PANCREATIC α-AMYLASE (P AMY)

ETHYLIDENE BLOCKED-pNPG$_7$
ENZYME COLORIMETRIC TEST
RX SERIES

INTENDED USE

A Pancreatic α-Amylase test system is a device intended for the quantitative *in vitro* determination of Pancreatic α -Amylase activity in serum, plasma and urine. This product is suitable for use on RX **series** instruments, which includes the RX **daytona** and the RX **imola**.

Cat. No.

AY 3855	R1. Enzyme/Antibodies	4 x 16 ml
	R2. Substrate	4 x 5 ml

GTIN: 05055273200614

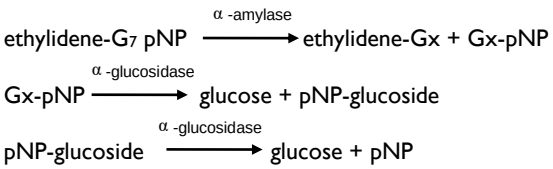
CLINICAL SIGNIFICANCE[1,2]

The amylases are a group of hydrolases that split complex carbohydrates constituted of α -D-glucose units. The two recognised types of amylases are ß-amylase (e.g. plant and bacterial exoamylase) and human α -amylases which can attack the α -1, 4-linkage anywhere along a polyglucan chain.

Human α-amylase consists of two major isoenzymes, pancreatic and salivary, which are encoded by two different genes. Pancreatic amylase is synthesised only in pancreatic tissue by acinar cells. Amylase measurements are used primarily for the diagnosis and treatment of pancreatitis (inflammation of the pancreas). The evaluation of the pancreatic isoamylase may have a greater clinical specificity for the diagnosis of pancreatic disorders than total amylase assessment.

PRINCIPLE[1,3,4]

Two monoclonal antibodies are incubated with the sample to inhibit the salivary amylase present but do not affect pancreatic amylase. This method uses ethylidene-p-nitrophenyl maltoheptaoside as the substrate. The substrate is then added, and any amylase present splits the substrate to produce oligosaccharides and pNP-G2, pNP-G3 and pNP-G4.
α -glucosidase is added as the indicator enzyme to release the p-nitrophenol (p-NP). The final result of the hydrolysis by amylase and α-glucosidase is free p-NP, which is detected by its absorbance at 405 nm. The terminal glucose is blocked preventing cleavage by the indicator enzyme.



SAMPLE COLLECTION AND PREPARATION[2,5]

Serum: Use serum free from haemolysis.
Heparinised Plasma: Anticoagulants other than heparin may diminish amylase activity.
Urine: 2nd morning urine.
Amylase is stable for 1 week at +15 to +25°C and 2 months at +3 to +8°C.

REAGENT COMPOSITION

Contents	Concentration in the Test
R1. Enzyme/Antibodies	
Hepes buffer	52.5 mmol/l, pH 7.15
Magnesium Chloride	12.6 mmol/l
Sodium Chloride	87 mmol/l
α -glucosidase	≥4 U/ml
Monoclonal Antibodies	42 µg/ml
R2. Substrate	
Ethylidene-G$_7$ pNP	22 mmol/l

SAFETY PRECAUTIONS AND WARNINGS

For *in vitro* diagnostic use only. Never pipette by mouth. Exercise normal precautions required for handling all laboratory reagents. The reaction releases p-nitrophenol as the end product which is harmful. Avoid contact with skin or mucous membranes. Flush affected areas immediately with polyethylene glycol 400 or large quantities of water.

Reagent R1 contains 0.1% sodium azide. In the event of contact with skin and mucous membranes, flush affected areas with large quantities of water and obtain medical attention. Sodium azide may react with lead or copper plumbing, to form potentially explosive azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide. Avoid contamination of reagent, samples and glassware by saliva or sweat because they have a high amylase content.

Health and Safety data sheets are available on request.

The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.

STABILITY AND PREPARATION OF REAGENTS

R1. Enzyme/Antibody Reagent
Contents ready for use. Stable up to the expiry date when stored at +2 to +8°C.

R2. Substrate
Contents ready for use. Stable up to the expiry date when stored at +2 to +8°C.

MATERIALS PROVIDED

Enzyme/Antibodies
Substrate

MATERIALS REQUIRED BUT NOT PROVIDED

Randox Assayed Multisera Level 2 (Cat. No. HN 1530) and Level 3 (Cat. No. HE 1532)
Randox Calibration Serum Level 3 (Cat. No. CAL 2351)
RX **series** Saline (Cat. No. SA 3854)

PROCEDURE NOTES

To avoid the potential for reagent carryover, it is recommended that the testing order of the reagents is confirmed. Please consult the reagent carryover document available on www.randox.com under support and documentation - Reagent product inserts or by contacting Randox Laboratories Technical Services, Northern Ireland +44 (0) 28 9445 1070.

The Chemistry parameters for Randox Dedicated RX **series** Assays are predefined on the hard drive of the analyser PC. The required programs should be downloaded to the analyser software. Please note that the predefined chemistry parameters use SI units. If alternative units are required, these can be edited by the user. In this case, the technical range should be edited in accordance with the users selected units. All necessary instructions are encoded on the bar code. If the barcode cannot be read by the analyser, enter manually the series of numbers given beneath the barcode. If problems continue, contact Randox Laboratories Technical Services, Northern Ireland +44 (0) 28 94451070.

CALIBRATION

The use of Saline and Randox Calibration Serum Level 3 is recommended for calibration. A 2-point calibration is recommended with change of reagent lot or as indicated by quality control procedures.

STANDARDISATION

Randox Calibration Serum Level 3 is traceable to Amylase reference materials IFCC456 and BCR476.

QUALITY CONTROL

Randox Assayed Multisera, Level 2 and Level 3 are recommended for daily quality control. Two levels of controls should be assayed at least once a day. Values obtained should fall within a specified range. If these values fall outside the range and repetition excludes error, the following steps should be taken:

1. Check instrument settings and light source.
2. Check cleanliness of all equipment in use.
3. Check water. Contaminants i.e. bacterial growth may contribute to inaccurate results.
4. Check reaction temperature.
5. Check expiry date of kit and contents.
6. Contact Randox Laboratories Technical Services, Northern Ireland +44 (0) 28 94451070.

Quality control requirements should be determined in conformance with government regulations or accreditation requirements.

INTERFERENCE

There is approximately 3% residual activity from salivary α-amylase. In rare cases, elevated pancreatic α-amylase values could therefore be due to extremely high salivary α-amylase.

The analytes below were tested up to the following levels and were found not to interfere:

Haemoglobin	1000 mg/dl
Free Bilirubin	25 mg/dl
Conjugate Bilirubin	25 mg/dl
Triglycerides	1000 mg/dl
Intralipid®	800 mg/dl

Physiological changes in serum or plasma analyte concentrations can be caused by a number of substances. Comprehensive discussion of possible interfering substances, their serum or plasma concentrations, and their possible physiological involvements is beyond the scope of this document. The listed reference contains specific details on known potential interfering substances [6]. The user must remain vigilant to the possible effect on results of unknown interferences from medications or endogenous substances. All patient results must be evaluated in light of the total clinical status of the patient.

PANCREATIC α-AMYLASE/CREATININE QUOTIENT [7]

The pancreatic α-amylase-creatinine quotient is usually determined to allow for fluctuations occurring. In urine, this is calculated by determining the concentration of creatinine in a random sampled urine and the quotient is calculated as follows:-

$$\text{quotient [U/g]} = \frac{\text{pancreatic α-amylase [U/l]}}{\text{creatinine (g/l)}}$$

NORMAL VALUES [4]

	37°C
Serum/Plasma	13 - 53 U/l
Spontaneously Voided Urine	≤350 U/l
I-Amylase/Creatine Quotient	≤205 U/g

Note: EDTA Plasma values are approximately 7.5% lower than serum values.

It is recommended that each laboratory establish its own reference range to reflect the age, sex, diet and geographical location of the population.

SPECIFIC PERFORMANCE CHARACTERISTICS

The following performance characteristics were obtained using the RX **daytona** analyser at 37°C.

LINEARITY

This method is linear up to 1157 U/l. In the event of a rerun, the linearity is extended to 4049 U/l.

SENSITIVITY

The minimum detectable activity of Pancreatic Amylase with an acceptable level of precision was determined as 9.00 U/l.

PRECISION

Within run precision

	Level 1	Level 2	Level 3
Mean (U/l)	20.4	88.2	351
SD	0.875	1.76	9.51
CV (%)	4.30	1.99	2.71
n	20	20	20

Between run precision

	Level 1	Level 2	Level 3
Mean (U/l)	22.3	106	351
SD	1.13	5.25	8.71
CV (%)	5.06	4.97	2.48
n	20	20	20

CORRELATION

This method (Y) was compared with another commercially available method (X) and the following linear regression equation obtained:

Y = 0.95 X + 0.26
and a correlation coefficient of 1.00

40 patient samples were analysed spanning the range 10 to 90 U/l.

REFERENCES

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3. Suens, E., Tanner, P. *et al*, Clin. Chem. 1989; **35:** 662-664.
4. Junge, W., Waldenstrom, J., Bouman, A., Haux, P., Merlot, J., Topfer, G., Kurle-Weittenhiller, A and Klein, G. Evaluation of the Assays for Total and Pancreatic alpha-Amylase Based on 100% Cleavage of Et-G7-PNP at 6 European Clinical Centres. Poster presented at 12th IFCC European Congress of Congress of Clinical Chemistry, Basle, Switzerland, 1997.
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7. Cummings, S.T., Fraser, C.G., Ann. Clin. Biochem. 1989; **26:** 335-40

The presence of a vertical bar in the margin indicates a technical update from the previous revision.

EC REP

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