagenic substrate, 2-chloro-4-nitrophenyl-α- D-maltotrioside (CNP-G3). The reaction of amylase with this substrate results in the formation of 2-chloro-p-nitrophenol, that can be measured spectrophotometrically at 405nm. This reaction proceeds very rapidly, no coupling enzymes are required, and the reaction is not readily inhibited by endogenous factors.

Advantages of $CNPG_3$ as α-Amylase Substrate :

1 The substrate, $CNPG_3$ used in α- Amylase kit is more stable than the following substrates that have a complex carbohydrate moiety (i.e. Poly Glucose) attached with p-Nitro Phenol. E.g.: EPS, PNP-G4, PNPG7, EPS-G, BPNPG7 and ET-G7PNP.

2 The hydrolysis of $CNPG_3$ into CNP is a one step reaction and does not require any supporting enzymes where as the other substrates require ancillary enzymes. For e.g. PNP-G7 requires Galactosidase, and Glucoamylase as ancillary enzymes.

3 As α-AMYLASE directly acts on the substrate $CNPG_3$ more than 98% hydrolysis of the substrate is possible where as with other substrates the hydrolysis is much lesser.

4 $CNPG_3$ substrate is more stable in liquid form when compared to other substrates.

5 Using $CNPG_3$ there is no interference from Bilirubin (up to 50 mg/dl), Ascorbate (up to 500 mg/dl), Glucose (up to 5000 mg/dl).

6 $CNPG_3$ is less sensitive to interferences by the change in ionic strength of the buffer.

Principle:

The Direct Amylase assay involves the use of a chromogenic substrate CNP G_3 (2-choloro-4-nitrophenyl linked with Galactomaltoside) which acts upon α-Amylase to release more than 98% of 2-choloro-4-nitrophenyl (CNP), and forms 2-choloro-4-nitrophenyl-α-D-maltoside (CNP G_2), maltotriose (G_3) and Glucose (G).

The rate of formation of 2-choloro-4-nitrophenyl is proportional to the α-Amylase activity in the sample, which can be monitored by kinetic assay at 405 nm.

CNP G3 **α**-amylase CNP+CNP G_2 + G_3 + G

Storage and Stability:

The reagent should be stored at 2-8^{0}C and is stable till the expiry date indicated on the label.

DO NOT FREEZE THE REAGENT. Contamination of the reagent should be strictly avoided.

Composition of the reagent:

MES Buffer, pH 6.0	**:**	**100 mMol/L**
CNP G_3	**:**	**11 mMol/L**
KSCN	**:**	**250 mMol/L**
Calcium Acetate	**:**	**0.02 mMol/L**
Azide	**:**	**0.1 %**

Specimen collection and handling:

1. Unhemolysed serum and Heparinized Plasma are the specimens
2. E.D.T.A, Oxalate or Citrate inhibit Amylase activity hence cannot be used.
3. Amylase in serum is reported to be stable for one week at room temperature and for 2 months when stored refrigerated at 2-8^{0}C.

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Assay Procedure:

Pipette in to tubes	Test
Amylase Reagent (Ready to Use)	**1.0 ml**
Sample	**25 µl**

Mix thoroughly and aspirate in to the analyzer. After 60 Seconds delay, measure the change of optical density after next 60 Seconds against distilled water at 405 nm.

Calculations:

Calculate the average change in absorbance per minute (Δ Abs/minute)

Activity of Amylase in IU/L = Δ Abs/min X 3178 (Kinetic Factor)

Note: Kinetic Factor 3178 is derived based on the Absorbance coefficient of 2-chloro-p-nitrophenol at 405 nm

Kinetic Factor Derivation:

Formula:

$$\frac{\text{Assay volume (ml)} \times 1000}{12.9 \times \text{light path (cm)} \times \text{sample volume}}$$

Assay volume	=	Total reaction volume expressed in ml
1000	=	To convert IU/ml to IU/L
12.9	=	Absorbance coefficient of 2-chloro-p-nitrophenol at 405 nm
Light path	=	Length of the light path expressed in cm (Usually 1.0 cm)
Sample volume	=	Sample volume expressed in ml

As per the above formula:

$$\text{Factor} = \frac{1.025 \times 1000}{12.9 \times 1 \times 0.025} = 3178$$

Normal Values:

Serum : 25-110 IU/L

Since the expected values are affected by age, sex, diet and geographical location, each laboratory should strongly urge to establish its own reference range for this procedure.

As Amylase is generally measured by the hydrolysis of different non natural substrates, values obtained can change widely for each Substrate. Therefore reference values mentioned for other Substrates should not be compared or correlated with the reference values mentioned for $CNPG_3$ Substrate.

Note:

1. **Saliva and sweat contain α-Amylase. To avoid possible contamination do not pipette by mouth and avoid contact of the reagent and pipette tips with the skin.**
2. **The expected values of Amylase are dependent on the substrate used in the formulation. Results cannot be compared with the kits based on formulations using other substrates.**
3. **Reagent should not be used if its absorbance exceeds 0.800 at 405 nm, against distilled water.**
4. **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.**

Quality Control:

To ensure adequate Quality Control, it is recommended that each batch should include normal and abnormal commercial reference control serum. It should be realized that the use of quality control material checks both instrument and reagent functions together. Proper instrument function, temperature control, cleanliness of glassware, and accuracy of pipetting can contribute for effective performance of the reagent.

System Parameters:

Reaction Type	:	Kinetic
Reaction Direction	:	Increasing
Wavelength	:	405 nm
Flow cell temp	:	37°C
Zero setting with	:	Distilled Water
Delay Time	:	60 seconds
Measuring Time	:	60 seconds
Sample Volume	:	25 µl
Reagent Volume	:	1.0 ml
Factor	:	3178
Linearity	:	2000
Units	:	IU/L

Analytical Sensitivity:

Detection limit: 7 U/l

Precision and Correlation:

Inter-Assay and Intra-Assay % CV were evaluated at three different pooled serum samples.

Imprecision:

Reproducibility within run was determined using human samples and controls (n = 20). The following results were obtained:

Serum Within run

Sample	Mean	SD	CV%
Sample1	187	1.48	0.79
Sample2	446	2.10	0.47
Sample3	507	2.64	0.52

Reproducibility was determined using human samples and controls between day (n = 20).

The following results were obtained:

Serum Between day

Sample	Mean	SD	CV%
Sample1	196	2.83	1.44
Sample2	474	6.25	1.32
Sample3	542	6.73	1.24

Method comparison:

A comparison of the High-Q Amylase - ML (y) with a commercial obtainable assay (x) gave with 36 samples the following results

y = 0.972x + 1.282; r = 0.999

Ordering Information

Ref./Cat.	Pack Size	Presentation
P-AMY-12	4 x 3 ml	Mono Reagent
P-AMY-20	4 x 5 ml	
P-AMY-50	5 x 10 ml	

Product Features

- **Liquid Stable, Ready to use Mono Reagent.**
- **CNPG3 as a Direct Chromogenic Substrate.**
- **98% hydrolysis achieved in a single step.**
- **Reagent does not require auxiliary enzymes like Galactosidase and Glucoamylase.**
- **2 Minutes increasing kinetic reaction (60 Sec Delay + 60 Sec Measuring).**
- **Linearity 2000 IU/L.**
- **Measuring Wavelength 405 nm.**
- **Kinetic factor 3178 at 37° C.**
- **Serum / Heparinized Plasma as Specimens.**
- **Available as multipurpose reagents and dedicated system packs.**

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Symbols used with IVD devices

	Date of manufacture		Manufactured by
IVD	In vitro diagnostic device		Keep away from sunlight
	Do not freeze		This way up
	Use by (yyyy-mm-dd or mm/yyyy)	R	Reagent
CAL	Calibrator Material	LOT	Batch code
2°C 8°C	Temperature limitation (store at)	CONTROL	Control
	Consult instructions for use		Keep dry Keep away from rain
REF	Catalog Number		

eIFU Indicator

Pariksha's world inside
SCAN TO EXPLORE MORE

Manufactured in India by :
Pariksha Biotech Pvt Ltd,
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AN ISO 13485 Certified Company

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