DIALAB Produktion und Vertrieb von chemisch-technischen Produkten und Laborinstrumenten Gesellschaft m.b.H.
IZ NOE-Sued, Hondastrasse, Objekt M55, 2351 Wr. Neudorf, Austria
Phone: +43 (0) 2236 660910-0, Fax: +43 (0) 2236 660910-30, e-mail: office@dialab.at

alpha-Amylase
mod. IFCC

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

REF	Kit Size	Configuration
D03103B	1 x 1.25 L	1 x 1 L R1 + 1 x 250 mL R2
D94570	5 x 100 mL	4 x 100 mL R1 + 1 x 100 mL R2
D94571	5 x 50 mL	4 x 50 mL R1 + 1 x 50 mL R2
D00578	5 x 25 mL	4 x 25 mL R1 + 1 x 25 mL R2
D96569	5 x 10 mL	4 x 10 mL R1 + 1 x 10 mL R2
D54911	5 x 50 mL	4 x 50 mL R1 + 2 x 25 mL R2
D0406917	5 x 62.5 mL	4 x 62.5 mL R1 + 1 x 62.5 mL R2
DA0806	5 x 20 mL	4 x 20 mL R1 + 1 x 20 mL R2
DK0705	5 x 50 mL	4 x 50 mL R1 + 1 x 50 mL R2
DT1006	5 x 20 mL	4 x 20 mL R1 + 1 x 20 mL R2
DE1806	2 x 62.5 mL	2 x 50 mL R1 + 2 x 12.5 mL R2
DB20304	4 x 62.5 mL	4 x 50 mL R1 + 4 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
D08581	12 x 5 mL	Urine Ctrl. normal	Diacon Urine Level 1
D08581SV	1 x 5 mL	Urine Ctrl. normal	Diacon Urine Level 1
D08582	12 x 5 mL	Urine Ctrl. abnormal	Diacon Urine Level 2
D08582SV	1 x 5 mL	Urine Ctrl. abnormal	Diacon Urine Level 2

For professional in vitro diagnostic use only.

GENERAL INFORMATION

Method	Colorimetric, kinetic, increasing reaction, mod. IFCC
Shelf life	24 months
Storage	2 – 8°C
Wavelength	405 nm
Temperature	37 °C
Sample	Serum, heparin plasma or EDTA plasma, urine

INTENDED USE

Diagnostic reagent for quantitative in vitro determination of α -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. 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.

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]. The increase in absorbance represents the total (pancreatic and salivary) amylase activity in the sample [3,4].

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

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

(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	kU/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

Substrate Start:
Reagents are ready to use.

Sample Start:
Mix 4 parts of Reagent 1 with 1 part of Reagent 2.
(= working reagent)

STORAGE AND STABILITY

Conditions: Protect from light!
Close immediately after use
Avoid contamination
Do not freeze the reagents!

Substrate Start:
Storage: at 2 – 8 °C
Stability: up to the indicated expiration date

Sample Start (working reagent):
Stability: at 2 – 8 °C 6 months
at 15 – 25 °C 4 weeks
Protect from light!

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.
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

Stability [5]:

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.

Substrate Start:

Pipette into test tubes	Serum/Plasma		Urine	
	Blank	Sample	Blank	Sample
Reagent 1	1000 µL	1000 µL	1000 µL	1000 µL
Dist. water	20 µL	-	10 µL	-
Sample/Calibr.	-	20 µL	-	10 µL
Mix. Incubate for approximately 1 minute. Then add:				
Reagent 2	250 µL	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.				

Sample Start:

Pipette into test tubes	Serum/Plasma		Urine	
	Blank	Sample	Blank	Sample
working reagent	1000 µL	1000 µL	1000 µL	1000 µL
Dist. water	20 µL	-	10 µL	-
Sample/Calibr.	-	20 µL	-	10 µL
Mix. Read initial absorbance after 2 minutes (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)
Amylase activity [U/L] = ΔA/min x Factor

Factors:

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

With calibrator:

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

Unit Conversion
U/L x 0.01667 = µkat/L

QUALITY CONTROL AND CALIBRATION

All control sera with alpha-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) as well as the Dialab urine controls **Diacon Urine Level 1** (control urine normal) and **Level 2** (control urine abnormal).
Each laboratory should establish corrective action in case of deviations in control recovery.

Calibration
The use of an alpha-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 α-Amylase activities up to 2000 U/L.
In case of manual procedure, the test is suitable for α-amylase activities which correspond to a maximum ΔA/min of 0.35.
If such value is exceeded, the sample should be diluted 1+9
with NaCl solution (9 g/L) and results multiplied by 10.

SENSITIVITY/LIMIT OF DETECTION

The lower limit of detection is 3 U/L.

PRECISION

Intra-assay 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 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

SPECIFICITY/INTERFERENCES

no interference up to:

Ascorbic acid	30 mg/dL
Bilirubin	40 mg/dL
Hemoglobin	550 mg/dL
Triglycerides	1000 mg/dL

For further information on interfering substances refer to Young DS [7].

METHOD COMPARISON

A comparison between Dialab alpha-Amylase (y) and 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 between Dialab alpha-Amylase (y) and a commercially available test (x) using 51 samples gave following results: y = 1.031 x – 3.613 U/L; r = 0.994.

TRACEABILITY

This method has been standardized against the original IFCC formulation from 1998.

EXPECTED VALUES [6]*

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

* 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 (mod. IFCC) carry-over to reagents Magnesium (Xylidyl blue), Carbon Dioxide (PEP-C) Protein Total in Urine/CSF (Pyrogallol red). The actual carry-over depends on the analyser.

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

1. Lorentz K. α-Amylase. In: Thomas L, editor. Clinical laboratory diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft; 1998. p.192-202.
2. Moss DW, Henderson AR. Digestive enzymes of pancreatic origin. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company; 1999.p.689-98.
3. Kruse-Jarres JD, Kaiser C, Hafkenscheid JC, Hohenwallner W, Stein W., Bohner J et al. Evaluation of a new alpha-amylase assay using 4,6-ethylidene-(G7)-1-4-nitrophenyl-(G1)-alpha,D-maltoheptaoside as substrate. J Clin Chem Biochem 1989; 27:103-13.
4. Schumann G, Aoki R, Ferrero CA et al. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37 °C. Clin Chem Lab Med 2006; 44(9): 1146-1155.
5. Guder WG, Zawta B et al. The quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag; 2001; p.16-7, 50-1.
6. Junge W, Wortmann W, Wilke B, Waldenstroem J et al. Development and evaluation of assays for determination of total and pancreatic amylase at 37°C according to the principle recommended by the IFCC. Clin Biochem 2001; 34:607-15
7. Young DS. Effects of Drugs on Clinical laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.
8. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed 2007; 45(9): 1240-1243.

CE IVD 2°C 8°C