

AMY-P

α-Amylase EPS pancreatic

Order information

REF	CONTENT	Analyzer(s) on which cobas c pack(s) can be used
08056820190	α-Amylase EPS Pancreatic (450 tests)	System-ID 2018 001 cobas c 303, cobas c 503
Materials required (but not provided):		
10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 20401
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 20391
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 20391
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 20392
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 20392
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001

English

System information

AMYP2: ACN 20180 (Serum/plasma)

AMYP2U: ACN 20181 (Urine)

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.

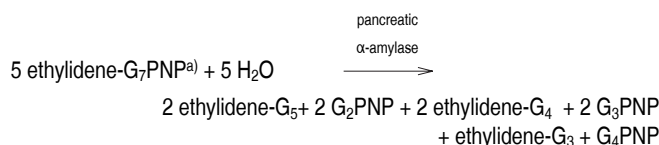
The kinetic method described here is based on inhibition of the activity of human salivary α-amylase by two different monoclonal antibodies and the well-proven cleavage of 4,6-ethylidene-(G₇)-1,4-nitrophenyl-(G₁)-α-D-maltoheptaoside (Ethylidene Protected Substrate = EPS) by pancreatic α-amylase followed 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}

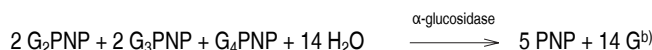
Colorimetric assay

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-G₇PNP).⁴

Simplified reaction scheme:



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-G₇ 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 for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:

Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:

Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:



Warning

H317 May cause an allergic skin reaction.

Prevention:

P261 Avoid breathing dust/fume/gas/mist/vapours/spray.

P272 Contaminated work clothing should not be allowed out of the workplace.

P280 Wear protective gloves.

Response:

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P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.

P362 + P364 Take off contaminated clothing and wash it before reuse.

Disposal:

P501 Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

Reagent handling

Ready for use

Storage and stability

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

On-board in use and refrigerated on the analyzer: 26 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.

Urine: Collect without additives. Pancreatic α -amylase is unstable in acid urine. Assay promptly or adjust pH to alkaline range (about pH 7) before storage.¹³

If stabilizers are added to the sample, the sample index feature must not be used.

See the limitations and interferences section for details about possible sample interferences.

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

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

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

Test definition

Reporting time	10 min		
Wavelength (sub/main)	700/415 nm		
Reagent pipetting		Diluent (H ₂ O)	
R1	78 μ L	–	
R3	16 μ L	–	
Sample volumes	Sample	Sample dilution	
		Sample	Diluent (NaCl)
Normal	3.1 μ L	–	–
Decreased	3.1 μ L	20 μ L	80 μ L
Increased	3.1 μ L	–	–

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

Calibration

Application for serum/plasma (ACN 20180)

Calibrators	S1: H ₂ O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	Automatic full calibration - after reagent lot change Full calibration - as required following quality control procedures

Application for urine (ACN 20181)

Transfer of calibration from serum/plasma application (ACN 20180)

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

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.

Serum/plasma:	PreciControl ClinChem Multi 1 PreciControl ClinChem Multi 2
Urine:	Quantitative urine controls are recommended for routine quality control.

The control intervals and limits should be adapted to each laboratory's individual requirements. It is recommended to perform quality control always after lot calibration and subsequently at least every 26 weeks. 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 c systems automatically calculate the analyte activity of each sample in the unit U/L (μ kat/L).

Conversion factor: U/L \times 0.0167 = μ kat/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.

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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 $\pm 10\%$ of initial value at a pancreatic α-amylase activity of 50 U/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.

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.

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).

Criterion: Recovery within $\pm 10\%$ of initial value at a pancreas α-amylase activity of 350 U/L.

Hemolysis: No significant interference up to an H index of 500 (approximate hemoglobin concentration: 311 μmol/L or 500 mg/dL).

Phosphate: No significant interference from phosphate up to a concentration of 60 mmol/L (186 mg/dL).

Urea: No significant interference from urea up to a concentration of 1500 mmol/L (9009 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 **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet for information. For further instructions refer to the operator's manual.

Limits and ranges

Measuring range

Serum, plasma and 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

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 3 U/L (0.05 μkat/L)

Limit of Detection = 3 U/L (0.05 μkat/L)

Limit of Quantitation = 3 U/L (0.05 μkat/L)

The Limit of Blank, the Limit of Detection and the Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from $n \geq 60$ measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the activity below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low activity samples.

The Limit of Detection corresponds to the lowest analyte activity which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte activity that can be reproducibly measured with a total error of 20 %. It has been determined using low activity α-amylase samples.

Expected values¹⁰

U/L

Serum/plasma	Men/Women	13-53 U/L
Spontaneously voided urine	Men	7-356 U/L
	Women	13-319 U/L
Pancreatic α-amylase/creatinine quotient	Men	35-199 U/g
	Women	52-259 U/g

μkat/L*

Serum/plasma	Men/Women	0.22-0.88 μkat/L
Spontaneously voided urine	Men	0.12-5.95 μkat/L
	Women	0.22-5.33 μkat/L
Pancreatic α-amylase/creatinine quotient	Men	0.58-3.33 μkat/g
	Women	0.87-4.33 μkat/g

*calculated by unit conversion factor

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.

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

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.

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

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. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability ($n = 84$) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

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Serum/plasma

Repeatability	Mean U/L	SD U/L	CV %
PCCC1 ^{c)}	38.3	0.314	0.8
PCCC2 ^{d)}	94.7	0.556	0.6
Human serum 1	7.31	0.252	3.4
Human serum 2	31.7	0.248	0.8
Human serum 3	325	1.16	0.4
Human serum 4	737	2.44	0.3
Human serum 5	1254	3.80	0.3

Intermediate precision	Mean U/L	SD U/L	CV %
PCCC1 ^{c)}	38.3	0.358	0.9
PCCC2 ^{d)}	93.5	0.695	0.7
Human serum 1	7.31	0.274	3.7
Human serum 2	31.7	0.293	0.9
Human serum 3	328	1.44	0.4
Human serum 4	737	2.85	0.4
Human serum 5	1254	5.05	0.4

c) PreciControl ClinChem Multi 1

d) PreciControl ClinChem Multi 2

Urine

Repeatability	Mean U/L	SD U/L	CV %
Control 1	39.2	0.308	0.8
Control 2	94.7	0.559	0.6
Human urine 1	7.05	0.261	3.7
Human urine 2	178	0.673	0.4
Human urine 3	325	0.988	0.3
Human urine 4	722	3.40	0.5
Human urine 5	1311	6.66	0.5

Intermediate precision	Mean U/L	SD U/L	CV %
Control 1	39.2	0.354	0.9
Control 2	94.5	0.727	0.8
Human urine 1	7.36	0.269	3.7
Human urine 2	178	0.979	0.5
Human urine 3	325	1.48	0.5
Human urine 4	722	4.85	0.7
Human urine 5	1311	7.41	0.6

The data obtained on **cobas c 503** analyzer(s) are representative for **cobas c 303** analyzer(s).

Method comparison

Pancreatic amylase values for human serum, plasma and urine samples obtained on a **cobas c 503** analyzer (y) were compared to those determined using the corresponding reagent on a **cobas c 501** analyzer (x).

Serum/plasma

Sample size (n) = 88

Passing/Bablok²¹ Linear regression
 $y = 1.005x + 0.0265 \text{ U/L}$ $y = 1.002x + 0.381 \text{ U/L}$

$\tau = 0.983$

$r = 1.000$

The sample activities were between 3.80 and 1456 U/L.

Urine

Sample size (n) = 69

Passing/Bablok²¹ Linear regression
 $y = 1.002x - 0.0394 \text{ U/L}$ $y = 0.998x + 0.567 \text{ U/L}$

$\tau = 0.992$

$r = 1.000$

The sample activities were between 5.40 and 1440 U/L.

Pancreatic amylase values for human serum, plasma and urine samples obtained on a **cobas c 303** analyzer (y) were compared to those determined using the corresponding reagent on a **cobas c 501** analyzer (x).

Serum/plasma

Sample size (n) = 73

Passing/Bablok²¹ Linear regression
 $y = 1.015x - 0.148 \text{ U/L}$ $y = 1.014x - 0.174 \text{ U/L}$

$\tau = 0.988$

$r = 1.000$

The sample activities were between 7.30 and 1420 U/L.

Urine

Sample size (n) = 70

Passing/Bablok²¹ Linear regression
 $y = 1.005x + 0.00463 \text{ U/L}$ $y = 1.014x - 0.829 \text{ U/L}$

$\tau = 0.997$

$r = 1.000$

The sample activities were between 3.60 and 1441 U/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.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

	Contents of kit
	Volume after reconstitution or mixing
	Global Trade Item Number

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