PANCREATIC α-AMYLASE (P AMY)

ETHYLIDENE BLOCKED-pNPG$_7$
ENZYME COLORIMETRIC TEST

HITACHI 704	**6 x 57 tests**
HITACHI 717	**6 x 80 tests**
HITACHI 902	**6 x 80 tests**
HITACHI 911/912	**6 x 80 tests**

INTENDED USE

For the quantitative *in vitro* determination of pancreatic α-amylase in serum, plasma and urine. This product is suitable for use on the Hitachi 704, 717, 902, 911 and 912.

Cat. No.

AY 7934	R1. Enzyme/Antibodies	6 x 20 ml
	R2. Substrate	3 x 10 ml

GTIN: 05055273200669

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, however the salivary amylase is found in numerous locations, such as salivary glands and female genital, pulmonary and malignant tissues.

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.

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

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

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

SPECIMEN 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 1 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)

PROCEDURE NOTE

Avoid contamination of reagent, samples and glassware by saliva or sweat because they have a high amylase content. Do not mouth pipette.

INSTRUMENT SETTINGS FOR HITACHI 704

Temperature:	25/30/37°C
TEST CODE	*(PAMY)
ASSAY CODE	5-25-32
SAMPLE VOLUME (µl)	14
R 1 VOL (µl)	350-20-0
R 2 VOL (µl)	70-20-0
WAVELENGTH (nm)	700-415
CALIB METHOD	2-0-0
STD 1 CONC POS	0-1
STD 2 CONC POS	0-0
STD 3 CONC POS	0-0
STD 4 CONC POS	0-0
STD 5 CONC POS	0-0
STD 6 CONC POS	0-0
UNIT	U/l
SD LIMIT	0.1
DUPLICATE LIMIT	100
SENSITIVITY LIMIT	0
ABS LIMIT	20000-Increase
PROZONE LIMIT	0-Lower
EXPECTED VALUE	*-*
INSTRUMENT FACTOR	1.00

* Data entered by operator

INSTRUMENT SETTINGS FOR HITACHI 717

Temperature:	25/30/37°C
PROGRAM 2 CHEMISTRY PARAMETERS	
TEST	*(PAMY)
ASSAY CODE (RATE-A)	5-40-50
SAMPLE VOLUME (µl)	10-2
R 1 VOLUME (µl)	250-20-NO
R 2 VOLUME (µl)	50-20-NO
WAVELENGTH (nm)	700-405
CALIB METHOD	2-0-0
STD 1 CONC-POS	*-*
STD 2 CONC-POS	0-0
STD 3 CONC-POS	0-0
STD 4 CONC-POS	0-0
STD 5 CONC-POS	0-0
STD 6 CONC-POS	0-0
SD LIMIT	0.1
DUPLICATE LIMIT	100
SENSITIVITY LIMIT	0
ABS. LIMIT (INC/DEC)	20000-Increase
PROZONE LIMIT	0-Lower
EXPECTED VALUE (U/l)	*-*
PANIC VALUE	*-*
INSTRUMENT FACTOR	1.00

* Data entered by operator

INSTRUMENT SETTINGS FOR HITACHI 902

1. Test Name	P AMY
2. Assay Code (Mthd)	RATE A
3. Assay Code (2.Test)	0
4. Reaction Time	10
5. Assay Point 1	25
6. Assay Point 2	31
7. Assay Point 3	0
8. Assay Point 4	0
9. Wave Leng. (SUB)	700
10. Wave Leng. (MAIN)	415
11. Sample Volume	10
12. R1 Volume	250
13. R1 Pos	*
14. R1 Bottle Size	S
15. R2 Volume	0
16. R2 Pos	0
17. R2 Bottle Size	S
18. R3 Volume	50
19. R3 Pos	*
20. R3 Bottle Size	S
21. Calib. Type (Type)	LINEAR
22. Calib.Type (Wght)	0
23. Calib. Conc. 1	0
24. Calib. Pos. 1	*
25. Calib. Conc. 2	*
26. Calib. Pos. 2	*
27. Calib. Conc. 3	0
28. Calib. Pos. 3	0
29. Calib. Conc. 4	0
30. Calib. Pos. 4	0
31. Calib. Conc. 5	0
32. Calib. Pos. 5	0
33. Calib. Conc. 6	0
34. Calib. Pos. 6	0
35. S1 ABS.	0
36. K Factor	10000
37. K2 Factor	10000
38. K3 Factor	10000
39. K4 Factor	10000
40. K5 Factor	10000
41. A Factor	0
42. B Factor	0
43. C Factor	0
44. SD Limit	0.1
45. Duplicate Limit	100
46. Sens. Limit	0
47. S1 ABS. Limit (L)	-32000
48. S1 SBS. Limit (H)	32000
49. ABS. Limit	20000
50. ABS. Limit (D/I)	INCREASE
51. Prz. Limit	0
52. Prz. Limit (U/D)	LOWER
53. Prz. (End Point)	35
54. Expect. Value (L)	*
55. Expect. Value (H)	*
56. Instr. Fact. (a)	1.0
57. Instr. Fact. (b)	0.0
58. Key Setting	*

* Data entered by operator

ASSAYS ON HITACHI 911/912

Prepare the reagent solutions as directed in the assay instructions. All necessary instructions are encoded on the bar code. If the bar code cannot be read by the analyser, enter manually the series of numbers given beneath the bar code. If problems continue, contact Randox Laboratories Technical Services, Northern Ireland +44 (0) 28 9445 1070.

CALIBRATION

When setting up this method, it is essential that a factor is established with a calibrator. We recommend Randox Calibration Serum Level 3.

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.

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.

SPECIFICITY

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

PANCREATIC α-AMYLASE/CREATININE QUOTIENT

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[7]

	37°C
Serum/Plasma	13 - 53 U/l
Spontaneously Voided Urine	≤350 U/l
α-Amylase/Creatinine 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 a Hitachi 717 analyser.

LINEARITY

This assay is linear up to 1500 U/l. Samples with activity greater than this should be diluted 1 + 4 with 0.9% NaCl solution and reassayed. Multiply the result by 5. In the event of a rerun (Hitachi 717, 911 and 912), the linearity is extended to 7,500 U/l.

SENSITIVITY

The minimum detectable concentration of Pancreatic α-amylase with an acceptable level of precision was determined as 6.11 U/l.

PRECISION

Intra Assay

	Level 1	Level 2	Level 3
Mean (U/l)	47.26	70.03	252
SD	0.43	0.72	1.23
CV(%)	0.90	1.03	0.49
n	20	20	20

Inter Assay

	Level 1	Level 2	Level 3
Mean (U/l)	48.11	71.59	256
SD	0.81	1.09	3.00
CV(%)	1.67	1.53	1.18
n	20	20	20

SPECIFIC PERFORMANCE CHARACTERISTICS

A correlation study was carried out at 37°C.

A comparison study was carried out using **41** normal and abnormal patient samples ranging from 5 to 289 U/l. The above method (Y) was compared with another commercially available method (X).

Linear regression analysis of this data resulted in the equation in the form of Y = 0.9949 X -2.64 and a correlation coefficient of 0.9984.

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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6. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Washington, DC: AACC Press; 2000. 335.
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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