AMYL2

cobas®

α-Amylase EPS ver.2

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	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	
12149443 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 × 5 mL)	Code 300	
10171778 122	Precipath U (20 x 5 mL)	Code 301	
10171760 122	Precipath U (4 × 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:

AMYL2: ACN 570

SAMY2: ACN 566 (STAT, reaction time: 7)

For **cobas c** 502 analyzer:

AMYL2: ACN 8570

SAMY2: ACN 8566 (STAT, reaction time: 7)

Intended use

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

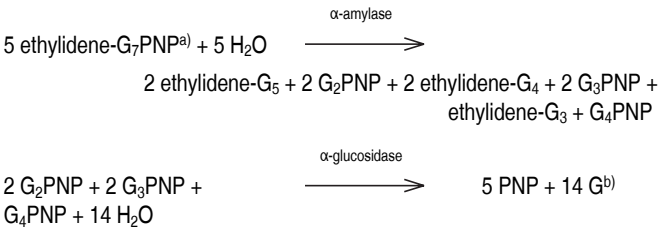
Numerous methods have been described for the determination of α-amylase. These either determine the decrease in the amount of substrate viscometrically, turbidimetrically, nephelometrically and amyloclastically or measure the formation of degradation products saccharogenically or kinetically with the 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. This assay follows the recommendation of the IFCC, but was optimized for performance and stability.

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:



a) PNP ≙ p-nitrophenol

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.

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); preservatives; stabilizers

R2 HEPES: 52.4 mmol/L; ethylidene-G_7-PNP: 22 mmol/L; pH 7.0 (37 °C); preservatives; stabilizers

R1 is in position B and R2 is in position C.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory

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

AMYL2

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[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: 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.

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]

Stability in *serum or plasma:*[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.

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 / 22-32 (STAT 7/ 22-32)
Wavelength (sub/main)	700/415 nm
Reaction direction	Increase
Unit	U/L (µkat/L)

Reagent pipetting		Diluent (H_2O)
R1	100 µL	–
R2	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 / 30-47 (STAT 7 / 30-47)
Wavelength (sub/main)	700/415 nm
Reaction direction	Increase
Unit	U/L (µkat/L)

Reagent pipetting		Diluent (H_2O)
R1	100 µL	–
R2	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 / 30-47 (STAT 7 / 30-47)
Wavelength (sub/main)	700/415 nm
Reaction direction	Increase
Unit	U/L (µkat/L)

Reagent pipetting		Diluent (H_2O)
R1	100 µL	–
R2	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 Roche system reagent using calibrated pipettes together with a manual photometer providing absolute values and substrate-specific absorptivity, ε.

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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

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 an amylase activity of 100 U/L (1.67 µkat/L).

Serum/plasma

Icterus:[15] 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:[15] No significant interference up to an H index of 500 (approximate hemoglobin concentration: 310 µmol/L or 500 mg/dL).

Lipemia (Intralipid):[15] No significant interference up to an L index of 1500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

In rare cases, samples with a combination of elevated turbidity (L-index) and high Amylase activity may cause a >React or >Abs flag.

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

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

Glucose: No interference from glucose up to 111 mmol/L (2000 mg/dL). Approximately 10 % higher recovery was found at glucose concentrations of 250 mmol/L (4500 mg/dL).

Ascorbic acid: No interference from ascorbic acid up to 5.68 mmol/L (100 mg/dL).

Drugs: No interference was found at therapeutic concentrations using common drug panels.[16,17]

Exception: Icodextrin-based drugs may lead to decreased amylase results.[18]

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

Urine

Ascorbic acid: No interference from ascorbic acid up to 2.27 mmol/L (40 mg/dL). Approximately 15 % lower recovery was found at ascorbic acid concentrations of 22.7 mmol/L (400 mg/dL).

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 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

Serum/plasma/urine

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[9]

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

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

The 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 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	83.2 (1.39)	0.8 (0.01)	0.9
Precipath U	182 (3.09)	1 (0.02)	0.6
Human serum 1	34.5 (0.576)	0.4 (0.007)	1.2
Human serum 2	97.9 (1.63)	0.7 (0.01)	0.7
Intermediate precision	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm U	84.0 (1.40)	1.1 (0.02)	1.3

Precipath U	184 (3.08)	3 (0.05)	1.5
Human serum 3	35.1 (0.586)	0.9 (0.015)	2.4
Human serum 4	98.9 (1.65)	1.6 (0.03)	1.6
Urine			
Repeatability	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Control level 1	50.6 (0.845)	0.5 (0.008)	0.9
Control level 2	164 (2.74)	1 (0.02)	0.6
Urine 1	21.4 (0.357)	0.2 (0.003)	1.1
Urine 2	68.5 (1.14)	0.7 (0.01)	0.9
Intermediate precision	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Control level 1	51.8 (0.865)	0.9 (0.015)	1.7
Control level 2	168 (2.81)	2 (0.03)	1.1
Urine 3	24.5 (0.409)	0.5 (0.008)	1.9
Urine 4	67.0 (1.12)	2.8 (0.05)	4.2

Method comparison

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 same reagent on a Roche/Hitachi 917 analyzer (x).

Serum/plasma

Sample size (n) = 79

Passing/Bablok[20]	Linear regression
y = 0.999x + 2.83 U/L	y = 0.998x + 4.75 U/L
τ = 0.969	r = 0.998

The sample activities were between 51.7 and 1409 U/L (0.863 and 23.5 µkat/L).

Urine

Sample size (n) = 88

Passing/Bablok[20]	Linear regression
y = 0.986x + 0.423 U/L	y = 0.982x + 2.03 U/L
τ = 0.987	r = 1.000

The sample activities were between 33.6 and 1248 U/L (0.561 and 20.8 µkat/L).

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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.

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AMYL2

α-Amylase EPS ver.2

CE

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