PANCREATIC α-AMYLASE (P AMY)

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

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. This product is suitable for use on RX **modena**.

Cat. No.

AY 8149	R1. Enzyme/Antibodies	7 x 11 ml
	R2. Substrate	7 x 4.8 ml

GTIN: 05055273215830

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 β-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 [1,4,3]

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.

$$\text{ethylidene-G}_7\text{ pNP} \xrightarrow{\alpha\text{-amylase}} \text{ethylidene-Gx + Gx-pNP}$$

$$\text{Gx-pNP} \xrightarrow{\alpha\text{-glucosidase}} \text{glucose + pNP-glucoside}$$

$$\text{pNP-glucoside} \xrightarrow{\alpha\text{-glucosidase}} \text{glucose + pNP}$$

SAMPLE COLLECTION AND PREPARATION [2,5]

Serum: Use serum free from haemolysis.

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
Sodium Azide	< 0.1% w/v
R2. Substrate	
Ethylidene-G$_7$ pNP	22 mmol/l
Sodium Azide	< 0.1% 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.

Reagents R1 and 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 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.

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. The reagent is stable on-board the RX **modena** analyser for 28 days.

R2. Substrate
Contents ready for use. Stable up to the expiry date when stored at +2 to +8°C. The reagent is stable on-board the RX **modena** analyser for 28 days.

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)
Randox Saline Diluent (Cat. No. SA 8396)

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 **modena** 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.

CALIBRATION

The use of Saline and Randox Calibration Serum Level 3 (Cat. No. CAL 2351) is recommended for calibration. The Randox Liquid Ethylidene pNPG7 37°C value should be used from the RX **series** section of the value sheet. A 2-point calibration is recommended every 28 days or with change of reagent lot.

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 (Cat. No. HN 1530 and HE 1532) are recommended for daily quality control. The Randox Liquid Ethylidene pNPG7 37°C value should be used from the RX **series** section of the value sheets. 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.

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 following analytes were tested up to the levels indicated at Pancreatic Amylase concentrations of 100 U/l and 530 U/l and found not to interfere:

	100 U/l	**530 U/l**
Haemoglobin	1000 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
Ascorbic Acid	6 mg/dl	6 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.

NORMAL VALUES [7]

	+37°C
Serum	13 - 53 U/l

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 **modena** analyser at +37°C.

REPORTABLE RANGE

Linearity data demonstrates that the reportable range for Pancreatic Amylase on the RX **modena** is 5 to 1649 U/l.

The RX **modena** analyser has an auto dilution feature that is automatically activated when measuring samples >1649 U/l, which are diluted and re-measured to obtain values within the measuring range.

SENSITIVITY

The limit of Quantitation (LoQ), the limit of Detection (LoD) and the limit of Blank (LoB) were determined consistent with CLSI guidelines EP17-A2. LoQ is the lowest concentration that can be detected with ≤20% imprecision. LoD is the lowest concentration that can be detected to determine the presence or absence of Pancreatic Amylase. LoB is the highest concentration that is likely to be observed in a blank sample.

	U/L
Limit of Blank	1
Limit of Detection	2
Limit of Quantitation	5

PRECISION

Precision estimates were completed according to CLSI documents EP5-A2. Each sample was assayed in duplicate twice per day for 20 days.

Within Run Precision

		Level 1	**Level 2**	**Level 3**	**Level 4**
Mean	(U/L)	54.4	96.3	294	527
SD		0.72	0.96	2.55	4.91
CV	(%)	1.3	1.0	0.9	0.9
n		80	80	80	80

Total Precision

		Level 1	**Level 2**	**Level 3**	**Level 4**
Mean	(U/L)	54.4	96.3	294	527
SD		1.22	3.25	7.45	13.3
CV	(%)	2.2	3.4	2.5	2.5
n		80	80	80	80

CORRELATION

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

Y = 1.02 X + 2.9
and a correlation coefficient of r = 0.999
Syx= 6.0
106 patient samples were analysed spanning the range 6.69 to 1414 U/L.

MATRIX COMPARISON

Lithium Heparin

Patient samples were drawn in matched pairs – one sample serum (x) and the second sample lithium heparin plasma (y). A minimum of 42 matched patient sample pairs were analysed in singlicate spanning the range 16 to 55 U/L and the following linear regression equation was obtained:

Y= 0.99 X – 0.01

Correlation coefficient of r = 0.985
Syx = 1.271

REFERENCES

1. Tietz, N.W., Burlina, A., Clin. Chem. 1988; **34** No. 10.
2. Tietz N.W. Fundamentals of Clinical Chemistry ed. 3 Philadelphia, W.B. Saunders C. 1987, pg 393.
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.
5. Jacobs, D.S., Kasten, B.Z. *et al*, Laboratory Test Handbook ed 2 Ohio, Lexi-Comp Inc., 1990 Pg 93.
6. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Washington, DC: AACC Press; 2000.
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

Randox Teoranta, Meenmore,
Dungloe, Donegal,
F94 TV06, Ireland

Revised 27 Jun 23 bm

THIS PAGE IS INTENTIONALLY BLANK

Randox Laboratories Ltd, 55 Diamond Road, Crumlin, County Antrim, BT29 4QY, United Kingdom
T +44 (0) 28 9442 2413 **F** +44 (0) 28 9445 2912 **E** applications@randox.com **I** www.randox.com