Intended Use
For **IN VITRO** quantitative determination of Amylase in serum using manual or automated applications.

Clinical Significance
Measurements of Amylase activity are primarily used for diagnosing diseases of the pancreas, as well as, for monitoring its causes and treatment. (1)

Method History
In 1958 Jasen and Wydeveld published an assay using p-nitrophenyl-alpha-D-maltohexaoside. (2) These substrates were later synthesized with a blocking group at the terminal end to eliminate alpha-glucosidase activity. (3, 4) A more simple and direct method based upon the direct hydrolysis of 2-chloro-p-nitrophenol linked with maltotriose has been recently introduced. (3) The direct reaction of alpha Amylase with the substrate results in the formation of 2-chloro-p-nitrophenol, which can be monitored by either a kinetic or end point assay. No coupling enzymes are required and the reaction is not readily inhibited by endogenous factors.

Method Principle

α-Amylase
5 CNP-G3 ----------> 3 CNP + 1 CNPG 2 + 2 G3 + 2 G

As shown above, α-Amylase hydrolyzes the 2-chloro-p-nitrophenyl- α-D-maltotrioside (CNP-G3) to release 2-chloro-p-nitrophenol and form 2-chloro-p-nitrophenyl-α-D-maltoside (CNPG2), maltotriose (G3) and glucose (G). The rate of formation of the 2-chloro-p-nitrophenol can be detected spectrophotometrically at 405 nm to give a measurement of α-Amylase activity in the sample.

Reagent Content
Each liter contains:

2-chloro-p-nitrophenyl- α-D-maltotrioside	2.25mmol
Sodium Chloride	70 mmol
Calcium Acetate	6 mmol
Buffer, Ph 6.0	100 mmol
Potassium Thiocyanate	900 mmol
Sodium Azide	0.1%

Warning: Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. On disposal, flush with a large volume of water to prevent azide buildup.

Reagent Preparation
The reagent is packaged ready for use. No preparation is required.

Precautions
Avoid contact of reagent with skin and eyes. Should contact occur, wash affected area with plenty of cold water. **DO NOT PIPETTE REAGENTS BY MOUTH.**

Reagent Storage And Stability
Store the Amylase reagents at 2-8˚C. When stored as directed, this reagent is stable until the expiration date stated on the label. Upon opening, store the Amylase Working Reagent capped at 2-8˚C. When stored as directed, the Amylase Working Reagent is stable for thirty days after opening.

Specimen Collection And Preparation
Test sera should be fresh, clear and unhemolyzed. When blood is drawn, it should be processed as soon as possible and the serum should be isolated from the clot without delay. In separated unhemolyzed serum the enzyme concentration is stable for one week at 25˚C and over two months at 2-8˚C. (1)

Calibration
α-Amylase activity is calculated based on the millimolar absorptivity of 2-chloro-p-nitrophenol. The millimolar absorptivity of 2-chloro-p-nitrophenol varies with pH, temperature and wavelength. The millimolar absorptivity at pH 6.0 and 37˚C, when measured at a wavelength of 405nm, is 12.9.

Limitations
1) The α-Amylase is linear to 2000 U/L. If a sample exceeds 2000 u/L, it should be diluted with an equal volume of saline and re-assayed. Multiply the value from the resulting calculation by 2.
2) Hemolyzed samples should not be used.

Quality Control
To monitor the performance of the Working Reagents and the procedure used, we recommend the regular use of normal and abnormal control serum.

Interfering Substances
Several substances have been reported to alter the Amylase activity in serum. (4) All common anticoagulants inhibit Amylase activity with the exception of heparin. Therefore, all Amylase assays should be done only in serum or heparinized plasma. (1)

Expected Values
A reference range for Amylase values in normal serum between 25 and 125 U/L has been reported in the literature. (4) As the expected values are affected by a number of factors, including age, sex and diet, it is recommended that each laboratory establish its own range of expected values.

Procedure
Important: Read entire procedure instructions before proceeding with assay.

Materials Required (Not Provided)

Spectrophotometer	
Cuvettes	cm light path
Timer	to time incubation time
Pipette	1.0 ml for reagent
Pipette	0.05 ml for sample

Materials Provided
Amylase Reagent

Analytical Parameters

Wavelength	405
Temperature	37˚C
Pathlength	1 cm
Reaction Mode	Rate
Reaction Time	5 minutes
Reagent Volume	1.0 ml
Sample Volume	0.05 ml
Total Volume	1.05 ml
Sample-to-reagent ratio	1:41

Assay Procedure
1. Set spectrophotometer wavelength at 405nm and zero the instrument with the cuvette containing water.
2. Pipette 1.0 of Working Reagent into each of two cuvettes marked "sample" and "control".
3. Incubate cuvettes for 2.0 minutes at 37˚C.
4. Pipette 0.05 ml of control or sample into their respective cuvettes. Mix all cuvettes well.
5. Replace the cuvettes in spectrophotometer and continuously monitor the change in absorbance for at least 3 minutes
6. Read the "control" and "sample" absorbencies.
7. Calculate the Amylase concentration (U/L) in the sample(s), as shown in results and calculations.

Calculations and Results
One international unit (U/L) is defined as the amount of enzyme that catalyzes the conversion of one micromole of substrate per minute under the defined conditions:

$$\text{Amylase} = \frac{\text{delta A/min} \times \text{Assay Vol (ml)} \times 1000}{12.9 \times \text{light path (cm)} \times \text{sample vol (ml)}}$$

Where:

delta A/min	=	change in absorbance per minute
Assay volume	=	total reaction volume in ml
1000	=	converts u/ml to u/L
12.9	=	extinction coefficient of CNP
Light path	=	1 cm reaction cuvette
Sample Volume	=	ml of sample used in assay

Example:

$$\text{Amy (u/L)} = \frac{0.02 \times 2.25 \text{ ml} \times 1000}{12.9 \times 1 \text{ cm} \times 0.05 \text{ ml}} = 87 \text{ U/L}$$

0.025	=	change in absorbance/minute
2.25 ml	=	total reaction volume
1000	=	conversion factor
12.9	=	extinction coefficient of CNP
1 cm	=	cuvette light path
0.05 ml	=	sample volume

Example:

$$\text{Amy (u/L)} = \frac{0.025 \times 2.25 \times 1000}{12.9 \times 1 \text{ cm} \times 0.05 \text{ ml}} = 87 \text{ U/L}$$

Method Performance Characteristics

Sensitivity: The sensitivity of this method is 0.00026-0.00031 absorbance units per U/L.
Linear Range: In this method there is no significant nonlinearity over the range of 0-2000 U/L.
Precision: Within-run and day-to-day precision is summarized below.

Precision Study

Amylase	TOTAL		WITHIN-RUN	
MEAN	SD	CV	SD	CV
U/L	U/L	%	U/L	%
105	2.46	2.35	2.34	2.21
258	4.98	1.94	4.91	1.90
450	11.7	2.61	7.48	1.65

Correlation
A comparison of this method using an automated analyzer and a reference method based upon the use of PNPG7 as substrate resulted in the following regression statistics.

Range	=	10 - 255
N	=	48
Y	=	1.07 x - 1.44
r	=	0.994

References
1. Fundamentals of Clinical Chemistry. Edited by Norbert W. Tietz. (1976) WB Saunders Co., Philadelphia.
2. Jansen AP, Wydeveid A. Alpha-(p-nitrophenyl)maltohexaoside as substrate for the assay of amylase. Nature 182:525-526(1958).
3. Chaven, RG, et al., U.S. Patent 4,963,479.
4. Young DS, Pestaner LC, Gibberman V. Effects of drugs on clinical laboratory tests. Clin Chem 21 No. 5(1975).

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