AMYLASE IFCC

REF:

INTENDED FOR USE

Enzymatic in vitro test for the quantitative determination of α-amylase in human serum, plasma and urine.

Summary:

The α -amylases (1,4- α -D-glucanohydrolases, EC 3.2.1.1) catalyze the hydrolytic degradation of polymeric carbohydrates such as amylose, amylopectin and glyco-gen by cleaving 1,4- α -glucosidic bonds. In polysaccharides and oligosaccharides, several glycosidic bonds are hydrolyzed simultaneously. Maltotriose, the smallest such unit, is converted into maltose and glucose, albeit very slowly.

Two types of a-amylases can be distinguished, the pancreatic type (P-type) and the salivary type (S-type). Whereas the P-type can be attributed almost exclusively to the pancreas and is therefore organ-specific, the S-type can originate from a number of sites. As well as appearing in the salivary glands it can also be found in tears, sweat, human milk, amniotic fluid, the lungs, testes and the epithelium of the fallopian tube.

Because of the sparsity of specific clinical symptoms of pancreatic diseases, α-amylase determinations are of considerable importance in pancreatic diagnostics. They are mainly used in the diagnosis and monitoring of acute pancreatitis. Hyper-amylasemia does not only occur with acute pancreatitis or in the inflammatory phase of chronic pancreatitis, but also in renal failure (reduced glomerular filtration), tumors of the lungs or ovaries, pulmonary inflammation, diseases of the salivary gland, diabetic ketoacidosis, cerebral trauma, surgical interventions or in the case of macro amylasemia. To confirm pancreatic specificity, it is recommended that an additional pancreas specific enzyme - lipase or pancreatic-α-amylase- also be determined.

Numerous methods have been described for the determination of α-amylase. These either determine the decrease in the amount of substrate viscometrically, turbidi-metrically, nephelometrically and amyloclastically or measure the formation of de-gradation products saccharogenically or kinetically with the aid of enzyme-catalyzed subsequent reactions. The kinetic method described here is based on the well-proven cleavage of 4,6-ethylidene-(G7)-1 ,4-nitrophenyl (G1)-,D-maltoheptaoside (Ethylidene Protected Substrate = EPS) by α -amylase and subsequent hydrolysis of all the degradation products to p-nitrophenol with the aid of α -glucosidase (100% chromophore liberation). The results of this method correlate with those obtained by HPLC.

PRINCIPLE :

Enzymatic colorimetric assay according to the IFCC-method.

Defined oligosaccharides such as 4,6-ethylidene-(G_7) p-nitro phenyl-(G_1)-α D-maltoheptaoside (ethylidene-G_7PNP) are cleaved under the catalytic action of α-amylases. The G_2PNP, G_3PNP and G_4PNP fragments so formed are completely hydrolyzed to p-nitrophenol and glucose by α-glucosidase.

$$5\ \text{ethylidene-}G_7\text{PNP} + H_2O \xrightarrow{\alpha\text{-amylase}} 2\ \text{ethylidene-}G_5 + 2\ G_2\text{PNP} + 2\ \text{ethylidene-}G_4 + 2\ G_3\text{PNP} + \text{ethylidene-}G_3 + G_4\text{PNP}$$

$$2\ G_2\text{PNP} + 2\ G_3\text{PNP} + G_4\text{PNP} + 14\ H_2O \xrightarrow{\alpha\text{-glucosidase}} 5\ \text{PNP} + 14\text{G}$$

(PNP = p-nitrophenol; G = glucose)

The color intensity of the p-nitrophenol formed is directly proportional to the α-amylase activity and is measured photometrically.

REAGENTS COMPOSITON :

R1	Hepes* buffer, pH 7.15 NaCl MgCl2 CaCl2 α-Glucosidase mod Preservative	52.5 mmol/l 87 mmol/l 12.6 mmol/l 0.075 mmol/l > 8 KU/l
R2	Hepes* buffer, pH 7.15 4.6-ethylidene-G7PNP	52.5 mmol/l 22 mmol/l

*Hepes = 2[4-(2-hydroxyethyl)-1-piperazinyl]-ethanesulfonic acid

Preparation and stability:

Substrate start/Hitachi:

R1: Ready for use

R2: Ready for use

Stability: Unopened kit components: Up to the expiration date at 2-8°C

Onboard Stability: R1: 28 days
R2: 28 days

Sample start:

4 Parts of R1 are mixed with one part R2. The resulting working reagent is stable:
30 days at +2°C to +8°C
10 days at +20°C to +25°C

Specimen:

Collect serum using standard sampling tubes.

Heparinized or EDTA- plasma.

Stability: 7 days at 20-25¢XC
1 month at 2-8¢XC

Urine

Collect without additives.

Stability: 2 days at 20-25¢XC
10 days at 2-8¢XC

α-Amylase is unstable in acid urine. Assay promptly or adjust pH to alkaline range (about pH 7) before storage.

Centrifuge samples containing precipitate before performing the assay.

Notes:

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.

Limitations - interference:

A slight change in the yellow coloration of solution 2 does not interfere with the performance of the test.

Do not pipette by mouth, and ensure that the reagent does not come into contact with the skin. (Saliva and sweat contain α -amylase!)

Criterion: Recovery within „b10% of initial value.

Icterus: No significant interference up to an index I of 60
(approximate unconjugated bilirubin concentration: 60 mg/dl)

Hemolysis: No significant interference up to an index H of 500 (approximate haemoglobin concentration: 500 mg/d).

Lipemia (Intralipid): No significant interference up to an index L of 1000 (approximate triglycerides concentration: 2000 mg/dl). There is poor correlation between turbidity and triglycerides concentration

Highly turbid and grossly lipemic samples may cause Abs. flags. Glucose: No interference from glucose up to 2000 mg/dl. Approximately 10% lower recovery was found at glucose concentrations of 4500 mg/dl.

Ascorbic acid: No interference from ascorbic acid up to 100 mg/dl. Approximately 10% lower recovery was found at ascorbic acid concentrations of 880 mg/dl.

31 commonly used pharmaceuticals were tested in vitro. No interference with the assay was found.

Procedure:

Applications for automated systems are available on request.

Materials provided

• Working solutions as described above

Additional materials required

• Calibrators and controls as indicated below

• 0.9% NaCl

Manual Procedure for sample start:

Wavelength:	405 nm (400-420nm)
Temperature:	37°C
Cuvette:	1 cm
Zero adjustment:	air or distilled water

	Serum / plasma	**Urine**
Working solution	1000 µl	1000 µl
Sample	50 µl	25 µl

Mix, read initial absorbance and start stopwatch simultaneously. Read again after exactly 1, 2 and 3 minutes.

Calculation:

Serum:

ΔA/min x 3800 = activity (U/l)

Urine:

ΔA/min x 10357 = activity (U/l)

Measuring /reportable range:

Measuring range: 3-1500 U/l (0.05-25.00 μkat/l)

Determine samples having higher activities via the rerun function. On instruments without rerun function, manually dilute the samples with 0.9% NaCl or distilled/deionized water (e.g. 1 + 4). Multiply the result by the appropriate dilution factor (e.g. factor 5).

Expected values:

	U/l	µkat/l
Serum/plasma	28 - 100	0.47 - 1.67
Spontaneously voided urine	≤ 460	≤ 7.67
α -amylase/Creatinin Quotient	≤ 310 U/g	≤ 5.17 µkat/g

EDTA plasma values are approximately 8% lower than serum values.
Note: The results (U/l or µkat/l) can be approximated to the reference range for α - amylase EPS by multiplying by the factor 2.2. This conversion factor only applies to human samples, not control sera.

α -Amylase/ creatinine quotient

To allow for fluctuations in the α-amylase activity in urine, it is advisable to determine the α-amylase/creatinine quotient. To do this, determine the α-amylase activity and creatinine concentration in spontaneously voided urine.

$$\text{Quotient [U/g] or µkat/mmol} = \frac{\text{α - amylase (U/l or µkat/l)}}{\text{creatinine [g/l or mmol/l]}}$$

Amylase/Creatinine Clearance Ratio (ACCR)

The ACCR is calculated from amylase activity and creatinine concentration. Both the serum and urine samples should be collected at the same time.

$$\text{ACCR [\%]} = \frac{\text{Urine amylase [U/l] x serum creatinine [mg/l]}}{\text{Serum amylase [U/l] x urine creatinine [mg/l]}} \times 100$$

ACCR approximately equal to 2-5%

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range. For diagnostic purposes the α -amylase results should always be assessed in conjunction with the patient¡¦s medical history, clinical examinations and other findings.

Analytical sensitivity (lower detection limit)

Detection limit: 3 U/l or 0.05 µkat/l

The lower detection limit represents the lowest α -amylase activity that can be distinguished from zero

Method comparison:

A comparison of the BIOMED AMYL-IFCC (y) with a commercial obtainable assay (x) gave the following result: y = 1.058 + 3.482 r = 1.000

Imprecision:

Reproducibility was determined using human samples and controls in an internal protocol (n = 20). The following results were obtained:

	Within run			Between day		
Probe	MW U/l	SD U/l	VK %	MW U/l	SD U/l	VK %
Probe 1	79.4	1.20	1.51	79.7	0.91	1.15
Probe 2	182	3.39	1.86	186	1.53	0.82
Probe 3	198	3.10	1.56	203	1.56	0.77

Quality Control:

Human Control Serum

The control intervals and limits must be adapted to the individual laboratory and country-specific requirements. Values obtained should fall within established limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

Calibration:

S1: 0.9% NaCl

Calibration frequency:

Two point calibration is recommended:

• after lot change

• as required following quality control procedures

Calibration verification: Not necessary

Disposal:

Please note the legal regulations.

Literature:

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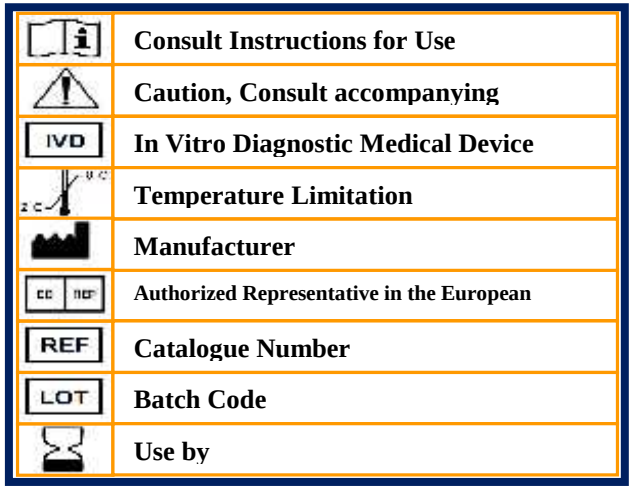

EGY- CHEM for lab technology
Badr City, Industrial Area Piece 170
250 Fadan In East of Elrubaki, EGYPT
Office Tel: +202 26236727 / +202 26236598
Factory Tel: +202 23108170 / +202 23108171
Fax: +202 26240986
www.egy-chem.com

EC REP
MDSS GmbH
Schiffgraben 41
30175 Hannover,
Germany