

# DIRECT AMYLASE

## INTENDED USE

For the quantitative measurement of  $\alpha$ -amylase ( $\alpha$ 1, 4-glucan-4-glucanohydrolase, E.C.3.2.1.1) activity in serum and plasma.

## SUMMARY

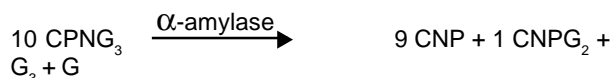
For many years, the levels of serum and plasma  $\alpha$ -amylase in patients have provided needed evidence for the diagnosis of acute pancreatitis.<sup>1-3</sup> Early assay techniques were based on either a change in the absorption maxima of the complex between starch and iodine as the  $\alpha$ -amylase degraded the starch; or a measurement of the increase in reducing groups as the starch was hydrolyzed by the  $\alpha$ -amylase.<sup>4</sup> These methods are not as reliable and easy to quantitate as spectrophotometric methods using a defined substrate.<sup>5</sup>

Some methods are based on the production of NADH proportionate to the activity of the  $\alpha$ -amylase. A defined substrate, such as maltotetraose, is degraded by  $\alpha$ -amylase to produce glucose which can be measured in a coupled enzyme assay. However, this method necessitates the removal of endogenous glucose which would give a high background to the assay.<sup>5</sup>

More recent methods are based on the production of p-nitro-phenol from defined oligosaccharide substrates with blocking groups attached on the terminal sugar. The action of the  $\alpha$ -amylase on the oligosaccharide yields a variety of chain lengths after hydrolysis. These methods then use a variety of coupling enzymes to hydrolyze the resulting short chain oligosaccharides to produce p-nitrophenol.<sup>6</sup> The coupling enzymes contain residual  $\alpha$ -amylase activity that may significantly reduce the stability of the reagent.

## PRINCIPLE

The Direct Amylase assay involves the use of a chromogenic substrate, 2-chloro-4-nitrophenol linked with maltotriose<sup>7</sup>.



As shown above,  $\alpha$ -amylase hydrolyzes the 2-chloro-4-nitrophenyl- $\alpha$ -D-maltotriose (CNPG<sub>3</sub>) to release 2-chloro-4-nitrophenol (CNP) and form 2-chloro-4-nitrophenyl- $\alpha$ -D-maltoside (CNPG<sub>2</sub>), maltotriose (G<sub>3</sub>) and glucose (G). The rate of formation of the 2-chloro-4-nitrophenol can be detected spectrophotometrically at 405 nm to give a direct measurement of  $\alpha$ -amylase activity in the sample. The reaction is not readily inhibited by endogenous factors.

## REAGENTS

### Composition

Component	Ingredients	Concentration
Direct Amylase Reagent	2-Chloro-4-Nitrophenyl- $\alpha$ -D-Maltotriose (CNPG <sub>3</sub> )	2.25 mM
	Sodium Chloride	350 mM
	Calcium Acetate	6 mM
	Potassium Thiocyanate	900 mM
	Sodium Azide	0.1 %
	Buffer	pH 6.0

### Precautions and Warnings

1. For In Vitro Diagnostic Use.
2. Do not pipette by mouth.
3. **Caution:** Contains Potassium Thiocyanate. Avoid inhalation or contact of reagent with skin and eyes. Wash skin or eyes with water and consult physician if contact occurs. Potassium Thiocyanate is not compatible with strong acids.
4. **Caution:** Contains sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azide. On disposal, flush drain with a large volume of water to prevent build up.
5. Avoid contamination of the reagent with salivary  $\alpha$ -amylase.
6. Do not use the reagent after the expiration date printed on the label.

### Preparation

The Direct Amylase reagent is supplied ready to use.

### Storage and Stability

Unopened reagent is stable until the expiration date printed on the label when stored at 2-8°C.

After opening, the reagent is stable for 60 days when properly capped immediately after each use and stored at 2-8°C.

Reagent is stable for 14 days on board the Roche/Hitachi 911 Analyzer.

### Indications of Deterioration

Absorbance greater than 0.50 vs. a water blank when measured at 405 nm in a 1 cm cuvette.

Inability to recover control values.

Presence of turbidity.

## SPECIMEN COLLECTION AND PREPARATION

Serum, sodium heparinized plasma or lithium heparinized plasma are the recommended sample types. Other anti-coagulants such as EDTA or citrate should not be used. Specimens should be collected as per the National Committee for Clinical Laboratory Standards Guideline H4-A3.<sup>8</sup>

Serum: Collect whole blood by venipuncture and allow to clot. Centrifuge and remove the serum as soon as possible after collection.<sup>10</sup> (Within 3 hours)

Plasma: Specimens may be collected in sodium or lithium heparin. Centrifuge and remove the plasma as soon as possible after collection.<sup>10</sup> (Within 3 hours)

If not analyzed promptly, samples may be stored ambient (20-25°C) for 14 days, refrigerated (2-8°C) for 21 days or frozen (-20°C) for 10 weeks. Samples may be frozen (-20°C), in a non-frost free freezer, and thawed for up to two freeze/thaw cycles. Refer to NCCLS Document H18-A for further instructions on specimen collection, handling, and storage.<sup>8</sup>

## PROCEDURE

### Manual Assay

1. Pipette 1.0 mL of the  $\alpha$ -amylase reagent into appropriate number of cuvettes for duplicate sampling and preparation of a blank.
2. Transfer the cuvettes to the spectrophotometer and equilibrate to 37°C  $\pm$  1°C.
3. Pipette 25  $\mu$ L of patient or control sample to the cuvette, cover and mix briefly by inversion.
4. Introduce 25  $\mu$ L of normal saline to the blank cuvette and mix by inversion.
5. Record absorbance at 405 nm for the blank, each of the samples and controls after 60 seconds and again after 120 seconds.
6. Subtract the absorbance value at 60 seconds from the 120 second absorbance value to obtain the ( $\Delta A$ /min for each of the blanks, controls and samples.

### Notes

1. The millimolar absorptivity of 2-chloro-4-nitrophenol varies with pH, temperature and wavelength.
2. Ensure that cuvette covers or films are not interchanged between samples.

### Automated Application

Follow the instrument manufacturer's instructions.

### Materials Provided

The  $\alpha$ -Amylase reagent is packaged in various formats. Any of the following items may be included in the package you receive.

Description	Configuration	Catalog No
Direct Amylase Reagent	1 x 1L	80-5260-00
Direct Amylase Reagent	3 x 100 mL	80-5259-00
Direct Amylase Reagent	1 x 50 mL	80-5261-00

### Materials Required but not Provided

Description	Configuration	Catalog No
Direct Amylase Verifier	2 x 3 mL	80-5773-00

1. Class A volumetric pipettes.
2. Spectrophotometer which maintains cuvette assay temperature at 37°C and is capable of measuring absorbance at 405nm.
3. Normal saline.
4. Cuvette covers or film.
5. Timer or stopwatch.
6. Amylase control sera or quality control material (See "Quality Control").

## Calibration

$\alpha$ -Amylase activity is calculated based on the millimolar absorptivity of 2-chloro-4-nitrophenol. The millimolar absorptivity of 2-chloro-4-nitrophenol varies with pH, temperature and wavelength. The millimolar absorptivity at pH 6.0 and 37°C, when measured in a 1 cm cuvette is 12.9. Instrument calibration should be at a wavelength of 405 nm. Refer to the instrument manufacturer's recommendation for calibration frequency.

## Quality Control

Reliability of test results should be routinely monitored with quality control materials or serum pools that reasonably represent performance on patient specimens. Controls or serum pools should be run with each assay to ensure that the reagents are functioning properly and that correct procedures have been followed. Quality control materials are intended for use only as monitors of accuracy and precision. An acceptable range for each lot of control material should be established by the laboratory. If control values are not within the expected range confirm procedures were performed correctly and follow normal troubleshooting measures. If the problem persists call Genzyme Technical Marketing in the U.S. at (800) 332-1042 or in the U.K. at (+44) (0) 1732 220022.

Quality control requirements should be established in accordance with local, state and/or Federal regulations or accreditation requirements.

## RESULTS

Calculate the  $\alpha$ -amylase activity of each sample and control using the following formula:

$$\alpha\text{-amylase (U/L)} = \frac{(\Delta A/\text{min}_s - \Delta A/\text{min}_b) \times \text{T.V.} \times 1000}{\epsilon \times \text{S.V.} \times d}$$

Where:

$\Delta A/\text{min}_s$  = Change in the absorbance per minute for the sample or control

$\Delta A/\text{min}_b$  = Change in the absorbance per minute for the blank

T.V. = Total volume of the assay (1.025 mL)

1000 = Conversion from U/mL to U/L

$\epsilon$  = Millimolar extinction coefficient of 2-chloro-4-nitrophenol at 405 nm, pH 6.0 and 37°C (12.9)

S.V. = Sample volume (0.025 mL)

d = Light path (1 cm)

$(\alpha\text{-amylase (U/L)}) = (\Delta A/\text{min}_s - \Delta A/\text{min}_b) \times K$

K = analyzer specific K factor determined with Genzyme's Direct Amylase Verifier, Cat. No. 80-5773-00.

## Limitations/Interfering Substances

1. Refer to the work of Young, et al<sup>14</sup> for a review of drug effects on amylase levels.
2. Amylase enzyme activity is temperature dependent<sup>10</sup>. Assays performed at temperatures <37°C or >37°C will show an apparent decrease or increase in amylase levels respectively.
3. Macroamylase has been shown to cause hyperamylasemia which may lead to overdiagnosis of

acute pancreatitis when using oligosaccharide substrates<sup>11</sup>.

- No significant interference was detected in the Direct Amylase assay up to and including the concentrations stated on the next page using the Roche/Hitachi 911 Analyzer.

<b>Substance Tested</b>	<b>Concentration with no significant (<math>\pm 10\%</math>) Interference</b>
Liposyn	3000 mg/dL (1%)
Triglyceride	3000 mg/dL
Ascorbic Acid	50 mg/dL
Bilirubin (unconjugated)	50 mg/dL
Bilirubin (conjugated)	50 mg/dL
Hemoglobin	500 mg/dL
Glucose	2000 mg/dL

Samples with hemoglobin interference higher than the upper limit may be diluted 1 part sample with 1 part physiological saline before assaying. Multiply the result by two to correct for the dilution.

### Expected Values

The expected range for Amylase is 23 - 88 U/L. It is recommended that each laboratory establish the normal range for its location.

## PERFORMANCE CHARACTERISTICS

### Sensitivity

An absorbance change of 0.0003 OD units per minute corresponds to an  $\alpha$ -amylase activity of 1 U/L at 37°C for an extinction coefficient of 12.9.

### Precision

Within run precision of the Direct Amylase reagent was determined using four levels of frozen serum pools analyzed 20 times in a single run on the Roche/Hitachi 911 Analyzer.

<b>Serum Pool</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>
n	20	20	20	20
Mean (U/L)	61	272	902	1509
% CV	1.34	0.61	0.51	0.62

Between run precision of the Direct Amylase reagent was determined using four levels of frozen serum pools. Each pool was run in duplicate for 20 times over at least five days on the Roche/Hitachi 911 Analyzer.

<b>Serum Pool</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>
n	40	40	40	40
Mean (U/L)	60	273	917	1507
% CV	1.7	0.8	0.9	0.6

### Accuracy

In comparative performance studies, 50 samples ranging in activity from 28-304 U/L were evaluated by the Genzyme  $\alpha$ -Amylase Test Reagent and another commercially available  $\alpha$ -Amylase method on the Roche/Hitachi 911 Analyzer. The correlation coefficient for the study was  $r = 0.9985$ . The regression equation was  $y = 0.90x - 2.50$ .

### Linearity

The  $\alpha$ -Amylase Test Reagent is linear to 2000 U/L on the Roche/Hitachi 911 Analyzer. If a sample exceeds 2000 U/L, it should be diluted with an equal volume of normal saline and re-assayed. Multiply the value from the resulting calculation by 2 to correct for the dilution.

## REFERENCES

- Ranson, JHC, Curr. Prob. Surg., 16:1 (1979).
- Salt, WB II and Schenker, S., Medicine, 55:269 (1976).
- Stefanini, P., Ermini, M., and Carboni, M., J. Am. Surg., 110:866 (1965).
- Henry, RJ, and Chiamori, N., Clin Chem., 6:434 (1960).
- Kaufman, RA and Tietz, NW, Clin Chem., 26:846 (1980).
- Blair, HE, U.S. Patent No. 4,649,108.
- Chavez, RG, et al, U.S. Patent 4,963,479.
- NCCLS document "Procedures for the Collection of Diagnostic Blood Specimens by Skin Puncture", 3rd Ed., 11:11 (1991).
- Demetriou, J., et al, "Clinical Chemistry: Principles and Techniques", 2nd Ed., Harper & Row (1974).
- Suelter, CH, "A Practical Guide to Enzymology", Wiley & Sons (1985).
- Rosenblum, JL, et al, Clin Chem., 38:9 (1992).
- Tietz, NW; "Clinical Guide to Laboratory Tests"; W.B. Saunders Co., 54 (1983).
- NCCLS document "Evaluation of Precision Performance of Clinical Chemistry Devices", 2nd Ed., 12:4 (1992).
- Young, DS, "Effects of Drugs on Clinical Laboratory Tests", 4th Ed., AACC Press, Washington DC; 3-43 to 3-47 (1995).
- IFCC document "The Theory of Reference Values", Stage 2, draft 2, J. Clin. Biochem., 21:749 (1983).

Manufactured by:



### Genzyme Corporation

One Kendall Square  
Cambridge, MA  
02139-1562  
USA  
Tel: 1-800-332-1042  
Fax: 1-617-252-7759

[www.genzymediagnosics.com](http://www.genzymediagnosics.com)

### Genzyme Diagnostics

50 Gibson Drive  
Kings Hill, West Malling  
KENT ME19 4AF  
United Kingdom  
Tel: +44 (0)1732-220022  
Fax: +44 (0)1732-220024