Amylase – Instructions for use

3 x 50 mL • REF A-R0000000001

3 x 14 mL • REF R3330000007

Manufactured exclusively for:

ISE S.r.l.
CUSTOMISED SOLUTIONS FOR YOUR LABORATORY

IVD CE

INTENDED USE

Product for use in the quantitative determination in vitro of the Amylase activity in human serum. The results of the test must always be interpreted in conjunction with the clinical context. For professional use only.

CLINICAL SIGNIFICANCE

For many years, the levels of serum and plasma a-amylase in patients have provided needed evidence for the diagnosis of the 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 a-amylase degraded the starch; or a measurement of the increase in reducing groups as the starch was hydrolysed by the a-amylase (4). These methods are not as reliable and easy to quantitative 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 a-amylase to produce glucose which can be measured in a coupled enzyme assay. However, this method necessitates the removal of endogeneous glucose which would give a high background to the assay (5). More recent methods are based on the production of p-nitro-phenol from defined oligosaccharide substrates with blocking groups attached on the terminal sugar. The action of the a-amylase on the oligosaccharides 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-nitropheno (5). The coupling enzymes contain residual a-amylase activity that may significantly reduce the stability of the reagent.

PRINCIPLE

The α-Amylase assay involves the use of a chromogenic substrate, 2-chloro-4-nitrophenol linked with maltotriose (7). α-amylase hydrolyzes the 2-chloro-4-nitrophenyl-α-D-maltotrioside (CNPG3) to release 2-chloro-aminophenol (CNP) and form 2-chloro-4-nitrophenyl- α-D-maltoside (CNPG2), maltotriose (G3) 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 endogeneous factors

REAGENTS

A-R0000000001 - 3 x 50 mL
Reagent: n° 3 vials x 50,0 mL ready for use
R3330000007 - 3 x 14 mL
Reagent: n° 3 vials x 14,0 mL ready for use

Concentrations

Reagents:			
	Conc.	**U.M.**	
CNPG3	2,27	mM	
Sodium Chloride	300	mM	
Calcium acetate	5,00	mM	
Potassium sulphacyanide	750	mM	*GHS08
Sodium Azide	< 0,1	%	
MES pH 6.0 ± 0.2	80,0	mM	

*Signal word: **WARNING**
Contains: potassium thiocyanate (CAS 333-20-0)
H373 - May cause damage to organs through prolonged or repeated exposure.
P260 - Do not breathe dust/fume/gas/mist/vapours/spray.
P314 - Get medical advice/attention if you feel unwell.
P501 - Dispose of contents/container in accordance with local/regional/national/international regulations.
EUH032 - Contact with acids liberates very toxic gas

Precautions

In addition to risk indications related to the active components, reagents may contain inactive components such as preservatives and detergents. The total concentration of these components is lower than the limits reported in the EC 1272/2008 regulation and subsequent amendments and additions. However, It is recommended to handle reagents according to the standards of good laboratory practice.

Reports of serious incidents

Kit for professional laboratory use, used only by qualified and properly trained technical personnel, under the supervision of a doctor in charge of the laboratory. In addition to risk indications related to the active components, reagents may contain inactive components such as preservatives and detergents. The total concentration of these components is lower than the limits reported in the EC 1272/2008 Regulation and subsequent amendments and additions. However, it is recommended to handle reagents according to the standards of good laboratory practice

Storage and stability

Store at 2 - 8°C and protect from direct light. When correctly stored, the reagents are stable up the expiry date reported on the label. A slight variation in the composition of the reagent may occur between batches, but this has no effect on the test results. After opening, the vials are stable 30 days if recapped immediately and protected from contamination, evaporation, direct light and stored at correct temperature.

SAMPLE COLLECTION

Type of sample and storage

Serum or heparanized plasma are recommended sample types. Other anti-coagulants such as EDTA or citrate should not be used. Centrifuge and remove the serum as soon as possible after collection. If not analysed promptly, samples should be stored at 2-8°C. a-amylase is reported to be stable for up to one week at room temperature (20-25°C) and several months when capped and stored at 2 - 8°C.

Precaution

All human samples must be handled and disposed of as potentially infectious materials.

Procedure

Quality control

Control sera with a Known level of α-Amylase are commercially available for quality control, including certificates of analysis showing values and confidence limits. Normal and pathological control sera are available as "Normal control serum" cod. R0400000006 and "Pathological control serum" code R0400000106. The values obtained must be contained within the acceptability range. In case of incorrect results check the following points:

- Cleanliness of glassware.
- Wavelength setting.
- Expiration date of reagents.

Automation

Although this device has been developed and manufactured to be used by manual method and instrumental systems, it can also be used in combination with other instrumental devices that are able to meet the requirements indicated in the paragraph "Reaction conditions / Technique".
All applications that are not explicitly approved cannot be guaranteed in terms of performance and will therefore have to be evaluated by the user.

Calibration

For calibration use the "Multicalibrator" code R0300000006. For ISE srl instruments calibration is recommended every 10 days.

Traceability:

The α-amylase concentration is reported in the package insert supplied with the Calibrator Serum.

Reaction conditions

Wavelength (primary):	405 nm
Wavelength (secondary):	500 nm
Temperature:	37°C

Technique – Procedure with Serum as starter

Bring the reagent to the reaction temperature.

	U.M.	**Calibration Serum**	**Simple**
Reagent	µL	1000	1000
Calib.Serum	µL	25	-
CSimple	µL	-	25

Mix gently then incubate at reaction temperature for 60 seconds
Read the absorbances of the sample and calibration serum at 405 nm. Repeat readings every 30 seconds or every 60 seconds. At least 3 repetitions of reading in the chosen times are recommended. Determine the average between Δ D.O. /min. The reaction volumes can be varied proportionately, the calculation remaining unchanged.

Results

The concentration of α-Amylase is obtained through the following formula:

$$\frac{\Delta \text{ D.O. Simple}}{\Delta \text{ D.O. Calib.Serum}} \times \text{Conc. Calib.Serum (U/L)} = \text{U/L Amylase}$$

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Calculation of results obtained against multiplication factor
Δ D.O./min x K-factor* = U/L for Amylase

Explanation of the formula:

$$\frac{Vt \times 1000}{M.E.C. \times O.P. \times Vc} = K - factor * \times \Delta\ D.O./min. = U/L\ Amylase$$

*K-factor = 3178
dove:
U/L = Activities in international units per liter
Δ D.O./min. = Change in absorbance per minute
Vt = total reaction volume (µL)
1000 = conversion of concentration per litre
C.M.E. = . Extinguishing micromolar of 12,9 cm^2/µmol a 405 nm
P.O. = optical path (1.0 cm)
Vc = sample volume in the final reaction mixture (µL)

Materials included in the kit
The reagents are described above.

Materials required but not supplied in the kit
Calibrators and controls.

NORMAL VALUE
- Serum or Plasma: < 94.0 U/L
- Urine: < 515 U/L

Each laboratory should calculate its own normal values on the basis of its local population

ANALYTICAL CHARACTERISTICS / PERFORMANCE
Linearity
The α-amylase test reagent is linear to 1900 U/L. If a sample exceeds 1900 U/L it should be diluted with an equal volume of normal saline assayed. Multiply the values from the resulting calculation by 2 to correct for the dilution.

Specificity
This method is specific for Amylase. Any non specific reaction is terminated during the lag time before taking the reading.

Accuracy-Recovery
The recovery of pure Amylase added to normal sample at known titer was 99.6%.

Precision of the method

Within-run precision					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	U/L	45.8	2.77	6.06	20
High	U/L	302	6.88	2.28	20
Between-run precision					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	U/L	45.8	2.04	4.47	20
High	U/L	302	15.3	5.07	20

Sensitivity
At λ 405 nm a concentration of about 8.327 U/l in the conditions established for this test.

Comparative method
The I.S.E. S.r.l. method was compared to a method in use.
A linear regression slope Y = 1.16935 x – 1.99462 was determined. A Correlation coefficient r = 0.99829 was calculated.

Disposal of reagent
Disposal of reagents must be performed in accordance with the EC regulations regarding waste, or the local national or regional legislation.

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Symbols used in IFU and Packaging	
IVD In vitro diagnostic medical device vitro	Manufacturer
REF Catalogue Number	Instruction for use
LOT Lot Number	Temperature limitation
Expiration date	

References

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REVISION	DATE	CHANGE
Rev.C	01/2024	Edit header and "Reagents" paragraph.