AMYLASE CNPG Liquiform

Insert

Ref.: 142

Purpose . System for determination of α-amylase in blood, urine and other biological fluids.

[Only for in vitro diagnostic use]

Principle . The α-amylase hydrolyzes the substrate 2-chloro-p-nitrophenyl-α-D-maltotrioside (CNPG3), releasing 2-chloro-4-nitrophenol (CNP) and forming 2-chloro-4-nitrophenyl-α-D-maltoside (CNPG2), maltotriose (G3) and glucose (G). The rate of formation of 2-chloro-4-nitrophenol may be measured photometrically and provides a direct measure of the activity of α-amylase in the sample.

$$10\ \text{CNPG3} \xrightarrow{\alpha\text{-amylase}} 9\text{CNP} + 1\text{CNPG2} + 9\text{G3} + 1\text{G}$$

System Features . The latest methods for determination of α-amylase is based on the production of p-nitrophenol from the hydrolysis of well defined substrates of oligosaccharides with blocking groups bound to terminal carbohydrate residues. The hydrolytic action of α-amylase on these oligosaccharides produces various chains of different sizes, being required to couple auxiliary enzymatic reactions to release p-nitrophenol. In many cases, the presence of amylase traces as contaminants of auxiliary enzymes greatly decreased the stability of these substrates.

The proposed assay uses a chromogenic substrate, 2-chloro-p-nitrofenol, bound to maltotriose. The α-amylase acts directly on this substrate, releasing an amount greater than 90% of the chromophore CNP, whose formation rate can be measured in kinetic mode. It is used a substrate in liquid medium, which does not require the use of auxiliary enzymes to form the color product, thus obtaining a prolonged stability of the substrate.

The Labtest system is easily applicable in automated and semi-automatic analyzers capable of measuring a kinetic reaction at 405 nm. The high linearity of the assay decreases considerably the number of samples which need to be diluted.

Methodology . Substrate 2-chloro-p-nitrophenyl-α-D-maltotrioside (CNPG3).

Reagent

1. R 1 **- Substrate - Store at 2 - 8 ºC**

Contains ≤100 mM buffer at pH 6.2; 560 μM 2-chloro-p-nitrophenyl-α-D-maltotrioside; 350mM sodium chloride, 6.0 mM calcium acetate, 900 mM potassium thiocyanate and 14.6 mM sodium azide.

The unopened reagent, when stored under the specified conditions, is stable until the expiration date printed on label. During handling, the reagents are subject to contamination of microbial and chemical nature, which may result in reduced stability.

Precautions and warnings

Actions such as pipetting the substrate by mouth, blowing into the substrate, using material contaminated by saliva, sweat and talk near to uncapped bottle can contaminate the reagent with microscopic amounts of saliva or sweat, capable of irreversibly damage the substrate.
As with all enzymatic reaction, the strict observation of time and incubation temperature is of great importance for the quality of the results.

Stability studies showed that the absorbance of the substrate, measured against water, presents an increase of 0.0015 each month. Sudden increases in the absorbance of the substrate indicate contamination and its use should be discontinued.

Usual safety care should be applied when handling the reagent. The reagent contains sodium azide which is toxic. Do not ingest, and in the case of contact with eyes, wash immediately with plenty of water and seek medical advice. Azide can form highly explosive compounds when in contact with lead or copper pipes. Therefore, use large volumes of water to dispose the reagent.

The reagent also contains potassium thiocyanate, which is poisonous. Do not ingest it.

Materials required and not provided

1. Photometer with thermostated cuvette able to accurately measure the absorbance at 405 nm.
2. Pipettes to measure sample and reagent.
3. Chronometer.
4. Calibrator - Calibra H Line - Ref 80, Labtest.

Sample

Use serum, plasma (heparin), urine and fluids (ascitic, duodenal and pleural). Samples containing citrate, oxalate or EDTA produce falsely decreased results.

The enzymatic activity is stable for 7 days between 15 - 25 ºC and for several months between 2 - 8 ºC. Do not use samples with signs of microbial contamination.

Urine samples must be collected within 2 to 24 hours.

When determining amylase activity in urine sample, you must also determine creatinine in the same sample.

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The result should be reported as Amylase/Creatinine ratio (U/g) in order to compensate for variations in amylase activity in samples obtained in each urination. Urine samples should be stored at 2 - 8 ºC. Do not add preservative.

A Standard Operating Procedure (SOP) for collection, preparation and storage of samples should be elaborated. We emphasize that the errors due to the sample could be much larger than errors occurring during the analytical procedure.

Since no known test can ensure that blood samples will not transmit infections, all should be considered potentially infectious. Therefore, when handling samples, you must follow the rules established for biosafety.

For disposal of chemicals and biological material we suggest applying the applicable local, state or federal environmental protection standards.

Interference

Bilirubin up to 10 mg/dL, hemoglobin up to 200 mg/dL and triglycerides up to 1800 mg/dL do not significantly interfere with the reaction.

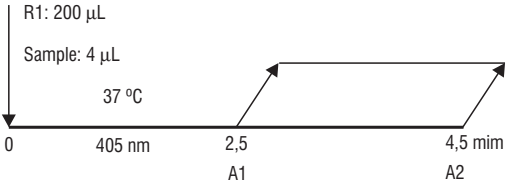
Drugs such as cholinergic, narcotics (morphine) and alcohol produce falsely elevated serum amylase.

The presence of macroamylase in the serum sample, resulting from complexation of amylase and high molecular weight proteins, may produce falsely elevated results in the absence of pancreatitis. In these cases, increased activity of amylase in the urine is not observed.

Procedure

Parameters for automated analyzers

Parameters	Application
Reaction Type	Kinetic
Reaction Direction	Increasing
Primary λ	405 nm
Secondary λ	700 nm
Temperature	37 ºC
Calibration	2 points Point 0: Blank (Deionized water / Saline) Point 1: Calibrator 1
Calibration Model*	Linear
Sample Volume**	4 µL
R1 Volume**	200 µL
Reading 1 (Abs 1)	150 seconds after the addition of R1 + sample
Reading 2 (Abs 2)	270 seconds after the addition of R1 + sample



*The definition of calibration model must be suitable for each type of equipment. If in doubt, please contact the Labtest Customer Service.

**The sample and reagents volumes can be modified proportionately without affecting the test performance. In case of reduced volumes, it is essential to observe the minimum volume required for the photometric measurement.

Manual Procedure
Optimum reaction conditions:
Wavelength: 405 nm;
Thermostated cuvette at 37 ±0.2 ºC with 1.0 cm thick solution;
Passband ≤2 nm;
Stray light ≤0.1.

When optimum reaction conditions mentioned above are met, you can choose to use the factor 6829.

Take two test tubes and proceed as follows:

	Test	Calibrator
Sample	0.02 mL	----
Calibrador*	----	0.02 mL
Reagent 1	1.0 mL	1.0 mL

*It is recommended to use the calibrator Calibra H - Labtest.

1. After the addition of reagent, mix and immediately transfer to the thermostated reaction cuvette at 37 ±0.2 ºC. Wait 30 seconds.

2. Read the initial absorbance (A1) at 405 nm simultaneously firing the chronometer. Repeat the reading after 2 minutes (A2).

As with any measurement of enzyme activity, strict observation of time and incubation temperature is of great importance for the quality of the results.

To assess the linearity of the reaction, record the absorbance at 1 minute intervals and check whether the differences of absorbance are equivalent in every minute.

Calculations . As in most cases it is not possible to work under optimum reaction conditions; good laboratory practices recommend performing calibration test by using the calibrator indicated by the manufacturer of the reagent. The Labtest indicates the calibrator Calibra H - Ref. 80 for calibrating the Amylase CNPG system.

$\Delta A/minute\ Test\ or\ Calibrator = (A_2-A_1)/2$

$$Factor = \frac{Calibrator\ Activity}{\Delta A/min\ Calibrator}$$

$Amylase\ (U/L) = \Delta A/min\ Test \times Factor$

Example

Calibrator
$A_1 = 0.158$ $A_2 = 0.290$

$$\Delta A/min\ Test = \frac{0.158 - 0.290}{2} = 0.066$$

Test
$A_1 = 0.092$ $A_2 = 0.132$

$$\Delta A/min\ Calibrator = \frac{0.132 - 0.092}{2} = 0.020$$

Calibrator Activity (U/L) = 454

$$Factor = \frac{454}{0.066} = 6878$$

Amylase activity (U/L) = 0.040 x 6872 = 136 U/L

Urinary amylase/Time

$$Urinary\ amylase\ (U/h) = \frac{Amylase\ (U/L) \times urine\ volume\ (ml)}{1000 \times sampling\ time\ (h)}$$

Amylase/Creatinine Ratio

$$Amylase/Creatinine\ Ratio\ (U/g) = \frac{Amylase\ (U/L) \times 100}{Creatinine\ (mg/dL)}$$

Calibration . Use calibrator Calibra H - Ref 80, Labtest.

Calibration ranges
When internal quality control indicates.
When using new batch of reagents.
When using new reagent vials of the same batch, if a new calibration has been performed during the use of the preceding vial.

Linearity

The reaction is linear up to 1700 U/L. For higher values, dilute the sample with 150 mmol/L NaCl (0.85%). Perform a new measurement and multiply the result by the dilution factor.

Internal quality control . The laboratory should maintain a program of internal quality control clearly defining regulations, objectives, procedures, criteria for quality specifications and tolerance limits, corrective actions and activities recording. Control materials should be used to monitor the measurement inaccuracy and calibration deviations.

It is suggested that the specifications for the coefficient of variation and total error are based on the components of biological variation (BV)[1,2,3].

Amylase clearance/creatinine clearance ratio . In most cases of acute pancreatitis, concomitant elevations in serum amylase and urine occur, but in some cases the elevation of urinary amylase is not accompanied by a parallel increase in serum amylase. Therefore, the assessment of the amylase clearance/creatinine clearance ratio, expressed as a percentage, provides greater diagnostic value in cases of acute pancreatitis and recurrent pancreatitis.

Determine the amylase activity and the concentration of creatinine in serum and urine sample and apply the results in the following formula:

$$Ratio\ (\%) = \frac{Urine\ amylase\ (U/L) \times Serum\ creatinine\ (mg/dL)}{Serum\ amylase \times Urine\ creatinine\ (mg/dL)} \times 100$$

Reference range . These values should be used only as a guide. It is recommended that each laboratory establish its own reference range in the population serviced.

Serum/Plasma
25 - 125 U/L

Urine
Up to 30 U/h

Urine amylase/Urine creatinine ratio
To 400 U/g

Amylase clearance/Creatinine clearance ratio
10 to 4.0%

Conversion of U/L to IS Units: µKat/L = U/L x 0.0167

Performance Characterization[4]

Accuracy . In three samples with values of 182, 542 and 886 U/L were added different quantities of analyte to yield recoveries between 104.8 and 107.6%. The average total systematic error obtained was 6.4%, which meets the desirable specification based on the components of Biological Variation of total error, which is $\leq \pm 7.4\%$[2].

Studies comparing methods . The proposed method was compared with another product of similar methodology, and the following results were obtained:

	Comparative Method	Amylase CNPG
Samples	40	40
Concentration range (U/L)	30 - 999	33 - 1027
Regression equation	Labtest Method = 1.0104 x Comparative Method - 1.80	
Correlation coefficient	0.996	

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By using the regression equation, the systematic error (bias) estimated is equal to 2.55% for a sample with amylase activity equal to 50 U/L, 0.46% for a sample with amylase activity equal to 120 U/L, and 0.14% for a sample with amylase activity of 200 U/L. These errors are smaller than the analytical systematic error of the desirable specification based on biological variation, which is ≤±7.4%.

Imprecision studies . The accuracy studies were performed using samples with activity values equal to 52, 120 and 202 U/L.

Imprecision - Within run

	N	Mean (U/L)	SD (U/L)	%CV
Sample 1	20	52	0.77	1.55
Sample 2	20	120	1.60	1.09
Sample 3	20	202	2.71	0.54

Imprecision - Run-to-run

	N	Mean (U/L)	SD (U/L)	%CV
Sample 1	20	52	0.89	2.22
Sample 2	20	120	1.68	1.83
Sample 3	20	202	2.47	1.64

The total inaccuracy obtained for samples meets the desirable specification for total inaccuracy based on biological variation, which is ≤4.4%[6].

The total error (random error + systematic error) estimated for activities of 50, 120 and 200 U/L are equal to 6.22, 3.48 and 2.85%, respectively.

The results indicate that the method meets the desirable specification for the total error (≤±14.6%) based on desirable components of the Biological Variation[2].

Methodological sensitivity . A protein sample containing 47 U/L related to amylase activity was used to calculate the detection limit of the assay, having found a value of 2.4 U/L, equivalent to 3 times the standard deviation of 20 sample replicates.

Effect of matrix dilution . Two samples with values equal to 1613 and 1629 U/L were used to evaluate the system response to matrix dilution with 150 mmol/L NaCl (0.85%). By using dilution factors ranging from 2 to 4, recoveries between 105 and 114% were found. The results indicate that the method meets the desirable specification for the total error (≤±14.6%) based on desirable components of the Biological Variation[2].

Notes

1. The material cleaning and drying are fundamental factors to the reagent stability and to obtain correct results.

2. The deionized or distilled water in the laboratory to prepare reagents, use in the measurements and for final glass washing must have resistivity ≥1 megaohm, or conductivity ≤1 microsiems and silicates concentration must be ≤0.1mg/L.

Reference

1. Westgard JO, Barry PL, Hunt MR, Groth T. Clin Chem 1981;27:493-501.

2. Ricos C, Desirable Specifications for Total Error, Imprecision, and Bias, derived from intra- and inter-individual biologic variation. Disponível em: http://westgard.com/biodatabase1.htm (acesso em 24/01/2012).

3. Basques JC. Especificações da Qualidade Analítica. Labtest Diagnóstica 2005.

4. Labtest: File data.

5. Genzyme Diagnostics. α-Amylase Teste Reagent,1996.

6. Kaufman RA, Tietz NW.ClinChem 1980;26:846-53.

7. Pesce AJ Kaplan LA. Methods in Clinical Chemistry, St, Louis: The C.V. Mosby Co., 1987;817-830.

8. Roseblum JL. Clin Chem 1992;38:920.

Presentation

Product	Reference	Contents
Amylase CNPG Liquiform	142-2/30	R 1 2 x 30 mL

Application procedures using Calcium Arsenazo Liquiform are available **for various automated systems.**

The number of tests in automated systems **depends of the programmed parameters.**

Consumer information

[Warranty conditions]

Labtest Diagnóstica warrants the performance of this product under the specifications until the expiration date shown in the label since the application procedures and storage conditions, indicated on the label and in this insert, have been followed correctly.

CE

Labtest Diagnóstica S.A.

CNPJ: 16.516.296 / 0001 - 38

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Símbolos utilizados com produtos diagnósticos in vitro

Símbolos usados con productos diagnósticos in vitro

Symbols used with ivd devices

	**Conteúdo suficiente para < n > testes** Contenido suficiente para < n > tests Contains sufficient for < n > tests		**Risco biológico** Riesgo biológico Biological risk
	**Data limite de utilização (aaaa-mm-dd ou mm/aaaa)** Estable hasta (aaaa-mm-dd o mm/aaaa) Use by (yyyy-mm-dd or mm/yyyy)		**Marca CE** Marcado CE CE Mark
	**Material Calibrador** Material Calibrador Calibrator Material		**Tóxico** Tóxico Poison
	**Material Calibrador** Material Calibrador Calibrator Material		**Reagente** Reactivo Reagent
	**Limite de temperatura (conservar a)** Temperatura limite (conservar a) Temperature limitation (store at)		**Fabricado por** Elaborado por Manufactured by
	**Representante Autorizado na Comunidade Europeia** Representante autorizado en la Comunidad Europea Authorized Representative in the European Community		**Número do lote** Denominación de lote Batch code
	**Consultar instruções de uso** Consultar instrucciones de uso Consult instructions for use		**Controle** Control Control
	**Número do catálogo** Número de catálogo Catalog Number		**Controle negativo** Control negativo Negative control
	**Adições ou alterações significativas** Cambios o suplementos significativos Significant additions or changes		**Controle positivo** Control positivo Positive control
	**Produto diagnóstico in vitro** Dispositivo de diagnóstico in vitro In vitro diagnostic device		**Controle** Control Control
	**Liofilizado** Liofilizado Lyophilized		**Corrosivo** Corrosivo Corrosive
	**Período após abertura** Periodo post-abertura Period after-opening		**Uso veterinário** Uso veterinario Veterinary use
	**Instalar até** Instalar hasta Install before		

Ref.: 140214

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