BIOLABO
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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 LP99553	**R1** 2 x 50 mL

TECHNICAL SUPPORT AND ORDERS
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CE

IN VITRO DIAGNOSTIC USE

CLINICAL SIGNIFICANCE (1) (2)

α-amylase (AMY) 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, is useful as a diagnostic concept.

PRINCIPLE (4)

Several procedures are available for the assay of α-amylase activity in serum (Amyloclastic methods, saccharogenic methods). Both these methods have poor linearity, sensitivity and precision when compared CNPG3 method. Reaction scheme is as follows:

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

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 **AMY** Reagent

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

According to 1272/2008 regulation, this reagent is not classified as dangerous

EUH210: Safety data sheet of working reagent is available on request

SAFETY CAUTIONS

BIOLABO reagents are designated for professional, in vitro diagnostic use (Do not pipette by mouth).

- Verify the integrity of the contents before use.
- Material Safety Data Sheet (MSDS) is available on request or on www.biolabo.fr
- Waste disposal: Respect legislation in force in the country.

All specimens and reagents should be handled as potentially infectious, in accordance with good laboratory practices using appropriate precautions. Respect legislation in force in the country.

REAGENTS PREPARATION

Ready for use

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. Basic medical analysis laboratory equipment.
2. Thermostated Spectrophotometer or Biochemistry Analyzer

STABILITY AND STORAGE

Stored away from light, well caped in the original vial at 2-8°C, reagents are stable when stored and used as described in the insert:

Unopened:

- Until expiry date stated on the label of the kit.

Once opened:

- Transfer requested quantity, well recap vials and store at 2-8°C,
- Reagent (R1) is stable at least for 3 months when free from contamination
- Discard any cloudy reagent or if absorbance is > 0.600 at 405 nm.

SPECIMEN COLLECTION AND HANDLING (1) (2)

Serum
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 alkaline 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).

Manufacturer | Use by | IVD In vitro diagnostic | Temperature limitation | REF Catalogue number | See insert | LOT Batch number | Store away from light | Σ sufficient for | → dilute with | H2O Demineralized water | Biological hazard

QUALITY CONTROL

- REF 95010 BIOLABO EXATROL-N Level I
- REF 95011 BIOLABO 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. Repeat the test with the same control
2. If control is still out of range, prepare a fresh control serum and repeat the test
3. If control is still out of range, verify analysis parameters: wavelength, temperature, specimen/reagent ratio, time counting, calibration factor
4. If control is still out of range, use a new vial of reagent and reassay
5. If control is still out of range, please contact BIOLABO technical support or your local Agent

EXPECTED VALUES (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 at 37°C on KENZA 240TX

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)	80	157	473
S.D. (IU/L)	2.9	3.7	8
C.V. %	3.7	2.4	1.7

Between run N = 20	*Low level*	*Normal level*	*High level*
Mean (IU/L)	76	152	461
S.D. IU/L	3	4	11
C.V. %	3.6	2.9	2.4

Comparison studies with commercially available reagent:
Realised on human specimens (n=100) between 4.4 and 439 IU/L

y = 1.0109 x – 0.9039 r = 0.9977

Analytical Sensitivity: approx. 0.003 abs/min for 10 IU/L

Interferences:

Turbidity	No interference up to 0.256 abs
Total bilirubin	No interference up to 541 µmol/L
Direct bilirubin	No interference up to 477 µmol/L
Ascorbic acid	No interference up to 2500 mg/dL
Glucose	No interference up to 964 mg/dL
Haemoglobin	No interference up to 360 µmol/L

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 BIOLABO Multicalibrator (traceable to standard reference material *IRMM/IFCC-456*)

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

PROCEDURE

Detailed Kenza 240TX procedure is available on request

Wavelength: 405 nm
Temperature: 37°C

	Automated analyzer	Manual procedure
Reagent	300 µL	1000 µL
Standard / Control or specimen	7 µL	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).		

Notes:

1- Performances and stability data's have been validated on KENZA 240TX and KENZA 450TX.

2-With Manual Procedure on Spectrophotometer and on other automated analyzers, performances and stability should be validated by user.

3- Applications proposal are available on request.

CALCULATION

With seric multicalibrator

$$\alpha\text{-amylase Activity} = \frac{(\Delta Abs/min)\ Assay}{(\Delta Abs/min)\ Calibrator} \times \text{Calibrator Concentration}$$

With theoretical factor :

Activity (IU/L) = ΔAbs/min x Factor

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

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

Example, with manual Procedure,
(Pathlength 1 cm, 37°C, 405 nm):

IU/L = (ΔAbs/min) x 3178

$$\mu Kat/L = \frac{UI/L}{60}$$

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*