

AMY-P

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 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 AMY-P, test ID 0-662 (serum, plasma)

Test AMYUP, test ID 0-663 (urine)

Intended use

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

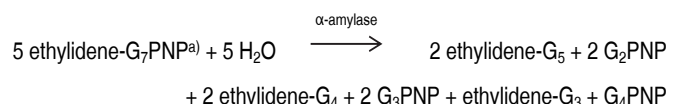
The kinetic method described here is based on inhibition of the activity of human salivary α-amylase by two different monoclonal antibodies and the well-proven cleavage of 4,6-ethylidene-(G₇)-1,4-nitrophenyl-(G₁)-α,D-maltoheptaoside (Ethylidene Protected Substrate = EPS) by pancreatic

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.

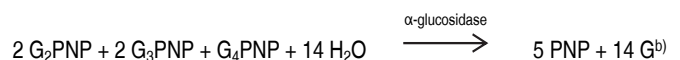
Test principle^{10,11}

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-G₇-PNP).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 at 409 nm.

Reagents - working solutions

R1 HEPES buffer: 52.4 mmol/L, pH 7.1; sodium chloride: 87 mmol/L; magnesium chloride: 12.6 mmol/L; calcium chloride: 0.08 mmol/L; α-glucosidase (microbial): ≥ 67 μkat/L; monoclonal antibodies (mouse): 97 mg/L; preservatives

SR HEPES buffer: 52.4 mmol/L, pH 7.1; ethylidene-G₇-PNP: 22 mmol/L; preservatives; stabilizers

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

Reagent R1 contains two monoclonal antibodies inhibiting human salivary α-amylase. The remaining activity of salivary α-amylase is approximately 3 %. In rare cases, elevated pancreatic α-amylase values may be obtained as a result of extremely high activities of salivary α-amylase.

AMY-P

α-Amylase EPS Pancreatic

cobas®
Enzymes

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^{10,12}

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

Only the specimens listed below were tested and found acceptable:
Serum (free from hemolysis)

Plasma (free from hemolysis): Li-heparin plasma.

Do not use other anticoagulants.

Serum, which has been separated from the cells as soon as possible after collection, is the specimen of choice.

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. Pancreatic 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*: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

Centrifuge samples containing precipitates before performing the assay.

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	51/63
Unit	U/L

Pipetting parameters

<i>Serum/plasma/urine</i>	Diluent (H ₂ O)	
R1	100 µL	
Sample	4 µL	10 µL

SR	20 µL
Total volume	134 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	74/94
Unit	U/L

Pipetting parameters

<i>Serum/plasma/urine</i>	Diluent (H ₂ O)	
R1	100 µL	
Sample	4 µL	10 µL
SR	20 µL	
Total volume	134 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 against the Roche system reagent using calibrated pipettes together with a manual photometer providing absolute values and the substrate-specific absorptivity, ε.

Quality control

Reference range	Precinorm U, Precinorm U plus or PreciControl ClinChem Multi 1
Pathological range	Precipath U, Precipath U plus or PreciControl ClinChem Multi 2
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 activity 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 - interference¹²

- Do not pipette by mouth and avoid all contact with the reagents, the reagent caps, and the samples. Saliva and sweat contain α-amylase and contact can cause inaccurate results, even in the presence of anti-salivary antibodies.

2. 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.¹⁵

Criterion: Recovery within ± 10 % of initial value.

Serum/plasma

Icterus:¹⁶ No significant interference up to an I index of 17 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 300 µmol/L or 17 mg/dL).

Hemolysis:¹⁶ No significant interference up to an H index of 100 (approximate hemoglobin concentration: 62 µmol/L or 100 mg/dL).

Lipemia (Intralipid):¹⁶ No significant interference.

Drugs: No interference was found at therapeutic concentrations using common drug panels.^{17,18} Exceptions: Icodextrin-based drugs may cause artificially low amylase results.¹⁹

Anticoagulants: Citrate and fluoride inhibit the reaction.

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

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

3-1500 U/L (0.05-25 µkat/L)

Determine samples having higher activities via the rerun function. Dilution of samples via the rerun function is a 1:10 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 10.

Lower detection limit

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 = 30).

Expected values¹⁰

Serum/plasma

Men/women 13-53 U/L (0.22-0.88 µkat/L)

Spontaneously voided urine

Men 7-356 U/L (0.12-5.95 µkat/L)

Women 13-319 U/L (0.22-5.33 µkat/L)

Pancreatic α-amylase/creatinine quotient

Men 35-199 U/g (0.58-3.33 µkat/g)

Women 52-259 U/g (0.87-4.33 µkat/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.

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

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 = 20) and intermediate precision (2 aliquots per run, 2 runs per day, 20 days). The following results were obtained:

Serum/plasma

	Level 1	Level 2
Mean	32 U/L (0.53 µkat/L)	184 U/L (3.1 µkat/L)
CV repeatability	1.2 %	0.91 %
CV intermediate precision	1.7 %	1.6 %

Urine

	Level 1	Level 2
Mean	59 U/L (0.99 µkat/L)	180 U/L (3.0 µkat/L)
CV repeatability	1.0 %	0.87 %
CV intermediate precision	1.2 %	1.1 %

Method comparison

Pancreatic α-amylase values for human serum, plasma and urine samples obtained on a COBAS INTEGRA 700 analyzer using the COBAS INTEGRA α-Amylase EPS Pancreatic reagent (y) were compared with those determined using the commercially available reagents for pancreatic α-amylase on an alternative clinical chemistry system (x). Samples were measured in duplicate. Sample size (n) represents all replicates.

Serum/plasma

	Alternative system
Sample size (n)	246
Corr. coefficient	(r) = 0.999 (r _s) = 0.994

Linear regression	y = 1.03x + 0.3 U/L
Passing/Bablok ²¹	y = 1.03x + 0.0 U/L

Sample concentrations were between 1.6 and 1310 U/L (0.03 and 21.9 µkat/L).

Urine

	Alternative system
Sample size (n)	106
Corr. coefficient	(r) = 0.999 (r _s) = 0.976

Linear regression	y = 1.00x + 1.4 U/L
Passing/Bablok ²¹	y = 1.00x + 1.2 U/L

Sample concentrations were between 0 and 765 U/L (0 and 12.8 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

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