Liquick Cor-AMYLASE EPS

DIAGNOSTIC KIT FOR DETERMINATION OF α-AMYLASE ACTIVITY

Kit name	Cat. No
Liquick Cor-AMYLASE EPS 500	2-335
Liquick Cor-AMYLASE EPS "bulk"	2-336

INTRODUCTION

α-Amylases are hydrolytic enzymes which hydrolyze 1,4-α-glucosidic bond in starch and other similar polysaccharides to maltose and other oligosaccharides. Several types of amylases can be distinguished, depending on the organ they are originating from. α-amylase is the most commonly measured in the diagnosis of acute pancreatitis, when its activity in serum is very high. Elevation of α-amylase activity in serum is also accompanied by increased excretion of enzyme in urine which can last longer than in the blood. Because of that activity in α-amylase in urine is used as a indicator of acute pancreatitis. Hyperamylasemia occurs also in chronic pancreatitis, failures of kidneys, lungs, diseases of the salivary glands, cerebral traumas, surgical interventions and macroamylasemia. To confirm pancreatic specificity it is recommended to determine also other pancreas specific enzyme like lipase.

METHOD PRINCIPLE

Enzymatic colorimetric method, with EPS substrate, in accordance to recommendations of IFCC – International Federation of Clinical Chemistry and Laboratory Medicine (modified IFCC method).
α-Amylase catalyzes hydrolysis of substrate 4,6-ethylidene-(G7)-p-nitrophenyl-(G1)-α,D-maltoheptaoside (EPS, Ethylidene Protected Substrate). Ethylidene group prevents the substrate from breaking down because of exo-enzymes activity, therefore in absence of α-amylase no increase of absorbance is observed.
α-Amylase hydrolyses the substrate into smaller fragments which are acted upon by α-glucosidase, causing the ultimate release of chromophore p-nitrophenol (pNP) and glucose.

$$\text{4,6-ethylidene-pNP-G7(EPS)} + H_2O \xrightarrow{\alpha\text{-amylase}} \text{4,6-ethylidene-Gx} + \text{p-nitrophenylo-G (7-x)}$$

$$\text{p-nitrophenylo-G (7-x)} + \text{(7-x)}\ H_2O \xrightarrow{\alpha\text{-glucosidase}} \text{p-nitrophenol} + \text{(7-x) glucose}$$

Increase of absorbance related to formation of p-nitrophenol is proportional to the α-amylase activity in sample and is measured spectrophotometrically at 405 nm wavelength.

REAGENTS

Package

	Liquick Cor-AMYLASE EPS 500	Liquick Cor-AMYLASE EPS "bulk"
1-AMYLASE EPS	3 x 400 ml	--*
2-AMYLASE EPS	1 x 300 ml	--*

* reagent volume is printed on the label

The reagents when stored at 2-8°C are stable up to expiry date printed on the package. The reagents are stable for 12 weeks on board the analyser at 2-10°C. Prevent the reagents from microbiological contamination and from saliva and sweat α-amylase. Protect from direct light. The reagents must be clear, do not use if turbid.

Working reagent preparation and stability

Assay can be performed with use of separate 1-AMYLASE EPS and 2-AMYLASE EPS reagents or with use of working reagent. For working reagent preparation mix gently **4 parts** of 1-AMYLASE EPS with **1 part** of 2-AMYLASE EPS. Avoid foaming.

Stability of working reagent: 4 weeks at 2-8°C
5 days at 18-25°C

Prevent the reagents from microbiological contamination and from saliva and sweat α-amylase. Protect from direct light.

Concentrations in the test

HEPES buffer, pH 7.2	52.5 mmol/l
sodium chloride	87 mmol/l
magnesium chloride	12.6 mmol/l
calcium chloride	0.075 mmol/l
α-glucosidase	≥ 4kU/l
4,6-ethylidene G7pNP (EPS)	> 4 mmol/l
stabilizers and preservatives	

Warnings and notes

- Product for in vitro diagnostic use only.
- A slight yellow colour of 2-AMYLASE EPS does not influence the reagent performance.

ADDITIONAL EQUIPMENT

- automatic analyser or photometer able to read at 405 nm;
- thermostat at 37ºC;
- general laboratory equipment;

SPECIMEN

Serum or plasma collected on heparin, free from hemolysis, urine.
Do not use anticoagulants: EDTA, citrates and oxalates as they inhibit amylase activity.
Serum / plasma can be stored for 7 days at 20-25ºC or for one month at. 4ºC.
Urine can be stored for 2 days at 20-25ºC or for 10 days at 4-8ºC. Amylase is very unstable in acid urine. Adjust pH to alkaline range before storage.
Nevertheless it is recommended to perform the assay with freshly collected samples!

PROCEDURE

These reagents may be used both for manual assay (Sample Start and Reagent Start method) and in several automatic analysers. Applications for them are available on request.

Manual procedure

wavelength	405 nm
temperature	37°C
cuvette	1 cm

Sample Start method

Pipette into the cuvettes:

	blank (B)	test (T)	calibrator (C)
working reagent	1000 µl	1000 µl	1000 µl

Bring up to the temperature of determination. Then add:

calibrator	-	-	20 µl
sample	-	20 µl	-
distilled water	20 µl	-	-

Mix well and after 2 minutes of incubation at adequate temperature (37ºC) read the absorbance, repeat the reading after next 1, 2 and 3 minutes.
Calculate the mean absorbance change per minute of tested sample (T) and calibrator (C):
ΔA/min (T) = [ΔA/min (T)] - [ΔA/min (B)]
ΔA/min (C) = [ΔA/min (C)] - [ΔA/min (B)]

Calculation

$$\text{amylase activity [U/l]} = \frac{\Delta A/\text{min (T)}}{\Delta A/\text{min (C)}} \times \text{calibrator concentration [U/l]}$$

Reagent Start method

Pipette into the cuvettes:

	blank (B)	test (T)	calibrator (C)
1-AMYLASE EPS	1000 µl	1000 µl	1000 µl

Bring up to the temperature of determination. Then add:

calibrator	-	-	30 µl
sample	-	30 µl	-
distilled water	30 µl	-	-

Mix well and after 1 min. of incubation add:

2-AMYLASE EPS	250 µl	250 µl	250 µl

Mix well and after 2 minutes of incubation at adequate temperature (37ºC) read the absorbance, repeat the reading after next 1, 2 and 3 minutes.

Calculate the mean absorbance change per minute of tested sample (T) and calibrator (C):

ΔA/min (T) = [ΔA/min (T)] - [ΔA/min (B)]

ΔA/min (C) = [ΔA/min (C)] - [ΔA/min (B)]

Calculation

$$\text{amylase activity [U/l]} = \frac{\Delta A/\text{min (T)}}{\Delta A/\text{min (C)}} \times \text{calibrator concentration [U/l]}$$

REFERENCE VALUES [5]

serum / plasma	28 – 100 U/l	0.47 – 1.7 µkat/l
urine	≤ 460 U/l	≤ 7.7 µkat/l

It is recommended for each laboratory to establish its own reference ranges for local population.

QUALITY CONTROL

For internal quality control it is recommended to use the CORMAY SERUM HN (Cat. No 5-172) and CORMAY SERUM HP (Cat. No 5-173) for determination in serum or CORMAY URINE CONTROL LEVEL 1 (Cat. No 5-161) and LEVEL 2 (Cat. No 5-162) for determination in urine with each batch of samples.

For the calibration of automatic analysers systems the CORMAY MULTICALIBRATOR LEVEL 1 (Cat. No 5-174; 5-176) or LEVEL 2 (Cat. No 5-175; 5-177) is recommended.

The calibration curve should be prepared every 12 weeks, with change of reagent lot number or as required e.g. quality control findings outside the specified range.

PERFORMANCE CHARACTERISTICS

These metrological characteristics have been obtained using the automatic analyser Biolis 24i Premium. Results may vary if a different instrument or a manual procedure is used.

- **Sensitivity:** 1.1 U/l (0.018 µkat/l).

- **Linearity:** up to 2000 U/l (33.3 µkat/l).
  If amylase activity exceeds 2000 U/l, dilute the sample with 0.9% NaCl 1:10 and repeat the assay. Multiply the result by the dilution factor.

- **Specificity / Interferences**
  Haemoglobin up to 0.156 g/dl, bilirubin up to 20 mg/dl, ascorbate up to 62 mg/l, triglycerides up to 1250 mg/dl and glucose up to 2000 mg/dl do not interfere with the test.

- **Precision**

Repeatability (run to run) n = 20	Mean [U/l]	SD [U/l]	CV [%]
level 1	71.9	0.76	1.05
level 2	384.2	1.58	0.41

Reproducibility (day to day) n = 80	Mean [U/l]	SD [U/l]	CV [%]
level 1	71.3	0.98	1.37
level 2	391.6	3.00	0.77

- **Method comparison**

A comparison between amylase values determined at Biolis 24i Premium (y) and at Advia 1650 (x) using 66 samples gave following results:

y = 1.0273 x - 2.8482 U/l;

R = 0.9999 (R – correlation coefficient)

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

1. Pesce, A.J., Kaplan, L.A.: "Methods in Clinical Chemistry", Mosby Ed. (1987).
2. Burtis C.A., Ashwood E.R.: "Tietz Textbook of Clinical Chemistry", W.B. Saunders Company Ed. (3_{rd} edition, 1999).
3. Lorentz K. Approved recommendation on IFCC methods for the measurement of catalytic concentration of enzymes. Part 9. IFCC method for alpha-amylase (1,4-alpha-D-glucan 4-glucanohydrolase, EC 3.2.1.1). International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). Committee on Enzymes. Clin Chem Lab Med. 1998 Mar;36(3):185-203.
4. Junge W, Wortmann W, Wilke B, Waldenström J, Kurrle-Weittenhiller A, Finke J, Klein G. Development and evaluation of assays for the determination of total and pancreatic amylase at 37 degrees C according to the principle recommended by the IFCC. Clin Biochem. 2001 May;34:607-15.
5. Alan H.B. Wu: Tietz Clinical Guide to Laboratory Tests, 4th ed. WB Saunders, 100-104, (2006).
6. Hohenwallner W, Hagele EO, Scholer A et al. Ber Oster Ges Klin Chem 1983;6:101-112.

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