

Amylase Reagent

Catalog #: 43704

for use with the

SDI CA480 Clinical Chemistry System

INTENDED USE

For the *in vitro* quantitative determination of amylase activity in serum.

SUMMARY AND EXPLANATION¹

Amylase determinations are performed in the diagnosis and treatment of diseases of the pancreas and the investigation of pancreatic function. Pancreatitis for example is associated with increases in amylase activity in serum.

METHODOLOGY¹

Historically, several methods for the assay of amylase activity have been used. Amylolytic methods measured the disappearance of substrate, as the iodine-starch methods. Saccharogenic methods measured the production of sugars, as maltose and glucose. These methods however, lack the linearity, sensitivity, and precision, of recent chromogenic methods, which yield colored products that can easily be measured spectrophotometrically. The SDI Amylase Reagent utilizes the chromogenic substrate E-pNP-G7 (or EPS).

Principle

The reaction of amylase with EPS substrate results in the cleavage of the substrate into smaller fragments. These smaller fragments are then reacted upon by alpha glucosidase, which causes the release of the chromophore, pNP. The rate of formation of this chromophore is proportional to the activity of amylase in the sample. Amylase activity can be measured by the rate on increase in absorbance at 405 to 420nm, caused by the formation of this chromophore.

REAGENT COMPOSITION

Active Ingredients	Concentrations
E-pNP-G7	1.1 mM
Alpha Glucosidase (microbial)	>1000 U/L
Sodium Chloride	51 mmol/L
Buffers and Stabilizers	
pH	7.0 ± 0.1.

Precautions:

This reagent is for *in vitro* diagnostic use only.

REAGENT PREPARATION

Reagent is supplied ready to use.

REAGENT STORAGE

1. Store the reagent at 2-8°C
2. The reagent is stable until the expiration date when stored at 2-8°C

REAGENT DETERIORATION

DO NOT USE REAGENT IF:

1. The initial absorbance, read against water at 405nm, is greater than 0.6 AU
2. The reagent fails to meet stated parameters of performance.

SPECIMEN COLLECTION AND HANDLING¹

1. Unhemolyzed serum is the specimen of choice
2. Anticoagulants, such as Citrate and EDTA, bind calcium, which is needed for amylase activity. Therefore, plasma with these anticoagulants should not be used.

3. Amylase in serum is reported stable for 2 months when stored refrigerated (2-8°C).

INTERFERENCE

Studies to determine the level of interference for hemoglobin, bilirubin, and lipemia were carried out, the following results were obtained:

Hemoglobin:

No significant interference (± 10%) from hemoglobin up to 400 mg/dL.

Bilirubin:

No significant interference (± 10%) from bilirubin up to 25.8 mg/dL.

Lipemia:

No significant interference (± 10%) from lipemia up to 1086 mg/dL measured as triglycerides.

See Young, et al.² or other interfering substances.

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED

1. SDI CA480 Clinical Chemistry System
2. Analyzer specific consumables, e.g.: sample cups
3. Control materials such as those provided by SDI Biomed

ASSAY PROCEDURE

System Parameters

AMYLASE (Liquid)	
TEMPERATURE:	37°C
WAVELENGTH:	405 nm
ASSAY TYPE:	Rate/Kinetic
DIRECTION:	Increase
SAMPLE / RGT RATIO:	1 : 40
e.g. Sample Vol.	0.025mL (25mL)
Reagent Vol.	1.0 mL
DELAY/LAG TIME:	60 Seconds
READ TIME:	1-3 Min

Procedure Notes:

Samples with values above 2000 U/L should be diluted 1:1 with saline, re-assayed and the results multiplied by two.

Calculation

One Unit (U/L) is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute under specified conditions. For example:

$$\text{Amylase (U/L)} = \frac{\text{DAbS./min} \times 1.025 \times 1000}{10.1 \times 0.025 \times 1.0}$$

$$= \text{DAbS./min} \times 4059$$

Where

DAbS./min.	= Average absorbance change per minute
1.025	= Total reaction volume (ml)
1000	= Conversion of U/mL to U/L
10.1	= Millimolar absorptivity at 405nm
0.025	= Sample Volume (mL)
1.0	= Light path in cm

Example:

If the average absorbance change per minute = 0.10 then $0.10 \times 4059 = 406$ U/L

NOTES:

1. To convert to nkat/L multiply U/L by 16.67
2. If any of the test parameters are altered, a new factor must be calculated using the above formula

CALIBRATION

The procedure is standardized by means of the millimolar absorbativity of pNP taken as 10.1 at 405nm (9.2 at 415nm) under the test conditions described

QUALITY CONTROL

The integrity of the reaction should be monitored by use of a two level control with known Amylase values

EXPECTED RANGE¹

25-125 U/L (37°C)

It is strongly recommended that each laboratory establish its own reference range

PERFORMANCE

Linearity:

When run as recommended the assay is linear from 0 to 2000 U/L

Method Comparison:

Studies performed between this procedure and a similar methodology yielded the following results:

Number of samples pairs:	41
Range of samples:	4 – 1101 (U/L)
Correlation Coefficient:	0.9999
Slope:	0.945
Intercept:	3.4 (U/L)

Precision:

Within Run	Level 1	Level 2	Level 3
n = 40			
Mean (U/L)	23.7	48.1	419.8
S.D. (U/L)	0.8	1.3	2.8
C.V. (%)	3.3	2.6	0.7

Total

n = 40 (10 days / 2 runs per day / 2 replicates per run)			
Mean (U/L)	23.7	48.1	419.8
S.D. (U/L)	0.9	1.7	3.0
C.V. (%)	3.7	3.5	0.7

REFERENCES

- 1 Tietz, N.W., Textbook of Clinical Chemistry, 2nd Edition, Philadelphia (PA), W.B. Saunders, p. 854-861 (1994).
- 2 Young, D.S., et al. Clin. Chem, 21:1D (1975).
- 3 Expert Panel of Enzymes of the International Federation of Clinical Chemistry, Clin. Chem. 24: 497-510 (1986).
- 4 Kaplan, L.A. and Pesce, J.J., Clinical Chemistry: Theory, analysis, and correlation, 3rd Edition, St. Louis (MO), Mosby, p. 567-568 (1996).

Manufactured for:



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