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α-AMYLASE - Total (4+1)

Diagnostic reagent for the in-vitro quantitative determination of Alpha Amylase in human serum and heparinized plasma on both automated and manual system.

REF: BS.1/AM05.010.0050	50 test	REF: BS.1/AM02.025.0050	50 test
REF: BS.1/AM05.020.0100	100 test	REF: BS.1/AM02.050.0100	100 test

CLINICAL SIGNIFICANCE [1,2]

Amylases are enzymes that break starch down to sugar molecules and is found primarily in the pancreas and salivary glands. Alpha-amylase is the major form of amylase found in humans. When released in the digestive tract, the enzyme hydrolyzes starch. Amylase determinations are useful in the diagnosis of diseases of the pancreas and parotids. Elevated serum levels are associated with acute pancreatitis and other pancreatic disorders as well as mumps and bacterial parotitis. Alpha-amylase is a calcium metalloenzyme, completely unable to function in the absence of calcium. In human physiology, both the salivary and pancreatic amylases are major digestive enzymes. Increased enzyme levels in humans are associated with salivary trauma; mumps due to inflammation of the salivary glands, pancreatitis and renal failure.

METHOD PRINCIPLE [3]

The present method is based on the use of a chromogenic substrate, 2- chloro-p-nitrophenol linked with maltotriose. The reaction of amylase with this substrate results in the formation of 2-chloro-p-nitrophenol, that can be measured spectrophotometrically at 405nm. This reaction proceeds very rapidly, no coupling enzymes are required, and the reaction is not readily inhibited by endogenous factors.

$$10\ \text{CNPG3} \xrightarrow{\alpha\text{-amylase}} 9\ \text{CNP} + \text{CNPG2} + 9\text{G3} + \text{G}$$

REAGENT COMPOSITION

Reagents:	Composition	
R1: Buffer Reagent		
- Goods Buffer pH 6.0	- 50	mmol/L
- Sodium chloride	- 300	mmol/L
- Calcium chloride	- 5	mmol/L
- EDTA	- 0.2	mmol/L
R2: Substrate		
- Goods Buffer pH 6.0	- 50	mmol/L
- Potassium thiocyanate	- 140	mmol/L
- GALG2-CNP	- 10.6	mmol/L

PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person. Reagent (R2) contains Potassium thiocyanate which is classified as dangerous substance (R22: harmful if swallowed).

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.
S57: use appropriate container to avoid environmental contamination.
S61: avoid release in environment.

For further information, refer to the Amylase reagent material safety data sheet.

REAGENT PREPARATION, STORAGE AND STABILITY

BioScien Amylase reagent working solution is prepared as by mixing 4 volumes of buffer (R1) and 1volume of substrate (R2), e.g. 400 µl R1 + 100 µl R2. After reconstitution working reagent is stable for 3 months at 2 – 8 ºC or 5 days at 15 - 25 ºC. All reagents are stable until expiration date stated on label when properly stored in an upright position and refrigerated at 2-8°C (do not freeze).

Deterioration
The ***BioScien*** Amylase reagent considered damaged if it is turbid, displays evidence of bacterial contamination or in case of reagent absorbance greater than 0.600. It is recommended that such reagent should be discarded.

SPECIMEN COLLECTION AND PRESERVATION [4,5]

Clean and dry glassware free from detergents must be used for sample collection. Use serum or Heparinized plasma or urine, unhemolyzed serum is the specimen of choice. Anticoagulants, such as Citrate and EDTA, bind calcium that is needed for amylase activity so plasma with these anticoagulants should not be used.

Stability in the specimen:

- One week 2-8°C
- One months at -20°C

SYSTEM PARAMETERS

Wavelength	405 nm (492 – 550 nm)
Optical path	1 cm
Assay type	Kinetic or Fixed Rate
Direction	Increase
Sample Reagent Ratio	1:40
e.g: Reagent volume	1 ml
Sample volume	25 µl
Temperature	37 °C
Pre-incubation time	1 min. at 37°C
Zero adjustment	Against Air
Sensitivity	2 U/L
Linearity	1500 U/L

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile Syringe
- Analytical tubes and automatic pipet
- Centrifuge and spectrophotometer

ASSAY PROCEDURE

Allow the reagents and samples to reach room temperature.

	Assay
Reagent R1: Buffer Reagent	800 µl
Reagent R2: Substrate	200 µl
Mix well and incubate for 1 minute at 37˚C.	
Specimen	25 µl

Kinetic Procedure
Read initial absorbance after 60 seconds. and start timer simultaneously. Read again after 1, 2 and 3 minutes. Determine the mean absorbance change per minute (ΔA/min).

Fixed Rate Procedure
Read the absorbance A1 after 1 minute then after 4 minutes read the absorbance A2.

CALCULATION

Kinetic Procedure
Alpha amylase (U/L) = ΔA/min x 3060

Fixed Rate Procedure
ΔA= A2-A1
Alpha amylase (U/L) = ΔA/min x 765.

QUALITY CONTROL

To ensure adequate quality control, it is recommended that normal and abnormal commercial control serum of known concentrations included in each run. If control values are found outside the defined range, check the instrument calibration, and reagent for problems. If control still out of range please contact ***Bioscien*** technical support.

PERFORMANCE CHARACTERISTICS

Precision	Within run (Repeatability)		Run to run (Reproducibility)	
	Normal level	High level	Normal level	High level
n	20	20	20	20
Mean U/L	70.4	183	70.4	183
SD.	0.186	0.219	0.181	0.234
CV. %	0.021	0.011	0.022	0.012

The results of the performance characteristics depend on the analyzer used.

Accuracy (Methods Comparison)
Result obtained from ***Bioscien*** Amylase reagent compared with commercial reagent of the same methodology performed on 20 human sera give a correlation of 0.98.

Sensitivity
When run as recommended, the minimum detection limit of the assay is 2 U/L.

Linearity
The reaction is linear up to Amylase concentration of 1500U/L.

INTERFERING SUBSTANCES [6,7]

A number of substances each been found to have a negligible effect on this procedure up to certain level.

Bilirubin conjugated	20 mg/dL
Bilirubin free	20 mg/dL
Hemoglobin	500 mg/dL
NaF	500 mg/dL
Ascorbic acid	500 mg/dL
Glucose	5 g/dL
Maltose	5 g/dL

EXPECTED VALUES [8]

	25˚C	30˚C	37˚C
Serum/plasma	up to 55 U/l	up to 73 U/l	up to 100 U/l
Random Urine	up to 273 U/l	up to 365 U/l	up to 450 U/l
24 hrs Urine	up to 205 U/24h	up to 295 U/24h	up to 410 U/24h

DYNAMIC RANGE

2 - 1500 U/L.

REFERENCES

1. Pierre, K.J., et al, Clin. Chem. 22:1219 (1976).
2. Kaufman, R.A., Tietz, N.W., Clin. Chem. 26/7:851 (1980).
3. E.S. Winn-Deen et al., Development of a direct assay for α-amylase, Clin. Chem. 34 (1988) p2005-2008.
4. Tietz, N.W. Textbook of Clinical Chemistry, Philadelphia, W.B. Saunders Company, pp. 725-734 (1986).
5. NCCLS document “Procedures for the Collection of Diagnostic Blood Specimens by Skin Puncture”, 3rd Ed. (1991).
6. Young, D.S., et al, Clin. Chem. 21:1D (1975).
7. Young, D.S. Effects of Drugs on Clinical Laboratory Tests, 4th Ed., (1995) p.3-43 to 3-47.
8. Tietz, N.W., Clinical Guide to Laboratory Tests, Philadelphia, W.B. Saunders Company, p. 54 (1983).

SYMBOLS IN PRODUCT LABELLING

IVD	For in-vitro diagnostic use	Σ	Number of <n> test in the pack
LOT	Batch Code/Lot number		Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		

EC REP

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