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 99523	**R1** 1 x 105 mL	**R2** 20 x 5 mL
REF 99123	**R1** 8 x 30 mL	**R2** 8 x 30 mL
REF 99223	**R1** 10 x 100 mL	**R2** 10 x 100 mL

TECHNICAL SUPPORT AND ORDERS
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CE

IVD USAGE IN VITRO

CLINICAL SIGNIFICANCE (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)

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

vial R1 **BUFFER**

Calcium Acetate	6.0	mmol/L
MES Buffer pH 6.0 at 25°C	100	mmol/L
Preservative		

vial R2 **SUBSTRATE**

CNPG3	2.25	mmol/L
Potassium thiocyanate	900	mmol/L
NaCl	350	mmol/L

Before reconstitution:
Xn, R20/21/22-R32 : Harmful by inhalation, in contact with skin and if swallowed
S36/37/39-38 : War suitable protective clothing, gloves and eyes/face protection. In case of insufficient ventilation wear suitable respiratory equipment

Once reconstituted: None

MATERIAL REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. Normal and pathological control sera.

SAFETY CAUTIONS

BIOLABO reagents are designated for professional, in vitro diagnostic use.

- Use adequate protections (overall, gloves, glasses). Do not pipette by mouth.
- In case of contact with skin or eyes, thoroughly wash affected areas with plenty of water and seek medical advice.
- Reagents contain sodium azide (concentration < 0.1%) which may react with copper and lead plumbing. Flush with plenty of water when disposing.
- Material Safety Data Sheet is available upon request.
- Waste disposal: Respect legislation in force in the country.

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

REAGENTS PREPARATION

Vial R2: Use a non-sharp instrument to remove aluminium cap.

REF 99523: Once opened add promptly 5 mL of reagent R1 (Buffer) to the contents of vial R2 (Substrate).

REF 99123 and 99223: Once opened add promptly the contents of vial R2 (Substrate) into vial R1 (Buffer).

To avoid contamination with salivary amylase, do not pipette by mouth. Mix gently and wait for complete dissolution before using reagents (approximately 2 minutes).

STABILITY AND STORAGE

Store at 2-8°C, well capped in the original vial and away from light.

- Unopened, reagents are stable upon expiry date stated on the label of the kit, when stored and used as described in the insert.
- Once opened and without contamination, contents of vial R1 is stable until expiry date stated on the label.
- Once reconstituted, working reagent is stable for:
  - ✓15 days at 18-25°C.
  - ✓90 days at 2-8°C.

Discard any reagent if cloudy or if absorbance > 0.600 at 405 nm.
Don't use working reagent after expiry date stated on the label of the kit.

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).

INTERFERENCES (3) (5)

Tested concentrations of bilirubin, hemoglobin, lipids do not interfere with α-amylase activity determination.
For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

CALIBRATION

Results will depend on the accuracy of the instrument calibration, the time counting, the respect of reagent/specimen ratio and the temperature control.

- Use the theoretical calibration factor (§ **CALCULATION**)
- Or REF 95015 BIOLABO Multicalibrator (calibration value determined with validated statistical technics and metrologically controlled instrument)
- or a multiparametric calibrator traceable to a reference method or material.

QUALITY CONTROL

- BIOLABO EXATROL-N (level I) REF 95010.
- BIOLABO EXATROL-P (level II) REF 95011.
- Other assayed control sera referring to the same method.
- 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.

PERFORMANCE CHARACTERISTICS

Within-run : N = 20	Normal level	High level
Mean IU/L	68.8	169.1
S.D. IU/L	2.3	1.5
C.V. %	3.3	0.9

Run to run : N = 20	Normal level	High level
Mean IU/L	100.7	509
S.D.IU/L	3.5	18.4
C.V. %	3.5	3.6

Detection limit: approximately 6 IU/L (0.1 µKat /L)

Sensitivity for 1 IU/L: 0.315 mAbs/minute at 405 nm.

Comparison with commercially available reagent:

y = 1.0281 x – 1.497 r = 0.9925

LINEARITY

The assay is linear up to 2000 IU/L (33 µKat/L).

If ΔAbs/min > 0.630, dilute specimen with saline solution and reassay taking into account the dilution factor. Linearity depends on the specimen/reagent ratio.

MANUAL PROCEDURE

Let stand reagents and specimens at room temperature.

Pipette into 1 cm pathlength thermostated cuvette :	
Reagent	1 mL
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).	

Notes : Specific procedures are available upon request for automated instruments. Please contact BIOLABO technical support.

CALCULATION

Calculate the result as follows:

With theoretical factor :

IU/L = (ΔAbs/min) x 3178

$$\mu kat/L = \frac{IU/L}{60}$$

With seric multicalibrator

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

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 | Use by | IVD In vitro diagnostic | Temperature limitation | REF Catalogue number | See insert | LOT Batch number | Store away from light | sufficient for | → dilute with