α-AMYLASE

CNPG3

Intended use:
Enzymatic in vitro test for the quantitative determination of a-amylase in human serum, plasma and urine.

Summary:
The a-amylases (1,4-a-D-glucanohydrolases, EC 3.2.1.1) catalyze the hydrolytic degradation of polymeric carbohydrates such as amylose, amylopectin and glycol- gen by cleaving 1,4-a-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, a-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, however, only occur with acute pancreatitis or in the inflam- matory 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 macroamylasemia. To confirm pancreatic specificity, it is recommended that an additional pancreas-specific enzyme - lipase or pancreatic-a-amylase- also be determined.
Numerous methods have been described for the determination of a-amylase. These either determine the decrease in the amount of substrate viscometrically, turbidimetrically, nephelometrically and amyloclastically or measure the formation of degradation products saccharogenically or kinetically with the aid of enzyme- catalyzed subsequent reactions. The kinetic method described here is based on the cleavage of 2-chloro-4-nitrophenyl-aD-maltotrioside (CNP-G3) by a-amylase.

Test principle:
Colorimetric test with 2-chloro-4-nitrophenyl-a-D-maltotriose (CNP-G3) as direct substrate. Colour is released directly as a result of a cleavage at the a-glycone:

$$\text{CNP - G3} \xrightarrow{\text{a-amylase}} \text{CNP + G3}$$

(CNP = chloro-nitrophenol; G = glucose)

The increase of absorption of chloro-nitrophenol is directly proportional to the a- amylase concentration. The hydrolysis pattern in the formulation of the reagent show about less than 10 % CNP-G2 and less than 1% CNP-G4 as by products.

Reagent concentration:
R1:

MES buffer, pH 6.0	100 mmol/l
NaCl	350 mmol/l
Ca-Acetate	6 mmol/l
Potassium thiocyanate	900 mmol/l
CNP-G3	2.27 mmol/l
Stabilizers and detergents	> 0.1 %

Preparation and stability:
R1: Ready for use
The reagent is stable:

Unopened	up to expiry date	at +2°C to +8°C
Opened:	14 days	at +2°C to +8°C

Specimen:
Serum/plasma
Collect serum using standard sampling tubes. Heparinized or EDTA- plasma.

Stability:	7 days	at +20°C -25°C
	1 month	at +2°C - 8°C

Urine
Collect without additives.

Stability:	2 days	at +20°C - 25°C
	10 days	at +2°C - +8°C

a-Amylase is unstable in acid urine. Assay promptly or adjust pH to alkaline range (about pH 7) before storage.
Limitations - interference:
Do not pipette by mouth, and ensure that the reagent does not come into contact with the skin. **(Saliva and sweat contain a-amylase!)**
Criterion: Recovery within ±10% of initial value.
Icterus: No significant interference up to 70 mg/dl bilirubin.
Hemolysis: No significant interference up to 170 mg/dl hemoglobin.
Lipemia (Intralipid): No significant interference up to 2600 mg/dl triglycerides. An increase of the initial absorbance of the reagent to A > 0.3 (405 nm) indicates a contamination of the reagent.

Testing 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:

Wavelength:	Hg 405 nm (400-420 nm)	
Temperature:	+37°C	
Cuvette:	1 cm light path	
Zero adjustment:	against water	
	Serum/plasma	Urine
R1	1000 µl	1000 µl
Serum/plasma	25 µl	---
Urine	---	125 µl

Mix and incubate 1 min at +37°C. Then read initial absorbance and start stopwatch simultaneously. Read again after exactly 1, 2 and 3 minutes. Determine the mean change of absorbance per minute (AA/min) and use this for the calculation.

Calculation:
Use absorption differences to calculate AA/min. Multiply with the following factors:

	Serum/plasma	Urine
Activity; +37°C (U/l)	3178 x A/min.	6356 x A/min

Measuring /reportable range:
Measuring range: Up to 1500 U/l
Dilute samples having higher activities with 0.9% NaCl (e.g. 1 + 9). Multiply the result by the appropriate dilution factor (e.g. factor 10).

Expected values:

	+37°C
Serum/plasma	< 96 U/l
Spontaneously voided urine	< 960 U/l
24 h urine	< 930 U/l

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 a-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: 5 U/l
Imprecision:
Reproducibility within run was determined using human samples and controls (n = 20). The following results were obtained:

Serum	**Within run**		
Sample	Mean (U/l)	SD (U/l)	CV %
Sample 1	187	1.48	0.79
Sample 2	446	2.10	0.47
Sample 3	507	2.64	0.52

Reproducibility was determined using human samples and controls between day (n = 20). The following results were obtained:#

Serum	**Between day**		
Sample	Mean (U/l)	SD (U/l)	CV %
Sample 1	196	2.83	1.44
Sample 2	474	6.25	1.32
Sample 3	542	6.73	1.24

Method comparison:
A comparison of the BIOANALYTIC AMYLASE (y) with a commercial obtainable assay (x) gave with 36 samples the following result:
y = 0.972x + 1.282; r = 0.999

α-AMYLASE

CNPG3

Quality Control:

Control Serum:

BIOCON N	5 x 5 mL	#B10814
BIOCON P	5 x 5 mL	#B10817

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

S2: BIOCAL H	5 x 3 mL	#B11895

Calibration frequency:

It is suggested to use Calibrator products produced by Bioanaliytic. It is suggested to use supplementary calibrator (pure water) to conduct 2-point calibration. The calibration curve is formed automatically. When lot number is changed or QC is invalid, calibration shall be conducted again. Recalibrate the assay every 14 days under ideal conditions,
or when the following occur:
Change in reagent lot or significant shift in control values;
Major preventative maintenance was performed on the analyser or a critical part was replaced(Halogen Lamp)

SYMBOLS

IVD	for in vitro diagnostic use only
LOT	lot of manufacturing
REF	code number
	storage at temperature interval
	expiration date (year/month)
	warning, read enclosed documents
	Read the directions

Literature:

1. Bertholf RL, Winn-Deen ES, Bruns DE. Amylase in urine as measured by a single -step chromolytic method. Clin Chem 1988; 34: 754-7
2. Fenton J, Foery R, Piatt L, Geschwindt K. A new chromogenic amylase method compared with two established methods. Clin Chem 1982; 28: 704-6.
3. Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-474
4. Greiling H, Gressner AM ed. Lehrbuch der Klinischen Chemie und Pathobiochemie, 3. Auflage. Stuttgart/New York: Schattauer Verlag, 1995.
5. Keller H ed. Klinisch- chemische Labordiagnostik für die Praxis, 2. Auflage. Stuttgart/New York: Georg Thieme Verlag, 1991 ;354-361.
6. Lorentz K. Approved Recommendation on IFCC Methods for the Measurement of Catalytic Concentration of Enzymes. Part 9. IFCC-Method for a-Amylase (1,4-a-D-Gluca 4-Glucano- Hydrolase, EC 3.2.1.1.). Clin Chem Lab Med 1998;38:195-203
7. Rauscher E et al. Fresenius Z Analyt Chem 1986; 324:304.
8. Salt WB II, Schenker S. Amylase-its clinical significance: a review of the literature [Review]. Medicine 1976; 55:269-281 .
9. Steinberg WM, Goldstein SS, Davies ND et al. Diagnostic assays in acute pancreatitis [Review]. Ann Intern Med 1985; 102:576 - 580.
10. Tietz NW ed.: Clinical Guide to Laboratory Tests. 3. Auflage. Philadelphia, PA: W B Saunders Company; 1995:46-51

Tietz NW, Huang WY, Rauh DF et al. Laboratory tests in the differential diagnosis of hyperamylasemia. Clin Chem 1986;32:301-307.

Order information (Cat No.) :

CC335	BAMY500	B25025	B28025	B31025	B34025	B80025
CR335	B21025	B25026	B28026	B31026	B34026	B80026
OL335	B21026	B25027	B28027	B32025	B35025	B80027
SH335	B21027	B25028	B28028	B32026	B35026	B80028
KL335	B21028	B27025	B30025	B33025	B36025	
AB335	B22025	B27026	B30026	B33026	B36026	
BAMY125	B24025	B27027	B30027	B33027	B37025	
BAMY250	B24026	B27028	B30028	B33028	B37026	

Manufacturer

Diaclinica Diagnostik Kimya.San.Tic.Ltd.Şti
Adress : İkitelli O.S.B Mutsan San.Sit. M4 Blok No:17-19 Başakşehir/İSTANBUL
Tel:+90(212) 549 33 88- Fax:+90 (212) 549 55 50
Web :www.diaclinica.com

ISO 9001:2015
ISO 13485:2016