DIALAB Produktion und Vertrieb von chemisch-technischen Produkten und Laborinstrumenten Gesellschaft m.b.H.
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Liquid Reagents – ready to use

alpha-Amylase

CNP-G3

Single Reagent

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

REF	Kit Size	Configuration
397756B	**1 x 1 L**	Single Reagent
397758	**5 x 100 mL**	Single Reagent
397759	**5 x 50 mL**	Single Reagent
300760	**5 x 25 mL**	Single Reagent
396754	**5 x 10 mL**	Single Reagent
355911	**10 x 50 mL**	Single Reagent
D0405917	**9 x 65 mL**	Single Reagent
3A0805	**5 x 20 mL**	Single Reagent
3T1005	**5 x 20 mL**	Single Reagent
3K0704	**5 x 50 mL**	Single Reagent
3E1805	**5 x 20 mL**	Single Reagent

Additionally offered:

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

TEST PARAMETERS

Method:	Colorimetric, Kinetic, Increasing Reaction, CNP-G3
Wavelength:	Hg 405 nm
Temperature:	37 °C
Sample:	Serum, Na- or Li-heparinized plasma
Linearity:	up to 2000 U/L (on Hitachi 911)

SUMMARY

For many years, the levels of serum α-amylase in patients have provided needed evidence for the diagnosis of acute pancreatitis [1-3]. Early assay techniques were based on either a change in the absorption maxima of the complex between starch and iodine as the α-amylase degraded the starch; or a measurement of the increase in reducing groups as the starch was hydrolysed by the α-amylase [4]. These methods are not as reliable and easy to quantitate as spectrophotometric methods using a defined substrate [5].
Some methods are based on the production of NADH proportionate to the activity of the α-amylase. A defined substrate, such as maltotetraose, is degraded by α-amylase to produce glucose which can be measured in a coupled enzyme assay. However, this method necessitates the removal of endogenous glucose which would give a high background to the assay [5].
More recent methods are based on the production of p-nitrophenol from defined oligosaccharide substrates with blocking groups attached on the terminal sugar. The action of the α-amylase on the oligosaccharide yields a variety of chain lengths after hydrolysis. These methods then use a variety of coupling enzymes to hydrolyze the resulting short chain oligosaccharides to produce p-nitrophenol [6]. The coupling enzymes contain residual α-amylase activity that may significantly reduce the stability of the reagent.

TEST PRINCIPLE

The direct amylase assay involves the use of a chromogenic substrate, 2-chloro-4-nitrophenol linked with maltotriose[7].

$$10\ \text{CNP-G}_3 \xrightarrow{\alpha\text{-Amylase}} 9\ \text{CNP} + 1\text{CNP-G}_2 + \text{G}_3 + \text{G}$$

As shown above, α-amylase hydrolyzes the 2-chloro-4-nitrophenyl-α-D-maltotrioside (CNP-G_3) to release 2-chloro-4-nitrophenol (CNP) and form 2-chloro-4-nitrophenyl-α-D-maltoside (CNPG_2), maltotriose G_3 and glucose (G). The rate of formation of the 2-chloro-4-nitrophenol can be detected spectrophotometrically at 405 nm to give a direct measurement of α-amylase activity in the sample. The reaction is not readily inhibited by endogenous factors.

REAGENT COMPOSITION

COMPONENTS	CONCENTRATION
MES buffer, pH 6.00	100 mmol/L
Sodium Chloride	350 mmol/L
Calcium Acetate	6 mmol/L
Potassium Thiocyanate	900 mmol/L
CNP-G3	2.27 mmol/L

REAGENT PREPARATION

The reagent is ready to use.

REAGENT STABILITY AND STORAGE

Conditions:	Protect from light Close immediately after use Avoid contamination Do not freeze the reagent
Storage:	at 2 – 8 °C
Stability:	up to the expiration date
After opening:	60 days*
On board stability (Hitachi 911):	2 weeks

* When properly capped immediately after each use and stored at 2 – 8 °C.

SAMPLE PREPARATION

Serum, sodium heparinized plasma or lithium heparinized plasma are the recommended sample types. Other anti-coagulants such as EDTA or citrate should not be used.
Refer to guidelines such as CLSI GP41-A6 Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture [8] and CLSI GP44-A4 Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests [9] for guidance.

SAMPLE STABILITY AND STORAGE [13]

in serum / plasma:	at 20 – 25 °C	7 days
	at 4 – 8 °C	7 days
	at -20 °C	1 year
	FREEZE ONLY ONCE!	

Discard contaminated specimens.

MATERIALS REQUIRED BUT NOT PROVIDED

NaCl solution (9 g/L)
General laboratory equipment

MANUAL TEST PROCEDURE

Bring reagents and samples to room temperature.

Pipette into test tubes	Blank	Sample
Reagent	1000 µl	1000 µl
dist. water or saline	25 µl	
Sample		25 µl
Mix, incubate for 1 min. at 37°C and read absorbance. Read absorbance again after exactly 1, 2 and 3 min. Determine ΔA/min during the linear part of the assay. Calculate: ΔA/min = [ΔA/min Sample] – [ΔA/min Blank]		

CALCULATION (light path 1 cm)

α-Amylase (U/L) = ΔA/min x Factor
Factor (37 °C) at 405 nm: 3178
The factor is based on the millimolar extinction coefficient of 2-chloro-4-nitrophenol at 405 nm, pH 6.0 and 37 °C: 12.9

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UNIT CONVERSION

U/L x 0.01667 = µkatal/L

REFERENCE RANGE [12] *

serum / plasma	20 – 104 U/L

* It is recommended that each laboratory establishes the normal range for its population.

PERFORMANCE CHARACTERISTICS

LINEARITY

The assay is linear up to 2000 U/L on the Hitachi 911 Analyzer. If a sample exceeds 2000 U/L, it should be diluted 1+1 with normal saline (9 g/L) and re-assayed. Multiply the result by 2.

PRECISION:

Intra-assay n = 20	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	61	0.81	1.34
Sample 2	272	1.66	0.61
Sample 3	902	4.60	0.51
Sample 4	1509	9.36	0.62

Inter-assay n = 40	Mean [U/L]	SD [U/L]	CV [%]
Sample 1	60	1.0	1.7
Sample 2	273	2.2	0.8
Sample 3	917	8.3	0.9
Sample 4	1507	9.0	0.6

SPECIFICITY/INTERFERENCES

no interference up to:

Ascorbic acid	50 mg/dL
Bilirubin (unconjugated)	50 mg/dL
Bilirubin (conjugated)	50 mg/dL
Hemoglobin	500 mg/dL
Triglycerides	3000 mg/dL
Glucose	2000 mg/dL

Samples with hemoglobin interference higher than the upper limit may be diluted 1 part sample with 1 part physiological saline. Multiply the result by two to correct for the dilution.
Macroamylase has been shown to cause hyperamylasemia which may lead to overdiagnosis of acute pancreatitis when using oligosaccharide substrates. [11]
Refer to Young et al [10] for a review of drug effects on amylase levels.

METHOD COMPARISON / ACCURACY

A comparison between Dialab α-Amylase (y) and a commercial obtainable assay (x) using 50 samples (28 – 304 U/L) gave following results:
y = 0.90 x – 2.50 U/L; r = 0.999.

QUALITY CONTROL

All control sera with Alpha Amylase values determined by this method can 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 an Alpha Amylase Calibrator is optional.
We recommend the Dialab multi calibration serum **Diacal Auto**.
This method is traceable to the molar extinction coefficient of 2-chloro-4-nitrophenol.

AUTOMATION

Special applications for automated analyzers can be made on request.

WARNINGS AND PRECAUTIONS

1. Reagent: Warning.
   H319: Causes serious eye irritation.
   P264: Wash hands thoroughly after handling.
   P280: Wear eye protection/face protection
   P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
   P337+P313: If eye irritation persists, get medical advice/attention.
2. The reagent contains Potassium Thiocyanate. Avoid inhalation or contact with skin and eyes. Wash skin or eyes with water and consult physician if contact occurs. Potassium Thiocyanate is not compatible with strong acids.
3. The reagent contains sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azide. On disposal, flush drain with a large volume of water to prevent build up.
4. Saliva and skin contain α-amylase. Therefore never pipette reagents by mouth and avoid skin contact with the reagents.
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!

WASTE MANAGEMENT

Please refer to local legal requirements.

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