α-Amylase CC* FS**

Diagnostic reagent for quantitative in vitro determination of α-Amylase in serum, plasma or urine on photometric systems

Order Information

Cat. No.	Kit size						
1 0501 99 10 021	R1	5 x	20 mL	+	R2	1 x	25 mL
1 0501 99 10 026	R1	5 x	80 mL	+	R2	1 x	100 mL
1 0501 99 10 023	R1	1 x	800 mL	+	R2	1 x	200 mL
1 0501 99 10 704	R1	8 x	50 mL	+	R2	8 x	12.5 mL
1 0501 99 10 930	R1	4 x	20 mL	+	R2	2 x	10 mL

Summary [1,2]

α-Amylases are hydrolytic enzymes which break down starch into maltose. In the human body α-amylases originate from various organs: the pancreatic amylase is produced by the pancreas and released into the intestinal tract; the salivary amylase is synthesized in the salivary glands and secreted into saliva. The amylase present in the blood is eliminated through the kidney and excreted into the urine. Therefore, elevation of serum activity is reflected in a rise of urinary amylase activity.
Measurement of α-amylase in serum and urine is mainly used for the diagnosis of pancreatic disorders as well as for detecting the development of complications. In acute pancreatitis the blood amylase activity increases within few hours after onset of abdominal pain, peaks after approx. 12 hours and returns to values within the reference range at the latest after 5 days. The specificity of α-amylase for pancreatic disorders is not very high as elevated levels are measured also in various non-pancreatic diseases, e.g. parotitis and renal insufficiency. Therefore, for confirmation of an acute pancreatitis measurement of lipase should be additionally performed.

Method

Enzymatic photometric test, in which the substrate 4,6-ethylidene-(G7)-p-nitrophenyl-(G1)-α-D-maltoheptaoside (EPS-G7) is cleaved by α-amylases into various fragments. These are further hydrolyzed in a second step by α-glucosidase producing glucose and p-nitrophenol. The increase in absorbance represents the total (pancreatic and salivary) amylase activity in the sample [3,4].

Principle

$$5\ \text{EPS-G7} + 5\ H_2O \xrightarrow{\alpha\text{-Amylase}} \begin{matrix} 2\ \text{Ethylidene-G5} + 2\ \text{G2PNP} \\ +\ 2\ \text{Ethylidene-G4} + 2\ \text{G3PNP} \\ +\ \text{Ethylidene-G3} + \text{G4PNP} \end{matrix}$$

$$2\ \text{G2PNP} + 2\ \text{G3PNP} + \text{G4PNP} + 14\ H_2O \xrightarrow{\alpha\text{-Glucosidase}} 5\ \text{PNP} + 14\ \text{G}$$

(PNP = p-Nitrophenol, G =Glucose)

Reagents

Components and Concentrations

R1:	Good's buffer	pH 7.15	0.1 mol/L
	NaCl		62.5 mmol/L
	$MgCl_2$		12.5 mmol/L
	α-Glucosidase		≥ 2 kU/L
R2:	Good's buffer	pH 7.15	0.1 mol/L
	EPS-G7		8.5 mmol/L

Storage Instructions and Reagent Stability

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 – 8°C, protected from light and contamination is avoided. Do not freeze the reagents!

Warnings and Precautions

1. Saliva and skin contain α-amylase therefore never pipette reagents by mouth and avoid skin contact with the reagents.
2. The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
3. Reagent 1 contains animal material. Handle the product as potentially infectious according to universal precautions and good clinical laboratory practices.
4. In very rare cases, samples of patients with gammopathy might give falsified results [8].
5. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patients' medical history, clinical examinations and other findings.
6. For professional use only!

Waste Management

Please refer to local legal requirements.

Reagent Preparation

Substrate Start

The reagents are ready to use.

Sample Start

Mix 4 parts of R1 + 1 part of R2
(e.g. 20 mL R1 + 5 mL R2) = mono reagent

Stability:			
	6 months	at	2 – 8°C
	4 weeks	at	15 – 25°C

The mono reagent must be protected from light!

Materials required but not provided

NaCl solution 9 g/L
General laboratory equipment

Specimen

Serum, heparin plasma or EDTA plasma, urine

Stability in serum or plasma [5]:

7 days	at	20 – 25°C
7 days	at	4 – 8°C
1 year	at	–20°C

Stability in urine [5]:

2 days	at	20 – 25°C
10 days	at	4 – 8°C
3 weeks	at	–20°C

Only freeze once! Discard contaminated specimens!

Assay Procedure

Application sheets for automated systems are available on request.

Wavelength	Hg 405 nm
Optical path	1 cm
Temperature	37°C
Measurement	Against reagent blank

Substrate Start

	Serum/ Plasma		Urine	
	Blank	**Sample**	**Blank**	**Sample**
Sample/Calibrator	-	20 µL	-	10 µL
Dist. water	20 µL	-	10 µL	-
Reagent 1	1000 µL	1000 µL	1000 µL	1000 µL
Mix, incubate for approx. 1 min., then add:				
Reagent 2	250 µL	250 µL	250 µL	250 µL
Mix, read absorbance after 2 min. and start stopwatch. Read absorbance again 1, 2 and 3 min thereafter.				

* complete color
** fluid stable

Sample Start

	Serum/Plasma		Urine	
	Blank	**Sample**	**Blank**	**Sample**
Sample/Calibrator	-	20 µL	-	10 µL
Dist. water	20 µL	-	10 µL	-
Mono reagent	1000 µL	1000 µL	1000 µL	1000 µL
Mix, read absorbance after 2 min. and start stopwatch. Read absorbance again 1, 2 and 3 min thereafter.				

Calculation

With factor

From absorbance readings calculate ΔA/min and multiply by the corresponding factor from table below:

ΔA/min x factor = Amylase activity [U/L]

	Substrate Start	Sample Start
Serum/Plasma	5670	4554
Urine	11250	9018

With calibrator

$$\alpha - \text{Amylase } [U/L] = \frac{\Delta A/\text{min Sample}}{\Delta A/\text{min Calibrator}} \text{ x Conc. Calibrator } [U/L]$$

Calculation factor

α-Amylase [U/L] x 0.0167 = α-Amylase [µkat/L]

Calibrators and Controls

For the calibration of automated photometric systems the DiaSys TruCal U calibrator is recommended. This method has been standardized against the original IFCC [International Federation of Clinical Chemistry and Laboratory Medicine] formulation from 1998. For internal quality control DiaSys TruLab N and P or TruLab Urine controls should be assayed. Each laboratory should establish corrective action in case of deviations in control recovery.

	Cat.-No.	Kit size
TruCal U	5 9100 99 10 063	20 x 3 mL
	5 9100 99 10 064	6 x 3 mL
TruLab N	5 9000 99 10 062	20 x 5 mL
	5 9000 99 10 061	6 x 5 mL
TruLab P	5 9050 99 10 062	20 x 5 mL
	5 9050 99 10 061	6 x 5 mL
TruLab Urine Level 1	5 9170 99 10 062	20 x 5 mL
	5 9170 99 10 061	6 x 5 mL
TruLab Urine Level 2	5 9180 99 10 062	20 x 5 mL
	5 9180 99 10 061	6 x 5 mL

Performance Characteristics

Measuring range

On automated systems the test is suitable for the determination of α-Amylase activities up to 2000 U/L.
In case of a manual procedure, the test is suitable for α-Amylase activities which correspond to a maximum of ΔA/min of 0.35.
If such values are exceeded the samples should be diluted 1 + 9 with NaCl solution (9 g/L) and results multiplied by 10.

Specificity/Interferences

No interference was observed by ascorbic acid up to 30 mg/dL, bilirubin up to 40 mg/dL, by hemoglobin up to 550 mg/dL and lipemia up to 1000 mg/dL triglycerides. For further information on interfering substances refer to Young DS [7].

Sensitivity/Limit of Detection

The lower limit of detection is 3 U/L.

Precision

Intra-assay precision n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	184	2.00	1.08
Sample 2	398	2.67	0.67
Sample 3	841	4.96	0.59

Inter-assay precision n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	180	1.82	1.01
Sample 2	383	3.74	0.97
Sample 3	817	7.48	0.92

Method Comparison

A comparison of DiaSys α-Amylase CC FS (y) with the recommended routine method [5] (x) using 51 samples gave following results:
y = 0.964 x - 2.455 U/L; r = 0.998

A comparison of DiaSys α-Amylase CC FS (y) with a commercially available test (x) using 51 samples gave following results:
y = 1.031 x - 3.613 U/L; r = 0.994

Reference Range [6]

	Women	Men
Serum/plasma	< 100 U/L (< 1.67 µkat/L)	< 100 U/L (< 1.67 µkat/L)
Urine	< 447 U/L (< 7.45 µkat/L)	< 491 U/L (< 8.18 µkat/L)

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Literature

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Manufacturer

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