UDI

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AMYLASE (COLOR/KINETIC)

REF 010

FOR IN VITRO DIAGNOSTIC USE

INTENDED USE

For the quantitative determination of amylase in serum, heparinized plasma and urine.

DIAGNOSTIC SIGNIFICANCE

The determination of amylase activity in serum and urine is most commonly performed for the diagnosis of acute pancreatitis. In acute pancreatitis, amylase levels are elevated for longer periods of time in urine than in serum. Therefore, determining the ratio of the amylase and creatinine clearances is important in following the course of the pancreatitis[1].

RANGE OF EXPECTED VALUES[2]

Serum : 16-108 U/L Urine: 0 - 14 U/Hour
Since the expected values are affected by age, sex, diet and geographical location, each laboratory is strongly urged to establish its own normal range.

METHOD PRINCIPLE

Wallenfels et al[3] introduced p-Nitrophenylglycosides as defined substrates for amylase determination in a procedure that eliminated interference from endogenous glucose and pyruvate. The present procedure is based on modification of Wallenfels, using as substrate p-Nitrophenyl-D-maltoheptoside (PNPG7) with the terminal glucose blocked to reduce spontaneous degradation of the substrate by glucosidase and glucoamylase[4]. The test is performed in a kinetic mode with a very short lag time and offers much greater stability than previous amylase methodologies.

Amylase hydrolyzes p-nitrophenyl D-maltoheptoside (PNPG7) to p-nitrophenylmaltotriose (PNPG3) and maltotetrose.

Glucoamylase hydrolyzes PNPG3 to p-Nitrophenylglycoside (PNPG1) and glucose. Then PNPG1 is hydrolysed by glucosidase to glucose and p-nitrophenol which produces a yellow color. The rate of increase in absorbance is measured at 405 nm and is proportional to the amylase activity in the sample.

PNPG7 $\xrightarrow{\text{AMYLASE}}$ PNPG3 + Maltotetrose
PNPG3 $\xrightarrow{\text{GLUCOAMYLASE}}$ PNPG1 + Glucose
PNPG1 $\xrightarrow{\text{GLUCOSIDASE}}$ p-Nitrophenol + Glucose

REAGENTS

1. AMYLASE SUBSTRATE (PNPG7): (Concentrations refer to reconstituted reagent) p-Nitrophenyl D-Maltoheptoside 0.9 mM, Glucosidase 25,000 U/L, Glucoamylase 10,000 U/L, Sodium Chloride 50 mM, Calcium Chloride 5 mM and Buffer 50 mM, pH 6.9 ± 0.01.

RECONSTITUTION

Reconstitute reagent with the volume of distilled water stated on the vial label.

PRECAUTION

DO NOT PIPETTE WATER BY MOUTH to avoid contamination with salivary amylase.

REAGENT STORAGE & STABILITY

1. Store dry reagent at 2-8 °C. Stable up to expiration date indicated on vial label.
2. Reconstituted reagent is stable for at least one day at room temperature (18-25 °C) and at least 14 days when refrigerated (2-8 °C).

REAGENT DETERIORATION

Do not use reagent if:
1. The absorbance of the reagent is greater than 0.70 when measured at 405 nm against water in a cuvette with a 1 cm path length.
2. The reagent fails to meet the stated parameters of performance.

SPECIMEN

SERUM / HEPARINIZED PLASMA / URINE
Anticoagulants, such as Citrate and EDTA, bind calcium, anion, needed for amylase activity. Therefore, plasma with any anticoagulant other than heparin should not be used.

Urine specimen should be adjusted to a pH of 7 and kept refrigerated until assayed.

Amylase in serum and urine is reported as stable for one week at room temperature (18-25 °C) and protected against evaporation and bacterial contamination[5].

MATERIALS PROVIDED

AMYLASE SUBSTRATE (PNPG7)

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

1. Accurate pipetting devices.
2. Test tubes / rack
3. Timing device.
4. Heating block /bath (37 °C).
5. Spectrophotometer capable of reading at 405 nm (400-420 nm). The cuvette compartment should be temperature controlled to maintain temperature (37 °C) during the assay.

PROCEDURE (AUTOMATED)

Refer the appropriate instrument application manuals available from us.

	TEST
Reconstituted amylase Reagent	1.0 ml
Pre-warm at 37oC for 5 minutes and add:	
Sample	0.025 ml
Mix and incubate at 37oC for 90 seconds and read the absorbance at 405 nm against distilled water. Continue readings every 30 seconds for 2 minutes and determine ΔA/Min.	

PROCEDURE (MANUAL)

Pipette into clean dry test tubes:

CALCULATIONS

ΔA/Min x 4824 = Amylase Activity in TEST (U/L)

EXAMPLE: If ΔA/min = 0.03 then 0.03 x 4824 = 145 U/L.

SI Units

To convert into SI Units (nKat/L) multiply the U/L value by 16.67

INTERFERENCES

A number of drugs and substances affect the determination of amylase[2, 6]. Young et al have published a comprehensive list of such substances[7].

LIMITATIONS

Samples that exceeded the linearity limit (2,000 U/L) should be diluted with an equal volume of saline and re-run. Multiply the results by two.

PERFORMANCE

1) LINEARITY : 2,000 U/L.

2. COMPARISON : UDI reagent tested on Manual Systems(y) was compared with CAPS survey results(x). The systematic difference between the results were within CLIA specified limits. N = 25

Correlation Coefficient	0.991
Regression Equation	y = 1.1x + 6.7

3) PRECISION:

	Mean (U/L)	S.D.	C.V.%
Within run	116.6	9.52	8.17
Run to run	80.4	4.55	5.66

QUALITY CONTROL

For accuracy and precision check, we recommend the use of normal and abnormal UDI controls based on human serum.

ORDERING INFORMATION:

UDITROL 'N'(Normal Serum Control)
REF#070N-010 2x5 ml

UDITROL 'A' (Abnormal Serum Control)
REF # 070A-010 2x5 ml

BIBLIOGRAPHY

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3. Wallenfels, K., et al, Carbohydrate Research 61:359 (1978).
4. Blair, H.E., U.S. Patent Pending.
5. Demetriou, J., et al, Clinical Chemistry: Principles and Techniques, 2nd Ed., (Henry, R.J, et al, eds.) Hagerstown (MD), Harper & Row (1974).
6. Bogoch, A., et al, Gastroenterology 26:697 (1954).
7. Young, D.S. et al, Clin. Chem. 21:1D (1975).

PRODUCT AVAILABILITY

AMYLASE (Color/Kinetic)

REF # 010-060 12 x 5 ml

EC REP
(Authorized representative in the European Community)

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CE

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