Pancreatic amylase CC* FS **

Diagnostic reagent for quantitative in vitro determination of pancreatic amylase in serum, plasma or urine on photometric systems

Order Information

Cat. No.	Kit size						
1 0551 99 10 021	R1	5 x	20 mL	+	R2	1 x	25 mL
1 0551 99 10 023	R1	1 x	800 mL	+	R2	1 x	200 mL
1 0551 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. As the pancreatic and the salivary amylase show a structural homology of 97%, the only method to distinguish both sufficiently is to use an assay based on monoclonal antibodies to inhibit the salivary enzyme. The amylase present in the blood is eliminated through the kidney and excreted into the urine. Therefore, an elevation of serum activity is reflected in the rise of urinary amylase activity. Measurement of α-amylase in serum and urine is mainly applied 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. Although the pancreatic amylase is much more specific for detection of pancreatic disorders than the total amylase, for confirmation of an acute pancreatitis an additional measurement of lipase is recommended.

Method

Enzymatic photometric test, in which the substrate 4,6-ethylidene-(G7)-p-nitrophenyl-(G1)-A-D-maltoheptaoside (EPS-G7) is cleaved by α-amylases into various fragments. These are further hydrolyzed in a second step by A-glucosidase producing glucose and p-nitrophenol [1,2]. As the salivary isoenzyme is inhibited selectively by a combination of two monoclonal antibodies during the preincubation phase, the increase in absorbance represents the pancreatic amylase activity in the sample [3-5].

Principle

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

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

(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		≥ 2,5 kU/L
	Monoclonal antibodies against salivary amylase (mouse)		≥ 31 mg/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. The remaining activity of salivary α-amylase is up to 3%. Very rarely extremely high activities of salivary α-amylase may lead to increased readings of pancreatic α-amylase. However saliva and skin do contain α-amylase, therefore never pipette 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 laboratory practice.
4. In very rare cases, samples of patients with gammopathy might give falsified results [10].
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 patient's medical history, clinical examinations and other findings.
6. For professional use only!

Waste Management

Please refer to local legal requirements.

Reagent Preparation

The reagents are ready to use.

Materials required but not provided

NaCl solution 9 g/L
General laboratory equipment

Specimen

Serum, heparin plasma or EDTA plasma, urine

Stability [8]:

in serum/plasma:	7 days	at	20 – 25°C
	7 days	at	4 – 8°C
	1 year	at	-20°C
in urine:	2 days	at	20 – 25°C
	10 days	at	4 – 8°C
	3 weeks	at	-20°C

Freeze only 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

	Blank	Serum / Plasma	Urine
Sample/Calibrator	-	20 µL	10 µL
Reagent 1	1000 µL	1000 µL	1000 µL
Mix, incubate for approx. 3 min., then add:			
Reagent 2	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

Calculation

With factor

Calculate ΔA/min from absorbance readings and multiply by the corresponding factor:

ΔA/min x 5670 = Pancreatic amylase activity [U/L]

With calibrator

$$\text{P – Amyl. [U/L]} = \frac{\Delta A/\text{min Sample}}{\Delta A/\text{min Calibrator}} \text{ x Conc. Calibrator [U/L]}$$

Conversion factor

Pancreatic amylase [U/L] x 0.0167 = Pancreatic amylase [µkat/L]

Calibrators and Controls

For the calibration of automated photometric systems, DiaSys TruCal U calibrator is recommended. This method is traceable to the molar extinction coefficient. For internal quality control DiaSys TruLab N and P 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

Performance Characteristics

Measuring range

On automated systems the test is suitable for the determination of pancreatic amylase activities up to 2000 U/L.
In case of a manual procedure, the test is suitable for pancreatic amylase activities which correspond to a maximum of ΔA/min of 0.350.
If such value is exceeded the sample should be diluted 1 + 10 with NaCl solution (9 g/L) and results multiplied by 11.

Specificity/Interferences

No interference was observed by ascorbic acid up to 30 mg/dL, bilirubin up to 40 mg/dL, lipemia up to 2,000 mg/dL triglycerides and Hemoglobin up to 150 mg/dL. For further information on interfering substances refer to Young DS [9].

Sensitivity/Limit of Detection

The lower limit of detection is 5 U/L.

Precision

Intra-assay precision n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	69.7	2.18	3.13
Sample 2	207	2.61	1.26
Sample 3	370	3.36	0.91

Inter-assay precision n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	68.3	1.48	2.17
Sample 2	204	1.61	0.79
Sample 3	371	3.14	0.85

Method Comparison

A comparison of DiaSys Pancreatic amylase CC FS (y) with a commercially available test (x) using 58 samples gave following results:
y = 0.97 x – 1.66 U/L; r= 0.994.

Reference Range [7]

	Women		Men	
	U/L	µkat/L	U/L	µkat/L
Serum/plasma	< 53	< 0.88	< 53	< 0.88
Urine	< 319	< 5.32	< 356	< 5.93

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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