α-AMYLASE Assay Kit

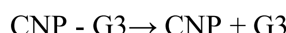
Cat.No: OttoBC125

Summary & Explanation

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, aamylase 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 pancreasspecific 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-aDmaltotrioside (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 aglycone:



CNP - G3→ CNP + G3

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.

Kit Companents

Content	Explanation	Shelf life
Reagent-1	1x30ml	6 months
Calibrator	1x0.5ml	6 months
Q.Control	1x0.5ml	6 months

Storage & Stability

Reagent: Stable up to expiry date when stored capped and at +4°C even after start using

Calibration and Quality Control: Reconstitute the contents of with 0.5 ml of redistilled water . Stable for 2 days when stored at +4°C

Reactivity

Universal

Specimen

Serum, Plasma (Edta or Heparin),Urine
Stability: +4°C 2 days, -80°C 6 months

Assay Range

5 U/l – 1500 U/l

Refference Range

Each laboratory is recommended to establish their own reference values.

Analytical Performance

Inter Assay Coefficent of Variation (CV) % 2.2
Intra Assay Coefficent of Variation (CV) % 2.3

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Procedure

Wavelength	405nm (±10nm)
Sample or Standard	5µl
Reagent-1	250µl
Zero Adjustment	Reagent blank

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:

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

Warning

For in vitro use only
Do not pipette by mouth
Do not use reagents beyond the expiry date.

References

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