AMYLASE CNPG LIQUIFORM VET

Instructions for Use

Ref.: **1088**

Purpose . System for determination of α-Amylase in samples.

Professional use only.

[Only for *in vitro* diagnostic use]

Principle . 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 can be measured photometrically and provides a direct measure of α-Amylase activity in the sample.

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

System Characteristics . The most recent methods for α-Amylase determination are based on the production of p-nitrophenol from the hydrolysis of well-defined oligosaccharide substrates, with blocking groups attached to the terminal carbohydrate residue. The hydrolytic action of α-Amylase on these oligosaccharides produces several chains of different sizes, and it is necessary to attach auxiliary enzymatic reactions to release p-nitrophenol. In many cases, the presence of traces of amylase as a contaminant of auxiliary enzymes considerably reduces the stability of these substrates.

The proposed assay uses a chromogenic substrate, 2-chloro-p-nitrophenol, linked to maltotriose. α-Amylase acts directly on this substrate by releasing an amount greater than 90% of the CNP chromophore, whose formation speed can be measured in kinetic mode. A substrate in a liquid medium is used. This does not require the use of auxiliary enzymes for the formation of the colored product, thus obtaining a prolonged stability of the substrate.

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

Methodology . 2-Chloro-p-nitrophenyl-α-D-maltotrioside substrate (CNPG3).

Reagent

1. R1 **- Substrate - Store at 2 -8 ºC**

It contains a buffer ≤100 mM, pH 6.2; 2-chloro-p-nitrophenyl-α-D-maltotrioside ≤560 μM; sodium chloride ≤350 mM; calcium acetate ≤6 mM; potassium thiocyanate 900 mM and sodium azide 14.6 mM.

Unopened reagent, when stored under the conditions stated, is stable until the expiration date printed on the label. While handling the reagents, they are subject to chemical and microbial contaminations that can cause reduced stability.

Precautions and special care

Actions such as pipetting the substrate by mouth, blowing on the substrate, using material contaminated with saliva, sweat and talking with the uncapped bottle can contaminate the reagent with microscopic amounts of saliva or sweat, capable of irreversibly deteriorating the substrate.

As in every enzymatic reaction, rigorous observation of the incubation time and temperature is of paramount importance for the quality of the results obtained.

Stability studies showed that the substrate absorbance, measured against water, increased by 0.0015 per month. Sudden increases in substrate absorbance indicate contamination and its use should be discontinued.

The usual safety precautions must be applied when handling the reagent. The reagent contains sodium azide which is toxic. Do not ingest and, in case of contact with the eyes, wash immediately with a large amount of water and seek medical assistance. Azide can form highly explosive compounds with lead or copper pipes. Therefore, use a large volume of water to dispose of the reagent.

The reagent also contains potassium thiocyanate, which is poisonous. Do not swallow it. In contact with acidic substances, it releases highly toxic gases.

Materials required and not provided

1. Photometer with a thermostated cuvette capable of accurately measuring the absorbance at 405 nm.
2. Pipettes for measuring sample and reagent.
3. Timer.
4. Calibrator - Calibra VET - Ref. 1015, Labtest.

Sample

Serum and plasma heparin. The enzyme activity is stable for 7 days between 20 - 25 ºC, 7 days between 4 - 8 ºC and 1 year at -20 ºC [11].

A Standard Operating Procedure (SOP) should be created for sample collection, preparation and storage. We emphasize that the errors due to the sample can be much greater than the errors that occurred during the analytical procedure.

All blood samples must be considered potentially infectious and handled in accordance with established biosafety standards.

To dispose of reagents and biological material, we suggest applying local, state or federal environmental protection standards.

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Interferences

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

Cholinergic and narcotic drugs (morphine) produce falsely high serum amylase results.

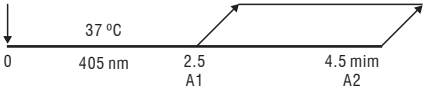
The presence of macroamylase in the serum sample, which results from the complexation of amylase with high molecular weight proteins, can produce falsely high results in the absence of pancreatitis.

Procedure

Parameters for Automatic Analyzers

Parameters	Application
Type of Reaction:	Kinetics
Reaction Direction	Growing
λ of Primary Wave	405 nm
λ Secondary Wave	700 nm
Temperature	37 ºC
Calibration	2 points Point 0: Blank (Deionized/Saline Water) Point 1: Calibrator 1
Calibration Model*	Linear
Sampel Volume**	4 µL
Volume of R1**	200 µL
Reading 1 (Absorbance 1)	150 seconds after adding R1 + sample
Reading 2 (Absorbance 2)	570 seconds after adding R1 + sample

R1: 200 µL
Sample: 4 µL



*The definition of a calibration model must be suitable for each model of equipment. Should you have any question, please contact Labtest Customer Service.

**Sample and reagent volumes can be modified proportionately without detriment to test performance. In case of volume reduction, it is essential to observe the minimum volume necessary for the photometric measurement.

Manual Procedure

Optimal Reaction Conditions:
Wave-length: 405 nm;
Cuvette thermostated at 37 ± 0.2 ºC with 1.0 cm of solution thickness;
Pass band ≤ 2 nm;
Stray Light ≤ 0.1.

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

Take 2 test tubes and proceed as follows:

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

*We recommend using the calibrator Calibra VET - Ref. 1015– Labtest.

1. After adding the reagent, homogenize it and transfer it immediately to the thermostated reaction cuvette at at 37 ± 0.2ºC. Wait 30 seconds.

2. Read the initial absorbance (A_1) at 405 nm, simultaneously starting the timer. Repeat the reading after 2 minutes (A_2).

As in every measurement of enzyme activity, strict observation of the incubation time and temperature is of paramount importance for the quality of the results obtained.

To assess the linearity of the reaction, record the absorbance at 1-minute Ranges and verify that the absorbance differences in each minute are equivalent.

Calculations . As in most cases it is not possible to work under optimal reaction conditions, good laboratory practices recommend performing the assay calibration using the calibrator stated by the reagent manufacturer. Labtest indicates the calibrator Calibra VET – Ref. 1015 for calibrating the Amylase CNPG Liquiform VET system.

A/minute Test or calibrator = $(A_2-A_1)/2$

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

Amylase (U/L) = A/min Test x Factor

Example

Calibrator
$A_1 = 0.158$ $A_2 = 0.290$

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

Test
$A_1 = 0.092$ $A_2 = 0.132$

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

Calibrator Activity (U/L) = 454

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

Amylase Activity (U/L) = 0.040 x 6878 = 136 U/L

Amylase/Creatinine Ratio

$$\text{Creatinine Amylase Ratio (U/g)} = \frac{\text{Amylase (U/L)x100}}{\text{Creatinine (mg/dL)}}$$

Calibration . Use calibrator Calibra VET - Ref. 1015, Labtest.
Calibration Range

When the internal quality control indicates.
When using a new batch of reagents.
When using new reagent bottles from the same batch, if a new calibration was performed while using the previous bottle.

Linearity

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

Internal Quality Control . The laboratory must maintain an internal quality control program that clearly defines the applicable regulations, objectives, procedures, criteria for quality specifications and tolerance limits, corrective actions and record of activities. Control materials must be used to monitor measurement imprecision 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].

Reference Range[9] . These values are to be used as a guide only. It is recommended that each laboratory establish its own reference range in the animal population served.

Species (U/L)

Canine	185-700
Feline	<500
Bovine	-
Equine	75-150

Conversion from U/L to SI Units: μKat = U/L x 0.0167

Performance Characterization[4]

Accuracy . In three samples with values of 182, 542 and 886 U/L, different amounts of the analyte were added, obtaining recoveries between 104.8 and 107.6%. The mean total systematic error obtained was 6.4%.

Method Comparison Studies . The proposed method was compared with a commercially available dry chemical product for veterinary use only. The following results were obtained using dog samples:

	Comparative Method	Labtest Method
Concentrarion Range (U/L)	362-1500	481-1348
Regression Equation	Labtest Method = 0.7472x Comparative Method +237.35	
Correlation Coefficient	0.9581	

Using the regression equation, the estimated systematic error (bias) is equal to 8.63 % for a sample with amylase activity equal to 700 U/L and 9.46% for a sample with amylase activity equal to 1,500 U/ L.

Precision Studies . Precision studies were performed using a sample with an enzymatic activity equal to 202 U/L.

Repeatability - Intra-assay Imprecision

	N	Average(U/L)	SD	CV (%)
Sample 1	20	202	2,71	0,54

Reproducibility Total Imprecision

	N	Average(U/L)	SD	CV (%)
Sample 1	20	202	2,47	1,64

Methodological Sensitivity . A protein sample containing 47 U/L referring to amylase activity was used to calculate the detection limit of the assay, and a value equal to 2.4 U/L, equivalent to 3 times the standard deviation of 20 replicates of the sample has been found.

Matrix Dilution Effects . Two samples with values equal to 1613 and 1629 U/L were used to evaluate the system response in matrix dilutions with NaCl 150 mmol/L (0.85%). Using dilution factors ranging from 2 to 4, recoveries between 105 and 114% were found.

Clinical Significance[10] . Serum amylase is present in several tissues, in greater amounts in the pancreas and duodenum. This enzyme acts directly in the intestine by hydrolyzing starch and glycogen. An increase in this enzyme may be indicative of gastrointestinal disease (intestinal perforation, small bowel obstruction), pancreatitis, pancreatic necrosis, obstruction of the pancreatic ducts, and pancreatic neoplasia.

Hyperamylasemia only occurs 12 hours after the process began, and may be an indication of injury to the acinar cells and obstruction of the pancreatic duct. However, serum amylase concentrations in dogs may increase due to extrapancreatic causes, due to isoenzymes located in the intestines, kidneys, uterus, ovaries, and testicles. Thus, it is assumed that only a 3-to 4-fold increase in the values of this enzyme can be diagnostic for acute pancreatitis.

Although serum amylase activity is readily available in routine (standard) biochemical tests, its usefulness in diagnosing pancreatitis is limited. Cats with spontaneous or experimental pancreatitis typically have serum amylase activity within the normal range or slightly increased, although a reduction has also been reported. Therefore, serum amylase activity is not helpful in diagnosing pancreatitis in felines.

Notes

1. Proper cleaning and drying of the material used are fundamental factors for the stability of the reagents and obtaining correct results.

2. The clinical laboratory aims to provide accurate and precise results. The use of water of inadequate quality is a potential cause of analytical errors. The water used in the laboratory must be of adequate quality for each application. Thus, to prepare reagents, use in measurements and for use in the final rinsing of the glassware, the water must have resistivity ≥1 megaohm.cm or conductivity ≤ 1 microsiemens/cm and silicate concentration <0.1 mg/L. When the deionizing column has its capacity saturated, several ions, silicates and substances with great oxidation or reduction power are released, which deteriorate the reagents in a few days or even hours, altering the results in an unpredictable way. Thus, it is essential to establish a water quality control program.

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References

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Presentation

Product	Reference	Content
Amylase CNPG Liquiform VET	1088-1/30	R 1 1 x 30 mL

Consumer Information

[Guarantee Terms and Conditions]

Labtest Diagnóstica guarantees the product performance the specifications, until the expiration date stated on the labels, provided that the care for use and storage stated on the labels and in these instructions are correctly followed.

Labtest Diagnóstica S.A.

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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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