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alpha-Amylase Pancreatic
ET-G7PNP

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

REF	Kit Size	Configuration
D00582	5 x 100 mL	4 x 100 mL R1 + 1 x 100 mL R2
D94577	5 x 50 mL	4 x 50 mL R1 + 1 x 50 mL R2
D00590	5 x 25 mL	4 x 25 mL R1 + 1 x 25 mL R2
D96568	5 x 10 mL	4 x 10 mL R1 + 1 x 10 mL R2
D56911	5 x 50 mL	4 x 50 mL R1 + 2 x 25 mL R2
D0404917	5 x 50 mL	4 x 50 mL R1 + 1 x 50 mL R2
DA0807	5 x 20 mL	4 x 20 mL R1 + 1 x 20 mL R2
DT1007	5 x 20 mL	4 x 20 mL R1 + 1 x 20 mL R2
DK0706	5 x 50 mL	4 x 50 mL R1 + 1 x 50 mL R2
DE1807	1 x 62.5 mL	1 x 50 mL R1 + 1 x 12.5 mL R2
DB20303	2 x 62.5 mL	2 x 50 mL R1 + 2 x 12.5 mL R2

Additionally available:

D98485	5 x 3 mL	Calibrator	Diacal Auto
D98485SV	1 x 3 mL	Calibrator	Diacal Auto
D98481	12 x 5 mL	Control normal	Diacon N
D14481	5 x 5 mL	Control normal	Diacon N
D98481SV	1 x 5 mL	Control normal	Diacon N
D98482	12 x 5 mL	Control abnormal	Diacon P
D14482	5 x 5 mL	Control abnormal	Diacon P
D98482SV	1 x 5 mL	Control abnormal	Diacon P

For professional in vitro diagnostic use only.

GENERAL INFORMATION

Method	Colorimetric, kinetic, increasing reaction, ET-G7PNP
Shelf life	24 months from production date
Storage	2 – 8°C
Wavelength	405 nm
Optical path	1 cm
Temperature	37 °C
Sample	Serum, heparin plasma or EDTA plasma, urine

INTENDED USE

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

DIAGNOSTIC SIGNIFICANCE [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, 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. 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.

TEST PRINCIPLE

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 hydrolysed in a second step by α-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].

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

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

(PNP = p-Nitrophenol, G = Glucose)

REAGENT COMPOSITION

COMPONENTS		CONCENTRATION	
Reagent 1:			
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
Reagent 2			
Good´s buffer,	pH 7.15	0.1	mol/L
EPS-G7		8.5	mmol/L

MATERIAL REQUIRED BUT NOT PROVIDED

- NaCl solution (9 g/L).
- Clinical chemistry analyser.

REAGENT PREPARATION

The reagents are ready to use.

STORAGE AND STABILITY

Conditions:	Protect from light!
	Close immediately after use
	Avoid contamination
	Do not freeze the reagents!
Storage	at 2 – 8 °C
Stability:	up to the indicated expiration date

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 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 [10].
5. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.
6. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
7. For professional use only!

SPECIMEN COLLECTION AND STORAGE

Use serum, heparin plasma or EDTA plasma, urine.

Stability [8]:

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

Freeze only once! Discard contaminated specimens.

TEST PROCEDURE

Bring reagents and samples to room temperature.

Pipette into test tubes	Blank	Serum / Plasma	Urine
Reagent 1	1000 µL	1000 µL	1000 µL
Sample/Calibrator	-	20 µL	10 µL
Mix. Incubate for approximately 3 minutes at 37 °C. Then add:			
Reagent 2	250 µL	250 µL	250 µL
Mix. Read initial absorbance after 2 min. (37°C) and start a stopwatch. Read absorbance again after exactly 1, 2 and 3 min.			

Automation

Special adaptations for automated analysers can be made on request.

INTERPRETATION OF RESULTS

Calculation

Calculate ΔA/min = [ΔA/min sample or cal.] – [ΔA/min blank] during the linear part of the assay.

With factor: (light path 1 cm)
Pancreatic amylase activity [U/L] = ΔA/min x Factor

Factors (37 °C):

Serum / Plasma	5670
Urine	11250

With calibrator:

$$\text{Pancreatic Amylase [U/L]} = \frac{\Delta\text{A/min Sample}}{\Delta\text{A/min Calibrator}} \times \text{Conc. of Cal [U/L]}$$

Unit Conversion

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

QUALITY CONTROL AND CALIBRATION

All control sera with pancreatic amylase values determined by this method and employing comparable substrate concentration may be used.
We recommend the Dialab serum controls **Diacon N** (control serum with values in the normal range) and **Diacon P** (control serum with values in the abnormal range).
Each laboratory should establish corrective action in case of deviations in control recovery.

Calibration

The use of pancreatic amylase calibrator is optional.
We recommend the Dialab multi calibration serum **Diacal Auto**.

PERFORMANCE CHARACTERISTICS

LINEARITY, MEASURING RANGE

On automatic systems the test is suitable for the determination of pancreatic amylase activities up to 2000 U/L.
In case of manual procedure, the test is suitable for pancreatic amylase activities which correspond to a maximum 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.

SENSITIVITY/LIMIT OF DETECTION

The lower limit of detection is 5 U/L.

PRECISION

Intra-assay 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 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

SPECIFICITY/INTERFERENCES

no interference up to:

Ascorbic acid	30 mg/dL
Bilirubin	40 mg/dL
Hemoglobin	150 mg/dL
Triglycerides	2000 mg/dL

For further information on interfering substances refer to Young DS [9].

METHOD COMPARISON

A comparison between Dialab alpha-Amylase Pancreatic (y) and a commercially available test (x) using 58 samples gave following results:
y = 0.97 x – 1.66 U/L; r = 0.994.

TRACEABILITY

This method is traceable to the molar extinction coefficient.

EXPECTED VALUES [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.

LIMITATIONS

- Eventual alpha-Amylase Pancreatic (ET-G7PNP) carry-over to reagents Magnesium (Xylidyl blue) and Protein Total in Urine/CSF (Pyrogallol red). The actual carry-over depends on the analyser.

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

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