BIOLABO
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MANUFACTURER:
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AMYLASE CNPG3

Reagent for quantitative determination of α-amylase activity [EC 3.2.1.1] in human serum and plasma, or urines

REF 99523	**R1** 1 x 105 mL	**R2** 20 x 5 mL
REF 99123	**R1** 8 x 30 mL	**R2** 8 x 30 mL

TECHNICAL SUPPORT AND ORDERS
Tel: (33) 03 23 25 15 50
support@biolabo.fr
Latest revision : www.biolabo.fr

CE

IVD

Made in France

I: corresponds to significant modifications

INTENDED USE

This reagent is designated for professional use in laboratory (manual or automated method).
I It allows the quantification of α-amylase activity in human serum and plasma, or urines.

GENERALITIES (1) (2)

α-amylase is most frequently measured in the diagnostic of acute pancreatitis. In this case, a transient rise in serum amylase activity occurs within 2 to 12 h of the onset and maximal levels are attained 12 to 72 h later. However, elevation of α-amylase activity in serum is also associated with other disorders (abdominal disorders, biliary tract diseases, diabetic ketoacidosis, severe glomerular dysfunction, salivary glands disorders…). The organ source can sometimes be identified by determining whether the major isoenzyme present is type P (pancreatic) or S (salivary). Diagnostic specificity and sensitivity of elevation of α-amylase activity in urine remain disputed. Renal clearance of amylase, as related to the reasonably constant clearance of creatinine, has been found useful as a diagnostic concept.

PRINCIPLE (4)

Kinetic CNPG3 method according to following reaction scheme:

$$10\ CNPG_3 \xrightarrow{\alpha\text{-amylase}} 9\ CNP + 1\ CNPG_2 + 9\ G_3 + G$$

CNPG3: 2-chloro-4-nitrophényl malto trioside
CNP : Chloro-nitro-phénol
G3: Maltotriose
G: Glucose

The rate of formation of CNP, directly proportional to the α-amylase activity in the specimen, is measured at 405 nm.

REAGENTS COMPOSITION

R1	**AMYLASE CNPG3**	Buffer
Calcium Acetate	6.0	mmol/L
MES Buffer pH 6.0 at 25°C	100	mmol/L
Preservative		
R2	**AMYLASE CNPG3**	Substrate
CNPG3	2.25	mmol/L
Potassium thiocyanate	900	mmol/L
NaCl	350	mmol/L

Before reconstitution :
Acute Tox. 4 : H302+H312+H332 – Harmful if swallowed, in contact with skin, or if inhaled.
Aquatic Chronic 3 : H412 – - Harmful to aquatic life with long lasting effects
P271 : Use in well-ventilated area, P302+P352 : IF ON SKIN : Wash with plenty of water. P501: Dispose of contents/container in accordance with dangerous waste disposal regulations. Classification due to Potassium Thiocyanate 75 - <100%.. For more details, refer to Safety Data Sheet (SDS)
Once reconstituted, working reagent is not classified as dangerous.

SAFETY CAUTIONS

- Refer to current Material Safety Data Sheet available on request or on www.biolabo.fr
- Verify the integrity of the contents before use.
- Waste disposal: Respect legislation in force in the country.
- All specimens or reagents of biological origin should be handled as potentially infectious. Respect legislation in force in the country.

I Any serious incident that has occurred in connection with the device is notified to the manufacturer and the competent authority of the Member State in which the user and/or patient is based.

REAGENTS PREPARATION

Vial R2: Use a non-sharp instrument to remove cap.
REF 99523: Add promptly 5 mL of buffer R1 into R2.
REF 99123: Add promptly the contents of R2 into buffer R1
Mix gently until complete dissolution.

STABILITY AND STORAGE

Stored away from light, well cap in the original vial at 2-8°C, reagent is stable when stored and used as described in the insert:
Unopened:

- Until expiry date stated on the label.

Once reconstituted:

- Working reagent is stable for15 days at 18-25°C or 90 days at 2-8°C without contamination.
- Discard any reagent if cloudy or if absorbance > 0.600 at 405 nm.
- Don't use working reagent after expiry date.

SPECIMEN COLLECTION AND HANDLING (1) (2)

Unhemolysed serum or heparinised plasma.
α-amylase activity is stable in serum/plasma for:

- at least 7 days at room temperature.
- 1 month at 2-8°C.

Urines: Adjust pH to alkalin range before storage.
α-amylase activity is stable in urines for 7 days at 2-8°C.
In case of delay in transporting urines to the laboratory, use a preservative as merthiolate or thimerozal (0.24mM or 0.1 g/L).

LIMITS (3) (5)

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

MATERIAL REQUIRED BUT NOT PROVIDED

1.Medical analysis laboratory equipment.
2.Spectrophotometer or Biochemistry Clinical Analyzer

QUALITY CONTROL

- REF 95010 EXATROL-N Level I
- REF 95011 EXATROL-P Level II
- External quality control program

It is recommended to control in the following cases:

- At least once a run
- At least once within 24 hours
- When changing vial of reagent
- After maintenance operations on the instrument

If control is out of range, apply following actions:

1. Prepare a fresh control serum and repeat the test
2. If control is still out of range, use a new vial of fresh calibrator
3. If control is still out of range, use a new vial of reagent and reassay

If control is still out of range, please contact BIOLABO technical support or your local Agent.

REFERENCE INTERVALS (1)

Serum (37°C)	α-amylase (IU/L)	α-amylase (µKat/L)
	22-80	[0.38-1.36]
Urines (37°C)	24-408 IU/24 h	[0.41-6.94]/24 h

Each laboratory should establish its own normal ranges for the population that it serves.

PERFORMANCES

On Kenza 240TX, 37°C, 405 nm.

Linearity Range: between 6 and 2000 IU/L

Detection limit: approx. 3 IU/L

Precision:

Within-run N = 20	*Low level*	*Normal level*	*High level*
Mean (IU/L)	36.9	250.4	466.4
S.D. IU/L	0.93	2.65	7.31
C.V. %	2.5	1.1	1.6

Between run N = 20	*Low level*	*Normal level*	*High level*
Mean (IU/L)	68	136	418
S.D. IU/L	1.52	2.93	8.96
C.V. %	2.2	2.2	2.1

Analytical Sensitivity: approx.. 0. 003 ΔAbs/min for 10 IU/L at 405 nm (manual procedure, 1 cm pathlength).

Comparison studies with commercially available reagent:

y = 1.0532 x – 2.8141 r = 0.9938

Interferences:

Turbidity	No interference up to 0.320 abs
Ascorbic acid	No interference up to 2500 mg/dL
Total bilirubin	No interference up to 400 µmol/L
Haemoglobin	Negative interference from 130 µmol/L
Glucose	No interference up to 1010 mg/dL

Other substances may interfere (see § Limits)

On the board stability: 2 months

Calibration Stability: 1 month

Make a new calibration when changing reagent batch, if quality control results are found out of the established range and after maintenance operations

CALIBRATION

- REF 95015 Multicalibrator traceable *to IRMM/IFCC-456*

The calibration frequency depends on proper instrument functions and on the preservation of reagent

MANUAL PROCEDURE

Manual method :

Let stand reagents and specimens at room temperature

Pipette into 1 cm pathlength thermostated cuvette :	
Reagent	1000 µL
Bring to temperature 37°C, then add :	
Specimen	25 µL
Mix. Record initial absorbance after 30 seconds, record absorbance at 405 nm every 30 seconds during 90 seconds.	
Calculate absorbance change per minute (ΔAbs/min).	

1- Performances with manual procedure should be validated by user.
2- Kenza applications and other applications proposal are available on request.

CALCULATION

With Seric Muticalibrator:

$$\text{ALT Activity} = \frac{(\Delta\text{Abs/min) Specimen}}{(\Delta\text{Abs/min) Calibrator}} \times \text{Calibrator Activity}$$

With Theoretical Factor:

Activity (U/L) = ΔAbs/min x Factor

$$\text{Factor} = \frac{VR \times 1000}{12.9 \times VE \times P}$$

With:
VR = Total reactional volume (mL)
VE = Specimen volume (mL)
12.9 = Molar extinction coefficient for CNP a 405 nm
P = Pathlength (cm).

Example, with Manual Procedure,

(Pathlength 1 cm, 37°C, 405 nm):

IU/L = (ΔAbs/min) x 3178

$$\text{µKat/L} = \frac{\text{UI/L}}{60}$$

REFERENCES

(1) TIETZ N.W. Text book of clinical chemistry, 3rd Ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders (1999) p. 689-698, 1284, 1286.
(2) Clinical Guide to Laboratory Test, 4th Ed., N.W. TIETZ (2006) p. 100-107.
(3) YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p. 3-43 to 3-47.
(4) E.S. WINN-DEEN . H.DAVID, G. SIGLER and R. CHAVEZ, Developpement of a direct assay for α-amylase, Clin. Chem. 34, (1988), p. 2005-2008.
(5) A. Ying Foo, Renze Bais, Clin Chim Acta, (1998) 272 : p.137-147

Manufacturer	Expiry date	In vitro diagnostic	Storage temperature	Dematerialized water	Biological risk
Product Reference	See Insert	Batch number	Store away from light	Sufficient for	Dilute with