α-Amylase EPS ver.2

Order information

COBAS INTEGRA α-Amylase EPS ver.2	300 Tests	Cat. No. **03183742** 122 System-ID 07 6609 7
Calibrator f.a.s.	12 × 3 mL	Cat. No. **10759350** 190
Calibrator f.a.s. (for USA)	12 × 3 mL	Cat. No. **10759350** 360 System-ID 07 3718 6
Precinorm U	20 × 5 mL	Cat. No. **10171743** 122 System-ID 07 7997 0
Precipath U	20 × 5 mL	Cat. No. **10171778** 122 System-ID 07 7998 9
Precinorm U plus	10 × 3 mL	Cat. No. **12149435** 122
Precinorm U plus (for USA)	10 × 3 mL	Cat. No. **12149435** 160 System-ID 07 7999 7
Precipath U plus	10 × 3 mL	Cat. No. **12149443** 122
Precipath U plus (for USA)	10 × 3 mL	Cat. No. **12149443** 160 System-ID 07 8000 6

● Indicates analyzer(s) on which **cobas c** pack can be used

COBAS INTEGRA 400/400 plus	COBAS INTEGRA 800
●	●

System information
COBAS INTEGRA α-Amylase EPS ver.2 (AMYL2)
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 (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]
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:

$$5\ \text{ethylidene-G}_7\text{PNP}^{a} + 5\ \text{H}_2\text{O} \xrightarrow{\alpha\text{-amylase}} 2\ \text{ethylidene-G}_5 + 2\ \text{G}_2\text{PNP} + 2\ \text{ethylidene-G}_4 + 2\ \text{G}_3\text{PNP} + \text{ethylidene-G}_3 + \text{G}_4\text{PNP}$$

$$2\ \text{G}_2\text{PNP} + 2\ \text{G}_3\text{PNP} + \text{G}_4\text{PNP} + 14\ \text{H}_2\text{O} \xrightarrow{\alpha\text{-glucosidase}} 5\ \text{PNP} + 14\ \text{G}^{b}$$

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 at 409 nm.

Reagents - working solutions

Components	Concentrations			
	R1	R2 = SR	Test	
HEPES	52.4	52.4	49.1	mmol/L
Sodium chloride	87		68	mmol/L
Calcium chloride	0.08		0.06	mmol/L
Magnesium chloride	12.6		9.8	mmol/L
α-Glucosidase	≥ 66.8		≥ 52.3	µkat/L
(microbial)			(≥ 3.1	kU/L)
Ethylidene-G_7-PNP		22	3.4	mmol/L
pH (37 °C)	7.00	7.00	7.00	

Both reagents contain nonreactive detergent and stabilizers.

Precautions and warnings

Pay attention to all precautions and warnings listed in this Method Manual, Chapter 1, Introduction.

Reagent handling

Ready for use.

Storage and stability

Shelf life at 2 to 8 °C	See expiration date on **cobas c** pack label
COBAS INTEGRA 400/400 plus systems	
On-board in use at 10 to 15 °C	12 weeks
COBAS INTEGRA 800 systems	
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]

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 400/400 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, and 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, and 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	Precinorm U or Precinorm U plus
serum, plasma	Precipath U or Precipath U plus
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. Other suitable control material can be used in addition.

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 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 400/400 plus/800 analyzers).

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

Limitations - interference[15]

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	No significant interference up to an I index of 52 (approximate conjugated bilirubin concentration: 889 µmol/L or 52 mg/dL).
	No significant interference with unconjugated bilirubin.
Hemolysis	No significant interference up to an H index of 260 (approximate hemoglobin concentration: 161 µmol/L or 260 mg/dL).
Lipemia	No significant interference.
Anticoagulants	Interference was found with citrate and fluoride.[12]
Drugs	No interference was found at therapeutic concentrations using common drug panels.[16,17] *Exception:* Icodextrin-based drugs may lead to decreased amylase values.[18]
Other	In very rare cases gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

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 Method Manual, Introduction, Extra Wash Cycles for further instructions. **Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.**

Measuring range

Serum/plasma/urine
3-2000 U/L (0.05-33 µkat/L)

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

Lower detection limit
3 U/L (0.05 µkat/L)
The 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 a zero sample (zero sample + 3 SD, within-run precision, 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 for serum and plasma

Representative performance data on 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 (within-run n = 21, between-run n = 21). The following results were obtained:

	Level 1	Level 2
Mean	76 U/L (1.3 µkat/L)	192 U/L (3.2 µkat/L)
CV within-run	1.4 %	1.2 %
Mean	73 U/L (1.2 µkat/L)	181 U/L (3.0 µkat/L)
CV between-run	1.4 %	1.4 %

Method comparison

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

Roche/Hitachi 917 analyzer	Sample size (n) = 64
Passing/Bablok[19]	Linear regression
$y = 0.98x + 0.51$ U/L	$y = 1.00x - 1.28$ U/L
$\tau = 0.987$	r = 1.000
SD (md 95) = 5.57	Sy.x = 5.59

Sample concentrations were between 22 and 1900 U/L (0.37 and 31.7 µkat/L).

COBAS INTEGRA 700 analyzer	Sample size (n) = 64
Passing/Bablok[19]	Linear regression
$y = 0.98x + 1.72$ U/L	$y = 0.97x + 3.01$ U/L
$\tau = 0.982$	r = 1.000
SD (md 95) = 12.22	Sy.x = 5.71

Sample concentrations were between 22 and 1930 U/L (0.37 and 32.2 µkat/L).

Specific performance data for urine

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

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run n = 21, between-run n = 21). The following results were obtained:

	Level 1	Level 2
Mean	39.4 U/L	201 U/L
	(0.66 µkat/L)	(3.4 µkat/L)
CV within-run	0.8 %	0.4 %
Mean	36.7 U/L	189 U/L
	(0.61 µkat/L)	(3.2 µkat/L)
CV between-run	1.0 %	1.0 %

Method comparison

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

Roche/Hitachi 917 analyzer	Sample size (n) = 59
Passing/Bablok[19]	Linear regression
$y = 0.98x - 0.32$ U/L	$y = 0.99x - 1.03$ U/L
$\tau = 0.988$	r = 1.000
SD (md 95) = 17.3	Sy.x = 6.54

The sample concentrations 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[19]	Linear regression
$y = 0.96x + 0.54$ U/L	$y = 0.95x + 1.92$ U/L
$\tau = 0.991$	r = 1.000
SD (md 95) = 18.6	Sy.x = 6.28

The sample concentrations were between 0.64 and 1853 U/L (0.01 and 30.9 µkat/L).

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