



AMYLASE REAGENT
C332-01

Contents	Product No.	Package
Amylase Reagent	C332-01	1 x 250 mL

REAGENT PREPARATION

This reagent is packaged ready for use.
No preparation is required.

REAGENT STORAGE AND STABILITY

Store the unopened reagent at 2-8°C.
When stored as directed, the reagent is stable until the expiration date stated on the label.

NOT FOR USE IN UNPROFESSIONAL SETTINGS

FOR TECHNICAL ASSISTANCE:
Email: catachem@catacheminc.com
Contact Form: www.catacheminc.com
Call: 203-262-0330



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- α -D-maltohexaoside. ⁽²⁾ These substrates were later synthesized with a blocking group at the terminal end to eliminate α -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 α Amylase with the substrate results in the formation of 2-chloro-p-nitrophenol, which can be monitored by a kinetic assay. No coupling enzymes are required and the reaction is not readily inhibited by endogenous factors.

Method Principle



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	2.25 mmol
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. The Catachem Amylase Reagent has been tested to reflect shipping conditions and is stable for the lifespan of the product if frozen up to 5 times or upon reaching temperatures up to 40°C for up to one week.

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 assay 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 serums.

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 use serum or heparinized plasma as a sample. ⁽¹⁾

Expected Values

A reference range for Amylase values in normal human serum between 25 and 125 U/L has been reported in the literature. ⁽⁴⁾ It is recommended that each laboratory establish its own range of expected values for the species under test.

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



**AMYLASE REAGENT
C332-01**

MANUAL/AUTOMATED APPLICATION

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	1:21

Assay Procedure

1. Set spectrophotometer wavelength at 405nm and zero the instrument with a 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. Individually Pipette 0.05 ml of control or sample into it's respective cuvettes. Mix well.
5. Replace the cuvette in the spectrophotometer and monitor the rate of change in absorbance for at least 3 minutes
6. Do this individually for all samples and controls.
7. Calculate the Amylase concentration (U/L) in the sample(s) and controls, 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:

$$\text{Amy (u/L)} = \frac{0.025 \times 1.05 \text{ ml} \times 1000}{12.9 \times 1 \text{ cm} \times 0.05 \text{ ml}} = \text{U/L}$$

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

Example:

$$\text{Amy (u/L)} = \frac{0.025 \times 1.05 \times 1000}{12.9 \times 1 \text{ cm} \times 0.05 \text{ ml}} = 40.7 \text{ U/L}$$

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).