Pointe Amylase (CNPG3) Reagent Set

HORIBA Instruments Incorporated
5449 Research Drive, Canton, MI 48188
Phone: 734-487-8300; (800) 445-9853

Intended Use
Pointe Amylase (CNPG3) Reagent Set is intended for research use only when performing quantitative kinetic determination of α-amylase activity in human urine using the Yumizen C230 and Yumizen C240 analyzers.

Clinical Significance
The determination of amylase activity in serum is 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.[1] Somogyi introduced a procedure in 1938 that standardized the amounts of starch and iodine.[2] His work became the basis for the widely-used Amyloclastic and Saccharogenic methods introduced in 1956[3] and 1960,[4] 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[5] 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[6] that are relatively fast but they require special instrumentation and have difficulty producing stable and reproducible starch solutions.

Several enzymatic procedures have been suggested[7,8] including one that used the defined substrate maltotetraose.[9] 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.[10]

Wallenfels et al[11] 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.

The present method is based on the use of a chromagenic substrate, 2-chloro-p-nitrophenol linked with maltotriose. 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.

Principle
$$10\ \text{CNPG3} \xrightarrow{\alpha\text{-amylase}} 9\ \text{CNP} + \text{CNPG2} + 9\text{G3} + \text{G}$$

α-Amylase hydrolyzes the 2-chloro-p-nitrophenyl-α-D-maltotrioside (CNPG3) to release 2-chloro-nitrophenol and form 2-chloro-p-nitrophenyl-α-D-maltoside (CNPG2), maltotriose (G3) and glucose (G). The rate of increase in absorbance is measured at 405 nm and is proportional to the α-amylase activity in the sample.

Reagents
MES Buffer, pH 6.0±0.1, 2-Chloro-p-Nitrophenyl-α-D-Maltotrioside 1.8 mM, Sodium Chloride 350 mM, Calcium Acetate 6 mM, Potassium Thiocyanate 900 mM, Sodium Azide 0.1% (See 'Precautions').

Reagent Preparation
The reagent is provided as a ready-to-use liquid. No preparation is required.

Reagent Storage
1. Store reagent at 2-8°C.
2. The reagent is stable until the expiration date if stored as directed.

Reagent Deterioration
Do not use if:
1. The absorbance of the working reagent is greater than 0.600 when measured at 405 nm against water in a cuvette with a 1 cm path length.
2. The reagent fails to meet stated parameters of performance.
3. The reagent is turbid or displays other evidence of bacterial contamination.

Precautions
1. This reagent contains potassium thiocyanate. POISON. Do not ingest.
2. This reagent contains sodium azide (0.1%) as preservative. Do not ingest. May react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with a large volume of water to prevent azide build up.
3. All specimens and controls should be handled as potentially infectious, using safe laboratory procedures. (NCCLS M29-T2)[12]

Specimen Collection and Handling
1. Fresh Urine is the specimen of choice. Specimens should be collected as per NCCLS document GP-16A3.[13]
2. Amylase in Urine is reported stable Urine samples are stable for up to 3 weeks when stored at -20°C, 10 days when stored at 4-8°C and 2 days at 20-25°C. [14]

Interferences
1. A number of drugs and substances affect the determination of amylase. Young et al have published a comprehensive list of such substances. [15, 16]
2. Presence of certain pathological conditions not associated with pancreatic disease can also give high Amylase results.[17]

Materials Provided
Amylase (CNPG3) reagent.

Materials Required but not Provided
1. Yumizen C230 / Yumizen C240 Analyzer
2. Yumizen C230 / Yumizen C240 Operation manual
3. Human Urine control set, catalog number P7582-CTL

Test Parameters

Chem:	AMYLASE	Chemistry:	Amylase
Chemistry No.:	204	Print Name:	AMY
Reaction Type:	Kinetic	Reaction Direction:	Positive
Pri. Wave:	405 nm	Sec. Wave	
Decimal.:	0	Samp. Type:	Urine
Blank Time:		Reaction Time:	3 11
Unit:	U/L	Incubation Time:	0

	Sample Vol.	Aspirated	Diluent		Reagent Vol.	Diluent	
Standard;	5	uL	uL	uL	R1: 200	uL	uL
Decreased;		uL	uL	uL			
Increased;		uL	uL	uL			

Linearity Range (Standard); 15-1300			Linearity Limit:			
Linearity Range (Decreased):			Substrate Depletion:	25000		
Linearity Range (Increased):			Mixed Blank Abs.:	-40000	40000	
R1 Blank Abs.:	-40000	40000	On-board Stability:	30	Day (s)	
Blank Response	-40000	40000	Reagent Alarm Limit:	10		
Twin Chemistry:						

Prozone Check:		
Q1:	Q2:	Q3:
Q4:	PC:	ABS:

Use Qualitative Result:		
	Range:	Flag:

Slope Offset:			
	Slope	Offset	Uint
	1	0	U/L

Pretreatment:			
Pretreat Sample Vol.:	uL	Pretreat Reagent Vol.:	uL

Ref. Range:					
Sample Type:	Gender:	Age Range:	Ref. Range:	Critical Range:	Unit:

Pointe
Amylase (CNPG3)
Reagent Set

Calibration Setup Parameters

Chem: AMYLASE
Calibration Setting
Math Model: K Factor
Factor: 3178.000 Replicates: 2
Acceptance Limits
Cal Time:
Slope Diff: SD:
Sensitivity: Repeatability: * User Defined
Deter Coeff:
Auto Calib.
☐ Cal Time

Calibrator	Conc.	Pos	Lot No.
Water	0.0	W	

Limitations
The linearity limit for amylase in samples is 1300 U/L.

Calibration
The procedure is standardized by means of the millimolar absorptivity of 2-chloro-p-nitrophenol that is 12.9 at 405 nm under the test conditions described.

Calculations (Example)

$$\frac{\Delta Abs./min \times TV \times 1000}{MMA \times SV \times LP} = U/L\ \alpha\text{-amylase in sample}$$

Where: ΔAbs./min = Absorbance difference per minute
TV = Total assay volume (1.025 ml)
1000 = Conversion of U/ml to U/L
MMA = Millimolar absorptivity of 2-chloro-p-nitrophenol (12.9)
SV = Sample volume (0.025 ml)
LP = Light path (1 cm)

$$\frac{\Delta Abs./min \times 1.025 \times 1000}{12.9 \times 0.025 \times 1.0} = \Delta Abs./min \times 3178 = U/L\ \alpha\text{-amylase}$$

Example: If ΔAbs./min = 0.03, then 0.03 x 3178 = 95 U/L

NOTE: To convert to SI Units (nKat/L) multiply the U/L value by 16.67.

Quality Control
The validity of the reaction should be monitored by use of control sample with known normal and abnormal amylase values. These controls should be run at least with every working shift in which amylase assays are performed. It is recommended that each laboratory establish its own frequency of control determination. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.

Expected Values
Urine: Spontaneously voided urine: ≤ 460 U/L; 24 hours urine collection: ≤ 410 U/24h.[18]
Since the expected values are affected by age, sex, diet and geographical location, each laboratory is strongly urged to establish its own reference range for this procedure.

Performance
1. Linearity: 15-1300 U/L
2. Comparison: A study was performed between the Yumizen 200 series and a similar analyzer and method, resulting in a correlation coefficient of 0.9999 and the linear regression equation was y=1.045 x + 4.8 (n=33).
3. Precision: Precision studies were performed using the Yumizen 200 series analyzer following a modification of the guidelines which are contained in NCCLS document EP5-T2.[19]

Within Run (n=20)

Mean	S.D.	C.V.%
43.9	0.4	1.0
189.2	1.1	0.6
136.9	0.9	0.6

4. Sensitivity: The limit of blank (LOB): 1.43 U/L

References

1. Wohlegemuth, J., Bio Chem. 29:1 (1908).
2. Somogyi, M., J. Biol Chem. 125:399 (1938).
3. Street, H.V., Close, J.R., Clin Chim Acta 1:256 (1956).
4. Henry, R.J., Chiamori, N., Clin. Chem. 6:434 (1960).
5. Rinderknecht, H.P., et al, Experentia 23:805 (1967).
6. Zinterhofer, L., et al, Clin. Chem. Acta 43:5 (1973).
7. Tietz, N.W., et al, Abs. of Proc. Of Int'l Seminar and Workshop on Enzymology, Chicago, IL (May 1972).
8. Schiwara, H.W., Artzl. Lab 17:340 (1971).
9. Pierre, K.J., et al, Clin. Chem. 22:1219 (1976).
10. Kaufman, R.A., Tietz, N.W., Clin. Chem. 26/7:851 (1980).
11. Wallenfels, K., et al, Carbohydrate Research 61:359 (1978).
12. NCCLS document "Protection of Laboratory Workers from Infectious Disease Transmitted by Blood, Body Fluids, and Tissue", 2nd Ed. (1991).
13. NCCLS document "Urinalysis; Approved Guideline", 3rd Ed. (2009).
14. Use of anticoagulants in diagnostic laboratory investigations. WHO publication WHO/DIL/LAB/99.1Rev.2, p.46 (2002).
15. Young DS. Effects of Drugs on Clinical Laboratory Tests. 4th Edition, Washington, DC, AACC Press (1995) 3: 46.
16. Young DS. Effects of Preanalytical Variables on Clinical Laboratory Tests. 2nd Edition, Washington, DC, AACC Press (1997) 3: 39
17. Young, D.S. and Friedman, D.S., Effects of Disease on Clinical Laboratory Tests, 3nd Ed., AACC Press (1997) 3: 34.
18. Junge W, Waldenström J, Bouman A et al. Evaluation of the Assays for Total and Pancreatic alpha-Amylase based on 100% cleavage of Et-G7-PNP at 6 European Clinical Centres (Poster Medlab 97); Bâle, Suisse: 12th IFCC European Congress of Clinical Chemistry (1997): 17-22.
19. NCCLS document "Evaluation of Precision Performance of Clinical Chemistry Devices", 2nd Ed. (1992).

Symbol Key

Use by (YYYY-MM-DD)	LOT Lot and batch code
REF Catalog number	Manufacturer
Temperature limitation	Consult instructions for use

Research use only

REF 12-A7564-120

Manufactured by
HORIBA Instruments Incorporated - Pointe Brand
5449 Research Drive Canton, MI 48188

Certified to Perform Reagents
The Pointe reagents are certified to be manufactured according to specified parameters. Any Pointe reagent product not meeting specifications through its listed expiration date will be remedied immediately without charge.

Rev. 02/24 P803-A7564-U-MIN