AMYL2

α-Amylase EPS ver.2

Enzymes

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
03183742 122	α-Amylase EPS ver.2 (300 tests)	System-ID 07 6609 7	COBAS INTEGRA 400 plus COBAS INTEGRA 800
10759350 190	Calibrator f.a.s. (12 × 3 mL)	System-ID 07 3718 6	
10759350 360	Calibrator f.a.s. (12 × 3 mL, for USA)	System-ID 07 3718 6	
12149435 122	Precinorm U plus (10 × 3 mL)	System-ID 07 7999 7	
12149435 160	Precinorm U plus (10 × 3 mL, for USA)	System-ID 07 7999 7	
12149443 122	Precipath U plus (10 × 3 mL)	System-ID 07 8000 6	
12149443 160	Precipath U plus (10 × 3 mL, for USA)	System-ID 07 8000 6	
10171743 122	Precinorm U (20 × 5 mL)	System-ID 07 7997 0	
10171735 122	Precinorm U (4 × 5 mL)	System-ID 07 7997 0	
10171778 122	Precipath U (20 × 5 mL)	System-ID 07 7998 9	
10171760 122	Precipath U (4 × 5 mL)	System-ID 07 7998 9	
05117003 190	PreciControl ClinChem Multi 1 (20 × 5 mL)	System-ID 07 7469 3	
05947626 190	PreciControl ClinChem Multi 1 (4 × 5 mL)	System-ID 07 7469 3	
05947626 160	PreciControl ClinChem Multi 1 (4 × 5 mL, for USA)	System-ID 07 7469 3	
05117216 190	PreciControl ClinChem Multi 2 (20 × 5 mL)	System-ID 07 7470 7	
05947774 190	PreciControl ClinChem Multi 2 (4 × 5 mL)	System-ID 07 7470 7	
05947774 160	PreciControl ClinChem Multi 2 (4 × 5 mL, for USA)	System-ID 07 7470 7	

English

System information

Test AMYL2, test ID 0-609 (serum, plasma)

Test AMYU2, test ID 0-509 (urine)

Intended use

In vitro test for the quantitative determination of the catalytic activity of α-amylase (1,4-α-D-glucan: glucanohydrolase; EC 3.2.1.1) in human serum, plasma, and urine on COBAS INTEGRA systems.

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

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, α-amylase determinations are of considerable importance in pancreatic diagnostics. They are mainly used in the diagnosis and monitoring of acute pancreatitis. Hyperamylasemia does not, however, only occur with acute pancreatitis or in the inflammatory phase of chronic pancreatitis, but also in renal failure (reduced glomerular filtration), tumors of the lungs or ovaries, pulmonary inflammation, diseases of the salivary gland, diabetic ketoacidosis, cerebral trauma, surgical interventions or in the case of macroamylasemia. To confirm pancreatic specificity, it is recommended that an additional pancreas-specific enzyme - lipase or pancreatic-α-amylase - also be determined.

Numerous methods have been described for the determination of α-amylase. These either determine the decrease in the amount of substrate viscometrically, turbidimetrically, nephelometrically and amyloclastically or measure the formation of degradation products saccharogenically or kinetically with the aid of enzyme-catalyzed subsequent reactions. The kinetic method described here is based on the well-proven cleavage of 4,6-ethylidene-(G_7)-1,4-nitrophenyl-(G_1)-α,D-maltoheptaoside (Ethylidene Protected Substrate = EPS) by α-amylase and subsequent 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.

Test principle[10,11]

Enzymatic colorimetric assay acc. to IFCC.

Defined oligosaccharides such as 4,6-ethylidene-(G_7)-p-nitrophenyl-(G_1)-α,D-maltoheptaoside (ethylidene-G_7PNP) are cleaved under the catalytic action of α-amylases. The G_2PNP, G_3PNP and G_4PNP fragments so formed are completely hydrolyzed to p-nitrophenol and glucose by α-glucosidase.

Simplified reaction scheme:

$$5\ \text{ethylidene-G}_7\text{PNP}^{a)} + 5\ H_2O \xrightarrow{\alpha\text{-amylase}} 2\ \text{ethylidene-G}_5 + 2\ G_2\text{PNP} + 2\ \text{ethylidene-G}_4 + 2\ G_3\text{PNP} + \text{ethylidene-G}_3 + G_4\text{PNP}$$

a) PNP = p-nitrophenol

$$2\ G_2\text{PNP} + 2\ G_3\text{PNP} + G_4\text{PNP} + 14\ H_2O \xrightarrow{\alpha\text{-glucosidase}} 5\ \text{PNP} + 14\ G^{b)}$$

b) G = Glucose

The color intensity of the p-nitrophenol formed is directly proportional to the α-amylase activity. It is determined by measuring the increase in absorbance at 409 nm.

Reagents - working solutions

R1 HEPES: 52.4 mmol/L; sodium chloride: 87 mmol/L; calcium chloride: 0.08 mmol/L; magnesium chloride: 12.6 mmol/L; α-glucosidase (microbial): ≥ 66.8 µkat/L; pH 7.0 (37 °C); detergent; stabilizers

SR HEPES: 52.4 mmol/L; ethylidene-G_7-PNP: 22 mmol/L; pH 7.0 (37 °C); detergent; stabilizers

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

Precautions and warnings

Pay attention to all precautions and warnings listed in Section 1 / Introduction of this Method Manual.

Reagent handling

Ready for use

Storage and stability

Shelf life at 2-8 °C	See expiration date on **cobas c** pack label

COBAS INTEGRA 400 plus system	
On-board in use at 10-15 °C	12 weeks
COBAS INTEGRA 800 system	
On-board in use at 8 °C	12 weeks

Specimen collection and preparation[9,12]

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

Only the specimens listed below were tested and found acceptable:
Serum
Plasma: Heparin (Li-, Na-, NH_4^+-) or EDTA (K_2-, K_3-) plasma

EDTA plasma values are approximately 5-10 % lower than serum values.

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.

Urine: Collect urine without additives. α-Amylase is unstable in acid urine. Assay promptly or adjust pH to alkaline range (just above pH 7) before storage.[13]

Centrifuge samples containing precipitates before performing the assay.

Stability in *serum:*[13]	7 days at 15-25 °C
	1 month at 2-8 °C
Stability in *urine:*[14]	2 days at 15-25 °C
	10 days at 2-8 °C

Materials provided

See "Reagents – working solutions" section for reagents.

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.

Application for serum, plasma and urine

COBAS INTEGRA 400 plus test definition

Measuring mode	Absorbance
Abs. calculation mode	Kinetic
Reaction mode	R1-S-SR
Reaction direction	Increase
Wavelength A/B	409/659 nm
Calc. first/last	50/69
Unit	U/L

Pipetting parameters

Serum/plasma/urine		Diluent (H_2O)
R1	100 µL	
Sample	4 µL	4 µL
SR	20 µL	
Total volume	128 µL	

COBAS INTEGRA 800 test definition

Measuring mode	Absorbance
Abs. calculation mode	Kinetic
Reaction mode	R1-S-SR
Reaction direction	Increase
Wavelength A/B	409/659 nm
Calc. first/last	73/98
Unit	U/L

Pipetting parameters

Serum/plasma/urine		Diluent (H_2O)
R1	100 µL	
Sample	4 µL	4 µL
SR	20 µL	
Total volume	128 µL	

Calibration

Calibrator	Calibrator f.a.s.
	Use deionized water as zero calibrator.
Calibration mode	Linear regression
Calibration replicate	Duplicate recommended
Calibration interval	Each lot and as required following quality control procedures

Traceability: This method has been standardized manually against Roche reagent according to IFCC.

Quality control

Quality control serum, plasma	Precinorm U, Precinorm U plus or PreciControl ClinChem Multi 1
	Precipath U, Precipath U plus or PreciControl ClinChem Multi 2
Quality control urine	Quantitative urine controls are recommended for routine quality control.
Control interval	24 hours recommended
Control sequence	User defined
Control after calibration	Recommended

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

COBAS INTEGRA analyzers automatically calculate the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help (COBAS INTEGRA 400 plus/800 analyzers).

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

Limitations - interference

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.

Serum/plasma

Icterus:[15] No significant interference up to an I index of 52 for conjugated bilirubin and 76 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 889 µmol/L or 52 mg/dL; approximate unconjugated bilirubin concentration: 1300 µmol/L or 76 mg/dL).

Hemolysis:[15] No significant interference up to an H index of 260 (approximate hemoglobin concentration: 161 µmol/L or 260 mg/dL).

Lipemia (Intralipid):[15] No significant interference up to an L index of 2200. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[16,17] Exceptions: Icodextrin-based drugs may cause artificially low amylase results.[18]

Anticoagulants: Interference was found with citrate and fluoride.[12]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[19]

Urine

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

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 COBAS INTEGRA analyzers. Refer to the CLEAN Method Sheet for further instructions and for the latest version of the Extra wash cycle list.

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

Limits and ranges

Measuring range

Serum/plasma/urine
3-2000 U/L (0.05-33 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 3 standard deviations above that of a zero sample (zero sample + 3 SD, repeatability, n = 21).

Expected values[9]

Serum/plasma		
Men/women	28-100 U/L	(0.47-1.67 µkat/L)
Spontaneously voided urine		
Men	16-491 U/L	(0.27-8.20 µkat/L)
Women	21-447 U/L	(0.35-7.46 µkat/L)
α-Amylase/creatinine quotient		
Men	58-283 U/g	(0.97-4.73 µkat/g)
Women	75-390 U/g	(1.25-6.51 µkat/g)

α-Amylase/creatinine quotient

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

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

Amylase/Creatinine Clearance Ratio (ACCR)[13]

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

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

ACCR is 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 COBAS INTEGRA 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 (1 aliquot per run, 1 run per day, 21 days). The following results were obtained:

Serum/plasma

Repeatability	Level 1	Level 2
Mean	76 U/L (1.3 µkat/L)	192 U/L (3.2 µkat/L)
CV	1.4 %	1.2 %

Intermediate precision	Level 1	Level 2
Mean	73 U/L (1.2 µkat/L)	181 U/L (3.0 µkat/L)
CV	1.4 %	1.4 %

Urine

Repeatability	Level 1	Level 2
Mean	39.4 U/L (0.66 µkat/L)	201 U/L (3.4 µkat/L)
CV	0.8 %	0.4 %

Intermediate precision	Level 1	Level 2
Mean	36.7 U/L (0.61 µkat/L)	189 U/L (3.2 µkat/L)
CV	1.0 %	1.0 %

Method comparison

α-Amylase values for human serum, plasma and urine samples obtained on a COBAS INTEGRA 700 analyzer using the COBAS INTEGRA α-Amylase EPS ver.2 (AMYL2) reagent (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi 917 analyzer (x) and to the previous reagent (AMYLL) on a COBAS INTEGRA 700 analyzer (x).

Serum/plasma

Roche/Hitachi 917 analyzer	Sample size (n) = 64
Passing/Bablok[20]	Linear regression
y = 0.98x + 0.51 U/L	y = 1.00x - 1.28 U/L
τ = 0.987	r = 1.000
SD (md 95) = 5.57	Sy.x = 5.59

The sample activities were between 22 and 1900 U/L (0.37 and 31.7 µkat/L).

COBAS INTEGRA 700 analyzer	Sample size (n) = 64
Passing/Bablok[20]	Linear regression
y = 0.98x + 1.72 U/L	y = 0.97x + 3.01 U/L
τ = 0.982	r = 1.000
SD (md 95) = 12.22	Sy.x = 5.71

The sample activities were between 22 and 1930 U/L (0.37 and 32.2 µkat/L).

Urine

Roche/Hitachi 917 analyzer	Sample size (n) = 59
Passing/Bablok[20]	Linear regression
y = 0.98x - 0.32 U/L	y = 0.99x - 1.03 U/L
τ = 0.988	r = 1.000
SD (md 95) = 17.3	Sy.x = 6.54

The sample activities were between 0.66 and 1767 U/L (0.01 and 29.5 µkat/L).

COBAS INTEGRA 700 analyzer	Sample size (n) = 59
Passing/Bablok[20]	Linear regression
y = 0.96x + 0.54 U/L	y = 0.95x + 1.92 U/L
τ = 0.991	r = 1.000
SD (md 95) = 18.6	Sy.x = 6.28

The sample activities were between 0.64 and 1853 U/L (0.01 and 30.9 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 Salt WB II, Schenker S. Amylase - its clinical significance: a review of the literature [Review]. Medicine 1976;55:269-281.

4 Steinberg WM, Goldstein SS, Davies ND, et al. Diagnostic assays in acute pancreatitis [Review]. Ann Intern Med 1985;102:576-580.

5 Tietz NW, Huang WY, Rauh DF et al. Laboratory tests in the differential diagnosis of hyperamylasemia. Clin Chem 1986;32:301-307.

6 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.

7 Rauscher E, von Bülow S, Hägele EO, et al. Ethylidene protected substrate for the assay of human α-amylase. Fresenius Z Anal Chem 1986;324:304-305.

8 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.

9 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.

10 Lorentz K. Approved recommendation on IFCC methods for the measurement of catalytic concentration of enzymes. Part 9. IFCC Method for α-Amylase. (1,4-α-D-Glucan 4-Glucanohydrolase, EC 3.2.1.1). Clin Chem Lab Med 1998;36(3):185-203.

11 Kurrle-Weittenhiller 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 ethylidiene-4-nitrophenyl-maltoheptaoside. Clin Chem 1996;42(S6):S98.

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

13 Tietz NW, ed. Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia PA: WB Saunders Company 1995;46-51.

14 Hohenwallner W, Hägele EO, Scholer A, et al. Bestimmung von alpha-Amylase mit p-Nitrophenylmaltoheptaosid als Substrat. Ber Öster Ges Klin Chem 1983;6:101-112.

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

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

17 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.

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

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

20 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
GTIN	Global Trade Item Number

FOR US CUSTOMERS ONLY: LIMITED WARRANTY

Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

CE

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com

Distribution in USA by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support 1-800-428-2336

Roche