TRUEchemie Alpha Amylase Test Kit (CNPG3)

for the quantitative determination of Amylase concentration in serum or plasma

INTENDED USE

The TRUEchemie Alpha Amylase Test Kit (CNPG3) is used for the quantitative determination of Amylase concentration in human serum or plasma.

PRINCIPLE

α- Amylase is secreted from the salivary glands and exocrine pancreas. α - Amylase catalyses the hydrolysis of α -1-4-glucosidic linkages of starch and other related polysaccharides to produce maltose and other oligosaccharides. The enzyme is relatively a small molecule, which is rapidly cleared by the kidney and excreted in the urine. α - Amylase is most frequently measured in the diagnosis of acute pancreatitis when serum levels may be grossly elevated and therefore is a useful diagnostic tool in critical care management. In acute pancreatitis α- Amylase levels reach a peak at 24 hours and remain elevated from 3-7 days. Hyperamylasemia is also associated with other acute abdominal disorders. Biliary tract diseases, diabetic ketoacidosis, severe glomerular dysfunction, salivary gland disorders, and ruptured ectopic pregnancy.

INTRODUCTION

The direct amylase assay involves the use of a chromogenic substrate CNPG3 (2-chloro-4-nitrophenyl linked with Galactomaltoside) which acts upon α - Amylase to release more than 95% of 2-chloro-4-nitrophenol (CNP), and form 2-chloro-4-nitrophenyl-α-D-maltoside (CNPG2), maltotriose (G3) and Glucose (G). The rate of formation of 2-chloro-4-nitrophenol is proportional to the α - Amylase activity in the sample, which can be monitored by kinetic assay at 405 nm.

$$10\ \text{CNPG3} \xrightarrow{\text{α- Amylase}} 9\text{CNP} + \text{CNPG2} + 9\text{G3} + \text{G}$$

PACK SIZE

Kit Size	2 x 10 ml
Cat. No.	ADX261
Kit contents	
1) Alpha Amylase Reagent	2 x 10 ml

REAGENT COMPOSITION

α- Amylase Reagent

MES pH 6.50	: 52 mmol/l
Sodium Chloride	: 87 mmol/l
Magnesium Chloride	: 12.6 mmol/l
Calcium Chloride	: 0.075 mmol/l
CNPG3	: 22 mmol/l
Preservatives	

REAGENT PREPARATION

Ready to use reagents.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use.
2. Handle in accordance with good laboratory procedures. Avoid ingestion and eye or skin contact.
3. Specimens should be considered infectious and handled appropriately.
4. The reagent contains sodium hydroxide that is corrosive. In case of contact with skin, flush with water. For eyes, seek medical attention.
5. The disposal of the residues has to be done as per local legal regulations.

REAGENT STORAGE & STABILITY

The unopened reagents are stable till the expiry date stated on the bottle and kit label when stored at 2-8°C. Do not use reagents over the expiration date.

SPECIMEN COLLECTION AND STORAGE

Unhemolysed serum or plasma is the specimen of choice.
EDTA, Oxalate or Citrate inhibit amylase activity hence cannot be used.
Amylase in serum is reported to be stable for one week at room temperature and for 2 months when stored refrigerated at 2-8 °C.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Pipettes to accurately measure required volumes.
2. Test tubes/rack
3. Timer
4. 37 °C heating block or water bath
5. Photometer capable of accurately measuring absorbance at 405 nm

TEST PROCEDURE

Primary wavelength	405 nm
Temperature	37 °C

Prewarm the reagent to reaction temperature.

	Blank (µL)	Sample (µL)
Distilled water	1000	--
Alpha Amylase Reagent	--	1000
Sample	--	25

Blank the Photometer with D.I Water.
Mix, read the absorbance after 30 Sec. and start the stopwatch. Read again the absorbance after every 30 Sec upto 90 Sec.

Calculations: ΔE = Initial absorbance - absorbance after every 30 Sec upto 90 Sec
(Avg ΔE/min) x 4640 = Amylase U/L

QUALITY CONTROL

Quality Controls are recommended to monitor the performance of automated assay procedures. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

EXPECTED VALUE

Normal values Serum, plasma: 25 - 140 U/L
It is strongly recommended that each laboratory establish its own normal range.

PERFORMANCE CHARACTERISTICS

Sensitivity: 10 U/L
Linearity: 2000 U/L under the described assay conditions. If the amylase activity is above 2000 U/L dilute the specimen suitably with normal saline. In such case the results obtained should be multiplied by dilution factor to obtain correct amylase activity. The linearity limit depends on the sample / reagent ratio, as well as the analyzers used.

PRECISION:

Intra-assay precision within run (n=10)	Mean (U/L)	SD (U/L)	CV (%)
Control Level - 1	74.5	0.3	0.5
Control Level - 2	505.4	0.7	0.1

Inter-assay precision run to run (n=12)	Mean (U/L)	SD (U/L)	CV (%)
Control Level - 1	72.6	0.4	0.6
Control Level - 2	509.7	1.2	0.2

The reagent was tested for 12 days, using two different Alpha Amylase concentrations. The coefficient of variation was <5%.

AUTOMATED PROCEDURE

Appropriate program sheet is available for different analyzers upon request.

METHOD COMPARISON

Results obtained using TRUEchemie Alpha Amylase reagent (y) did not show systematic differences when compared with another commercial reagent (x) with similar characteristics. The results obtained is below: The correlation coefficient (r^2) was 0.99 and the regression equation is y=0.993x+0.594. The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Bilirubin (mixed isomers)	: Less than 10% interference up to 600 µmol.
Haemolysis	: Less than 10% interference up to 5 g
Lipemia	: Less than 10% interference up to 5 g

WASTE MANAGEMENT

Please refer to local regulation requirements.

SYSTEM PARAMETERS

Mode	:	Kinetic
Factor	:	4640
Wave length	:	405 nm
Units	:	U/L
Flow cell Temp.	:	37 °C
Blank	:	Distilled Water
Reagent volume	:	1000 µL
Sample volume	:	25 µL
Lag time	:	30 Sec.
Read time	:	90 Sec.
Low Normal	:	25
High Normal	:	140
Sensitivity	:	10
Linearity	:	2000
Reaction Slope	:	Increasing

REFERENCES

1. Greiling H, Gressner AM, eds. Lehrbuch des Klinischen Chemie und Pathobiochemie, 3rd ed. Stuttgart/New York: Schattauer Verlag, 1995.
2. Burtis CA., Ashwood ER. Tietz Fund. Of Clin. Chem. 5th ed.; 30-54, 372-378 and 964.
3. Tietz NW, ed. Clinical Guide to Laboratory Tests, 3rd ed.Philadelphia, PA:WB Saunders,1995; 46-51.
4. Hohenwallner W, Hagele EO, Scholer A et al. Ber Oster Ges Klin Chem 1983; 6.101-112.
5. Junge W, Waldenstrom J, Bouman A et al. Evaluation of the Assays for Total and Pancreatic O-Amylase based on 100% Cleavage of ET-G7-PNP at 6 European Clinical Centres (Poster Medlab 97). Basel, Switzerland; 12 th IFCC European Congress of Clinical Chemistry, August 17-22, 1997.
6. ISO 15223-1:2021 Medical devices — Symbols to be used with information to be supplied by the manufacturer — Part 1: General requirements

Index of Symbols

Consult instructions for use	REF Catalogue number	Caution
IVD *In vitro* diagnostic medical device	LOT Batch code	Non-sterile
2°C 8°C Temperature limit 2-8 °C	Do not re-use	Use-by date
Manufacturer	Date of manufacture	Keep dry
Do not use if package is damaged	Keep away from sunlight	

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