AMYLASE (AMY)

Ethylidene Blocked-pNPG$_7$
RX DAYTONA PLUS

INTENDED USE

An α-amylase test system is a device intended for the quantitative *in vitro* determination of α-amylase activity in serum and urine. This product is suitable for use on the RX **daytona plus**, RX **series** analyser.

FOR PRESCRIPTION USE ONLY

Cat. No.		
AY 8335	R1. Enzyme Reagent	4 x 20 ml
	R2. Substrate	4 x 7 ml

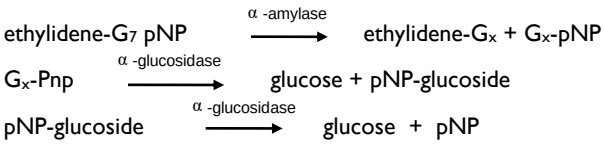
GTIN: 05055273208269

CLINICAL SIGNIFICANCE

Amylase measurements are used primarily for the diagnosis and treatment of pancreatitis (inflammation of the pancreas).

COLORIMETRIC METHOD[1,2]

The method uses ethylidene blocked p-nitrophenyl-maltoheptaoside as substrate. The indicator enzyme α-glucosidase, used to release the p-nitrophenol, is also employed in the method. The terminal glucose of the substrate is chemically blocked preventing cleavage by the indicator enzymes.



G = glucose
pNP = paranitrophenol
x = 2 to 5

SPECIMEN COLLECTION AND PREPARATION

Serum: Use serum free from haemolysis.
Urine: 2nd morning urine.

Amylase is stable for 1 week at +15 to +25°C and 2 months at +2 to +8°C.

REAGENT COMPOSITION

Contents		Initial Concentration of Reagents
R1.	**Enzyme Reagent**	
	Hepes buffer	52.5 mmol/l, pH 7.15
	Sodium chloride	87 mmol/l
	Magnesium chloride	12.6 mmol/l
	α-Glucosidase	≥ 4 U/ml
	Sodium Azide	0.09% w/v
R2.	**Substrate**	
	4,6-Ethylidene-G$_7$ pNP	22 mmol/l
	Sodium Azide	0.09% w/v

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.

Solutions R1and R2 contain Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.
Sodium Azide reacts with lead and copper plumbing, to form potentially explosive azides. When disposing of such reagents, flush with large volumes of water to prevent azide build up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide.

Safety data sheets are available on request.
Please dispose of all biological and chemical materials according to local guidelines.

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

STABILITY AND PREPARATION OF REAGENT

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

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

MATERIALS PROVIDED

Amylase Enzyme Reagent
Amylase 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 8396)

PROCEDURE NOTE

Avoid contamination of reagent, samples and equipment by saliva or sweat because they have a high amylase content.

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 **daytona plus** 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 9445 1070.

RX DAYTONA PLUS 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 9445 1070.

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

NORMAL VALUES [3,4]

	+37°C
Serum	28 - 100 U/l
Spontaneously Voided Urine	≤ 460 U/l
α-Amylase/Creatinine Quotient	≤ 310 U/g

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

SERUM
SPECIFIC PERFORMANCE CHARACTERISTICS
The following performance characteristics were obtained using the RX **daytona plus**.

INTERFERENCE
The following analytes were tested up to the levels indicated at Amylase concentrations of 40 U/L and 150 U/L and found not to interfere:

	40 U/l	150 U/l
Haemoglobin	250 mg/dl	1000 mg/dl
Total Bilirubin	60 mg/dl	60 mg/dl
Conjugate Bilirubin	60 mg/dl	60 mg/dl
Triglycerides	2000 mg/dl	2000 mg/dl
Intralipid®	2000 mg/dl	2000 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 [5]. 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.

REPORTABLE RANGE
Linearity data demonstrates that the reportable range for Amylase on the RX **daytona plus** is 2.5 to 1649 U/l.

SENSITIVITY
The limit of Quantitation (LoQ), the limit of Detection (LoD) and the limit of Blank (LoB) were determined consistent with CLSI guidelines EP17-A. LoQ is the lowest concentration that can be detected with ≤20% bias and ≤20% imprecision. LoD is the lowest concentration that can be detected to determine the presence or absence of Amylase. LoB is the highest concentration that is likely to be observed in a blank sample.

RX daytona plus

Limit of Blank (U/l)	0.000
Limit of Detection (U/l)	0.249
Limit of Quantitation (U/l)	2.50

PRECISION
Within Run Precision

	Level 1	Level 2	Level 3
Mean (U/l)	27.7	103	335
SD	0.91	1.91	4.04
CV (%)	3.28	1.85	1.21
n	80	80	80

Total Precision

	Level 1	Level 2	Level 3
Mean (U/l)	27.7	103	335
SD	1.12	3.00	8.36
CV (%)	4.04	2.90	2.49
n	80	80	80

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

Y = 1.05x – 2.97
and a correlation coefficient of r = 1.00

49 patient samples were analysed spanning the range 3 to 1249 U/L.

URINE
SPECIFIC PERFORMANCE CHARACTERISTICS
The following performance characteristics were obtained using the RX **daytona plus**.

LINEARITY
The method is linear up to 1139 mmol/L.

SENSITIVITY
The minimum detectable concentration of α-amylase with an acceptable level of precision was determined as 10.5 U/l.

PRECISION

Within Run Precision

	Level 1	Level 2	Level 3
Mean (U/l)	87.4	372	980
SD	1.71	6.78	19.2
CV (%)	1.95	1.82	1.96
n	20	20	20

CORRELATION

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

Y = 1.04x - 4.46
and a correlation coefficient of r = 1.00

60 patient samples were analysed spanning the range 18.5 to 939 U/L.

REFERENCES

1. Rauscher, E., *et coll*., Fresenius Z. Analyt. Chem. 324 (1986) 304-305.
2. Kruse-Jarres, J.D., *et coll*., J. Clin. Biochem. 27 (1989) 103.
3. 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.
4. Junge, W., *et coll*., Clin Biochem. 22 (1989) 109.
5. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Washington, DC: AACC Press; 2000.

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

EC REP

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Revised 08 Jan 24 ld

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