AMY-P

cobas®

α-Amylase EPS Pancreatic

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
20766623 322	α-Amylase EPS Pancreatic 200 tests	System-ID 07 6662 3	Roche/Hitachi **cobas c** 311, **cobas c** 501/502
10759350 190	Calibrator f.a.s. (12 x 3 mL)	Code 401	
10759350 360	Calibrator f.a.s. (12 x 3 mL, for USA)	Code 401	
12149435 122	Precinorm U plus (10 x 3 mL)	Code 300	
12149435 160	Precinorm U plus (10 x 3 mL, for USA)	Code 300	
12149443 122	Precipath U plus (10 x 3 mL)	Code 301	
12143443 160	Precipath U plus (10 x 3 mL, for USA)	Code 301	
10171743 122	Precinorm U (20 x 5 mL)	Code 300	
10171735 122	Precinorm U (4 x 5 mL)	Code 300	
10171778 122	Precipath U (20 x 5 mL)	Code 301	
10171760 122	Precipath U (4 x 5 mL)	Code 301	
05117003 190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 391	
05947626 190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 391	
05947626 160	PreciControl ClinChem Multi 1 (4 x 5 mL, for USA)	Code 391	
05117216 190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 392	
05947774 190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 392	
05947774 160	PreciControl ClinChem Multi 2 (4 x 5 mL, for USA)	Code 392	
04489357 190	Diluent NaCl 9 % (50 mL)	System-ID 07 6869 3	

English

System information

For **cobas c** 311/501 analyzers:

AMY-P: ACN 571

For **cobas c** 502 analyzer:

AMY-P: ACN 8571

Intended use

In vitro test for the quantitative determination of pancreatic α-amylase in human serum, plasma and urine on Roche/Hitachi **cobas c** systems.

Summary[1,2,3,4,5,6,7,8,9,10]

The α-amylases (1,4-α-D-glucanohydrolases, EC 3.2.1.1) catalyze the hydrolytic degradation of polymeric carbohydrates such as amylose, amylopectin and glycogen by cleaving 1,4-α-glucosidic bonds. In polysaccharides and oligosaccharides, several glycosidic bonds are hydrolyzed simultaneously. Maltotriose, the smallest such unit, is converted into maltose and glucose, albeit very slowly.

Two types of α-amylases can be distinguished, the pancreatic type (P-type) and the salivary type (S-type). Whereas the P-type can be attributed almost exclusively to the pancreas and is therefore organ-specific, the S-type can originate from a number of sites. As well as appearing in the salivary glands it can also be found in tears, sweat, human milk, amniotic fluid, the lungs, testes and the epithelium of the fallopian tube. Because of the sparsity of specific clinical symptoms of pancreatic diseases, enzymatic determinations are of considerable importance in pancreas diagnostics. The determination of pancreas-specific α-amylase instead of total α-amylase is of advantage here.

The determination of pancreatic α-amylase is suitable for the diagnosis and monitoring of acute pancreatitis and acute attacks during chronic pancreatitis. In terms of clinical sensitivity and specificity, the diagnostic value of pancreatic α-amylase is comparable to that of lipase, the generally recognized pancreas-specific enzyme. The sensitivity of pancreatic α-amylase is 38 % higher than that of total α-amylase in the diagnosis of acute pancreatitis when - as commonly used - three times the upper normal limit is taken as the criterion.

A variety of methods have been described for determining pancreatic α-amylase: radio- and enzyme-immunoassays as well as the partial inhibition of salivary α-amylase by an inhibitor derived from wheatgerm and calculation of the pancreatic α-amylase from the remaining and total amylase activities.

The kinetic method described here is based on inhibition of the activity of human salivary α-amylase by two different monoclonal antibodies and the well-proven cleavage of 4,6-ethylidene-(G_7)-1,4-nitrophenyl-(G_1)-α-D-maltoheptaoside (Ethylidene Protected Substrate = EPS) by pancreatic α-amylase followed by hydrolysis of all the degradation products to p-nitrophenol with the aid of α-glucosidase (100 % chromophore liberation). The results of this method correlate with those obtained by HPLC. This assay follows the recommendation of the IFCC, but was optimized for performance and stability.

Test principle (simplified)[10,11]

Colorimetric assay

After immunoinhibition with antibodies against human salivary α-amylase the pancreatic α-amylase is selectively determined with an enzymatic colorimetric method using the substrate 4,6-ethylidene-p-nitrophenyl-α-D-maltoheptaoside (ethylidene-G_7PNP).[4]

Simplified reaction scheme:

$$5\ \text{ethylidene-G}_7\text{PNP}^{a)} + 5\ H_2O \xrightarrow{\text{pancreatic α-amylase}} 2\ \text{ethylidene-G}_5 + 2\ G_2PNP + 2\ \text{ethylidene-G}_4 + 2\ G_3PNP + \text{ethylidene-G}_3 + G_4PNP$$

$$2\ G_2PNP + 2\ G_3PNP + G_4PNP + 14\ H_2O \xrightarrow{\text{α-glucosidase}} 5\ PNP + 14\ G^{b)}$$

a) PNP ≙ p-nitrophenol

b) G ≙ Glucose

The rate of p-nitrophenol formation is directly proportional to the catalytic pancreatic α-amylase activity. It is determined by measuring the increase in absorbance photometrically.

Reagents - working solutions

R1 HEPES buffer: 52.4 mmol/L, pH 7.1 (37 °C); sodium chloride: 87 mmol/L; magnesium chloride: 12.6 mmol/L; calcium chloride: 0.075 mmol/L; α-glucosidase (microbial): ≥ 67 µkat/L; monoclonal antibodies (mouse): 97 mg/L; preservatives

R3 HEPES buffer: 52.4 mmol/L, pH 7.1 (37 °C); 4,6-ethylidene-G_7 PNP: 22 mmol/L; preservatives; stabilizers

R1 is in position B and R3 is in position C.

Precautions and warnings

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory

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α-Amylase EPS Pancreatic

reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

Reagent handling

Ready for use

Storage and stability

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Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Diluent NaCl 9 %

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Specimen collection and preparation[11,12]

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.
Serum.
Plasma: Li-heparin plasma.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability:[13]	7 days at 15-25 °C 1 month at 2-8 °C

Urine.
Collect without additives.

Stability:[14]	2 days at 15-25 °C 10 days at 2-8 °C

Pancreatic α-amylase is unstable in acid urine. Assay promptly or adjust pH to alkaline range (about pH 7) before storage.[13]

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- See "Order information" section

General laboratory equipment

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum, plasma and urine

cobas c 311 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10/44-56		
Wavelength (sub/main)	700/415 nm		
Reaction direction	Increase		
Units	U/L (µkat/L)		
Reagent pipetting		Diluent (H_2O)	
R1	100 µL	–	
R3	20 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	4 µL	–	–
Decreased	8 µL	15 µL	135 µL
Increased	4 µL	–	–

cobas c 501 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10/57-70		
Wavelength (sub/main)	700/415 nm		
Reaction direction	Increase		
Units	U/L (µkat/L)		
Reagent pipetting		Diluent (H_2O)	
R1	100 µL	–	
R3	20 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	4 µL	–	–
Decreased	8 µL	15 µL	135 µL
Increased	4 µL	–	–

cobas c 502 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10/57-70		
Wavelength (sub/main)	700/415 nm		
Reaction direction	Increase		
Units	U/L (µkat/L)		
Reagent pipetting		Diluent (H_2O)	
R1	100 µL	–	
R3	20 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	4 µL	–	–
Decreased	8 µL	15 µL	135 µL
Increased	8 µL	–	–

Calibration

Calibrators	S1: H_2O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration • after reagent lot change • as required following quality control procedures

Traceability: This method has been standardized against the Roche system reagent using calibrated pipettes together with a manual photometer providing absolute values and the substrate-specific absorptivity, ε.

Quality control

For quality control, use control materials as listed in the "Order information" section.

In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined

limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

Roche/Hitachi **cobas c** systems automatically calculate the analyte activity of each sample.

Conversion factor: U/L x 0.0167 = µkat/L

Limitations - interference[12,15]

Serum/plasma

The residual activity of salivary α-amylase is approx. 3 %. In rare cases, very high activities of salivary α-amylase can hence lead to elevated values being measured for pancreatic α-amylase.

A slight change in the yellow coloration of solution 2 does not interfere with the performance of the test.

Do not pipette by mouth, and ensure that the reagent does not come into contact with the skin. (**Saliva and sweat** contain α-amylase!)

Criterion: Recovery within ± 10 % of initial value at a pancreatic α-amylase activity of 50 U/L (0.84 µkat/L).

Icterus:[16] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:[16] No significant interference up to an H index of 200 (approximate hemoglobin concentration: 124 µmol/L or 200 mg/dL).

Lipemia (Intralipid):[16] No significant interference up to an L index of 1500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Highly turbid and grossly lipemic samples may cause Abs. flags.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[17,18]

Exception: No interference from ascorbic acid up to 5.68 mmol/L (100 mg/dL). Icodextrin based drugs may lead to decreased amylase values.[19]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[20]

Patients with macroamylase may have elevated p-amylase results. The elevation is not due to an insufficient inhibition of salivary amylase in the serum immune complex. It is caused by a higher than normal level of p-amylase since the immune complex is not subject to glomerular filtration.

This elevated p-amylase is not diagnostic for pancreatitis. However, measurement of an elevated p-amylase in urine is confirmatory of pancreatitis, pancreatic trauma, or pancreatic carcinoma as the amylase released is not completely bound by the immune complex and thus subject to glomerular filtration.[19]

Urine

Drugs: No interference was found at therapeutic concentrations using common drug panels.[18]

Exception: Approximately 15 % lower recovery was found at ascorbic acid concentrations of 22.7 mmol/L (400 mg/dL).

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/Multiclean/SCCS or the NaOHD/SMS/SmpCln1+2/SCCS Method Sheets. For further instructions refer to the operator's manual. **cobas c** 502 analyzer: All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is not required.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

3-1500 U/L (0.05-25.0 µkat/L)

Determine samples having higher activities via the rerun function. Dilution of samples via the rerun function is a 1:5 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 5.

Lower limits of measurement

Lower detection limit of the test

3 U/L (0.05 µkat/L)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Expected values[10]

Serum/plasma	Men/women	0.22-0.88 µkat/L	13-53 U/L
Spontaneously voided urine	Men	0.12-5.95 µkat/L	7-356 U/L
	Women	0.22-5.33 µkat/L	13-319 U/L
Pancreatic α-amylase/creatinine quotient	Men	0.58-3.33 µkat/g	35-199 U/g
	Women	0.87-4.33 µkat/g	52-259 U/g

Pancreatic α-amylase/creatinine quotient

To allow for fluctuations in the pancreatic α-amylase activity in urine, it is advisable to determine the pancreatic α-amylase/creatinine quotient. To do this, determine the pancreatic α-amylase activity and creatinine concentration in spontaneously voided urine.

$$\text{Quotient [µkat/mmol or U/g]} = \frac{\text{pancreatic α-amylase [µkat/L or U/L]}}{\text{creatinine [mmol/L or g/L]}}$$

Amylase/Creatinine Clearance Ratio (ACCR)[14]

The ACCR is calculated from amylase activity and creatinine concentration. Both the serum and urine samples should be collected at the same time.

$$\text{ACCR [\%]} = \frac{\text{Urine amylase [U/L] x serum creatinine [mg/L]}}{\text{Serum amylase [U/L] x urine creatinine [mg/L]}} \times 100$$

ACCR approximately equal to 2-5 %.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in an internal protocol with repeatability (n = 21) and intermediate precision (3 aliquots per run, 1 run per day, 21 days). The following results were obtained:

Serum/plasma

Repeatability	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm U	42.6 (0.71)	0.4 (0.01)	0.9
Precipath U	97.8 (1.63)	0.7 (0.01)	0.7
Human serum 1	23.8 (0.40)	0.4 (0.01)	1.7
Human serum 2	41.8 (0.70)	0.6 (0.01)	1.5
Intermediate precision	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm U	42.7 (0.71)	0.6 (0.01)	1.3
Precipath U	99.6 (1.66)	1.5 (0.03)	1.5
Human serum 3	23.9 (0.40)	0.5 (0.01)	2.1
Human serum 4	50.3 (0.84)	0.6 (0.01)	1.2

Urine

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Repeatability	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm U	42.9 (0.72)	0.5 (0.01)	1.1
Precipath U	98.1 (1.64)	0.9 (0.02)	0.9
Urine 1	23.5 (0.39)	0.3 (0.01)	1.3
Urine 2	151 (2.52)	1 (0.02)	0.8
Intermediate precision	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm U	43.2 (0.72)	0.6 (0.01)	1.3
Precipath U	99.6 (1.66)	1.5 (0.03)	1.5
Urine 3	69.2 (1.16)	1.3 (0.02)	1.9
Urine 4	191 (3.19)	2 (0.04)	1.1

Method comparison

Pancreatic amylase values for human serum, plasma and urine samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi 917 analyzer (x).

Serum/plasma

Sample size (n) = 87

Passing/Bablok[21]	Linear regression
y = 1.004x + 0.421 U/L	y = 1.002x + 0.778 U/L
τ = 0.991	r = 1.000

The sample activities were between 23.6 and 1410 U/L (0.394 and 23.5 µkat/L).

Urine

Sample size (n) = 88

Passing/Bablok[21]	Linear regression
y = 0.982x - 0.712 U/L	y = 0.981x + 0.588 U/L
τ = 0.987	r = 1.000

The sample activities were between 65.0 and 1270 U/L (1.08 and 21.2 µkat/L).

References

1 Greiling H, Gressner AM, eds. Lehrbuch der Klinischen Chemie und Pathobiochemie, 3rd ed. Stuttgart/New York: Schattauer Verlag 1995.

2 Keller H, ed. Klinisch-chemische Labordiagnostik für die Praxis, 2nd ed. Stuttgart/New York: Georg Thieme Verlag 1991;354-361.

3 Tietz NW, Burlina A, Gerhardt W, et al. Multicenter Evaluation of a Specific Pancreatic Isoamylase Assay Based on a Double Monoclonal Antibody Technique Clin Chem 1988;34:2096-2102.

4 Junge W, Troge B, Klein G, et al. Evaluation of a New Assay for Pancreatic Amylase: Performance Characteristics and Estimation of Reference Intervals. Clin Biochem 1989;22:109-114.

5 Rizzotti P, Klein G. Evaluation of a specific immunoinhibition method for the determination of pancreatic α-Amylase. Eur J Clin Chem Clin Biochem 1994;32:97-106.

6 Jalali MT, Laing I, Gowenlock AH, et al. Specific radioimmunoassays for human pancreatic and salivary isoamylases. Clin Chim Acta 1985;150:237-246.

7 Harmoinen A, Jokela H, Koivula T, et al. Evaluation of a new inhibitor test for isoamylase on Hitachi 705 analyzer J. Clin Chem Clin Biochem 1986;24:903-905.

8 Gerber M, Wulff K. Fortschritte in der spezifischen Bestimmung der Pankreas-α-amylase. Laboratoriumsmedizin 1988;12:110-113.

9 Kruse-Jarres JD, Hafkenscheid JCM, Hohenwallner W, et al. Evaluation of a New α-Amylase Assay Using 4,6-Ethylidene-(G7)-1-4-nitrophenyl-(G1)-α-D-maltoheptaoside as Substrate. J Clin Chem Clin Biochem 1989;27:103-113.

10 Junge W, Wortmann W, Wilke B, et al. Development and evaluation of assays for the determination of total and pancreatic amylase at 37°C according to the principle recommended by the IFCC. Clin Biochem 2001;34:607-615. Erratum Clin Biochem 2003;36:161.

11 Kurrle-Weitenhiller A, Hölzel W, Engel D, et al. Method for the determination of total and pancreatic α-amylase based on 100 % cleavage of the protected substrate ethylidene-4-nitrophenyl-maltoheptaoside. Clin Chem 1996;42(S6):98.

12 Young DS. Effects of Preclinical Variables on Clinical Laboratory Tests. AACC Press 1997, 2nd edition 1997.

13 Tietz NW. Clinical Guide to Laboratory Tests. 3rd ed. Philadelphia, Pa: WB Saunders Co; 1995

14 Hohenwallner W, Hägele EO, Scholer A, et al. Ber Öster Ges Klin Chem 1983;6:101-112.

15 Gokal R, Moberly J, Lindholm B, et al. Metabolic and laboratory effects of icodextrin. Kidney Int 2002;62(81):62-71.

16 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.

17 Breuer J. Report on the Symposium "Drug effects in Clinical Chemistry Methods". Eur J Clin Chem Clin Biochem 1996;34:385-386.

18 Sonntag O, Scholer A. Drug interference in clinical chemistry: recommendation of drugs and their concentrations to be used in drug interference studies. Ann Clin Biochem 2001;38:376-385.

19 Lott J. Inflammatory diseases of the pancreas. CRC critical reviews in clinical laboratory sciences 1982;17:201-228.

20 Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.

21 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

CONTENT	Contents of kit
→	Volume after reconstitution or mixing

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