Manual Procedure

Automated procedure on request

MEDICHEM MIDDLE EAST
Clinical Chemistry Reagents
Liquid Stable Reagents

Cat. No. 14100	R1	1 x 10	ml
For 20 tests	R2	1 x 10	ml
Cat. No. 14101	R1	1 x 20	ml
For 40 tests	R2	1 x 20	ml

α-Amylase

Kinetic colorimetric method with Gal-G_2-α-CNP substrate

Liquid Reagents

Test principle

$$\text{Gal-}G_2\text{-}\alpha\text{-CNP} + H_2O \xrightarrow{\alpha\text{-Amylase}} \text{Gal-}G_2 + \text{CNP}$$

α-Amylase hydrolyzes the substrate (Gal-G_2-α-CNP) 2-chloro-4-nitrophenyl-L-O-β-D-galactopyranosyl maltoside to release directly 2-chloro -p-nitrophenol (CNP),which is measured colorimetrically at 405 nm. The rate of increase in absorbance is proportional to α-amylase activity in the sample.

Concentrations in the test

Reagent R1		
MES Buffer, pH = 6.0	50.0	mmol/L
Calcium chloride	10	mmol/L
Sodium chloride	600	mmol/L
preservative		
Reagent R2		
Gal-G_2-α-CNP	3.8	mmol/L
Stabilizer, preservative, detergent		

Stability and preparation of working reagent

Reagent R1: *liquid.*
Reagent R2: *liquid.*

All reagents are stable up to expiry date given on the label when stored at +2 ➔ +8 °C.

Working Reagent:

Mix 1 volume of reagent R1 with 1 volume of reagent R2.
Stability: 2 weeks at 2 - 8 °C.

Note: Don't use if the working reagent absorbance at 405 nm aginst water is more than 0.600.

Specimen collection and handling

1. Non-hemolyzed serum, heparinized plasma.
2. Anticoagulants, such as citrate and EDTA, bind calcium that is needed for amylase activity. Plasma with these anticoagulants should not be used.
3. Amylase in serum is reported stable for 1 week at 20 - 25°C and 1 month at 2 - 8 °C.
4. Urine: Dilute (1+2) with sodium chloride solution (0.9 %) and multiply result by 3.
5. Amylase in urine is reported stable for 2 days at +20 to +25°C, and 10 days at +2 to + 8 °C.
   Stability may decrease in urine samples with pH< 5.

Calibrator

MediCal U Cat .No 15011

Quality control

Meditrol N Cat .No 15171
Meditrol P Cat .No 15181
Applicable to IFCC method

Procedure

Wavelength	Hg 405 nm
Spectrophotometer	405 nm
Cuvette	1 cm light path
Temperature	37°C
Measurement	against air or distilled water
Reaction	kinetic – increase

Assay : Incubate Working Reagent at 37 °C before use:

Sample	20 µl
Working Reagent	1000 µl
Mix, incubate at 37°C for 2 min. Read change in the absorbance per1min. for 3 min. Determine the mean absorbance change per 1 min. (ΔA/min).	

Calculation

α-Amylase activity (U/L) = ΔA/min X 3800

Note: It is recommended that each laboratory (as per instrument performance) could make its own factor (F) by the use of a calibrator according to the following formula:

$$F = \frac{\text{Conc.}_{\text{Clibrator}}}{\Delta/\text{min}_{\text{Calibrator}}}$$

Linearity

Up to 1000 U/L.
If the result exceeds 1000 U/L, repeat the test using diluted serum (1+2) with sodium chloride solution (0.9 %) and multiply the result by 3.

Interferences

1. A number of drugs and substances affect the determination of amylase. Young *et al.,* have published a comprehensive list of such substances.
2. Macroamylase in the specimen can cause a measured hyperamylasemia that could lead to a false diagnosis of acute pancreatitis. However no clinical symptoms are usually associated with macroamylasemia.
3. Bilirubin up to 20 mg/dl, lipid up to 1000 mg/dl, glucose up to 1000 mg/dl, fructose up to 1.0 g/L, sucrose up to 1.0 g/L, ascorbic acid up to 6 g/L and hemoglobin up to 0.4 g/dl have a negligible effect on this procedure.

Precautions

1. Avoid contamination of pipette and reagent with saliva, sweat and skin contact due to the presence of α-amylase.
2. Avoid ingestion.
3. Reagents are acidic solutions, flush with water when contact occurs.
4. Reagents contain sodium azide. Don't swallow. Avoid any contact with skin and mucous membranes. May react with lead and copper plumbing to form explosive metal azides. Upon disposal, flush with large amounts of water to prevent azide build up.

Reference range

	Serum / Heparinized plasma	Urine very fresh
Adults	Up to 120 U/L	Up to 600 U/L

References

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2. Morishita, et al. Clinical Chemistry 46 : 7, 928 - 933.
3. Young, DS., Effects of Drugs on Clinical Laboratory Tests, fifth edition 2000, AACC Press, Washington, D.C.
4. Tietz , N.W. clinical Guide to laboratory tests., Philadelphia , W.B.Saunders company ,p.54 (1983)
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In vitro diagnostics

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