



# **α-AMYLASE**

## **(CNP-G3 Method)**

### **Liquid Reagent**

#### **INTENDED USE:**

This reagent kit is used for *in-vitro* quantitative determination of α-Amylase in human serum.

#### **TEST PRINCIPLE:**

Defined oligosaccharides such as 2-chloro-4-nitrophenyl— α-D Maltotriose are elevated under the catalytic action of α-amylases. The release of the chromogen CNP is then measured.

The intensity of chromogen CNP formed is directly proportional to the α-amylase activity and is measured kinetically at 405nm.

#### **REACTION :**

2-chloro-4-nitrophenyl—α -D Maltotriose  $\xrightarrow{\alpha\text{-amylase}}$   
chloro-nitro phenol (CNP) + CNP-G2+9G3 + G.

#### **KIT CONTENTS:**

**Reagent 1 :** Amylase Enzyme Reagent

**Product Insert:** 01 No.

#### **PREPARATION OF THE WORKING REAGENT :**

α-Amylase Liquid reagents are ready to use.

#### **STORAGE AND STABILITY:**



The reagent should be stored in 2-8°C and are stable till the expiry date mentioned in the labels. Do not freeze the reagent.

#### **SPECIMEN COLLECTION AND STORAGE:**

Unhemolysed serum is the specimen of choice. E.D.T.A., oxalate or citrate inhibit Amylase activity hence cannot be used.

Amylase in serum is reported to stable for one week at room temperature and for two months when stored at 2-8°C.

#### **PRECAUTIONS:**

1. For *in-vitro* diagnostics use only.
2. Do not inhale the reagent or pipette by mouth.
3. Do not freeze or expose the reagents to higher temperature as it may affect the performance of the kit.
4. Before the assay bring all the reagents to room temperature.
5. Avoid contamination of the reagent during assay process.
6. Use clean glassware free from dust or debris.
7. Do not use the reagent if the reagent is hazy or cloudy.

#### **PROCEDURE (Automated):**

Refer to specific instrument application instructions.

#### **TEST PROCEDURE (Manual):**

Pipette into test tubes	Test
Amylase Enzyme Reagent	1.0 ml
Sample	25 µl

Mix thoroughly and transfer the assay mixture immediately to the thermo stated cuvette and record the first absorbance

reading after 60 seconds and subsequently two more absorbance readings with 60 seconds interval at 405 nm.

#### **CALCULATIONS:**

Calculate the average change in absorbance per minute (ΔAbs/minute)

Activity of α-Amylase in IU/l = ΔAbs/minute X 3178

#### **NORMAL VALUES\*:**

Serum: 25 - 140 IU/l

\*It is recommended that each laboratory should establish its own normal range.

#### **PERFORMANCE:**

1. **Linearity:** 2000 IU/l

2. **Comparison:** r = 0.98

$$y = 0.97x + 4.4$$

3. **Precision:**

	Within Run			Run to Run		
	Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
<b>Low</b>	9.8	0.6	0.9	11.0	0.9	2.5
<b>High</b>	1450.0	6.9	0.8	1490.0	15.4	1.2

4. **Specificity:** No interference with 1 gm/l Haemoglobin & Bilirubin 30 mg/dl.

#### **CLINICAL SIGNIFICANCE:**

The α-amylases (1,4-α-D-Glucan-4-glucanohydrolase, EC 3.2.1.1) catalyze the hydrolytic degradation of polymeric carbohydrates such as amylose, amylopectin and glycogen 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. Because of the sparsely 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. Hyperamylasemia does not, however, only occur with acute pancreatitis or in the inflammatory phase of chronic pancreatitis, but also in renal failure (reduced glomerular filtration), tumours of the lungs or ovaries, pulmonary inflammation, diseases of the salivary gland, diabetic ketoacidosis, cerebral trauma, surgical interventions or in the case of macroamylasemia.

#### **AUTOMATED APPLICATIONS:**

α-Amylase Liquid Reagents can be used with Hitachi 700 series, RA50, 1000 XT, Express 550, Synchron CX4, LISA 200, BTR 810/820/830, Erbachem-5, Ranlab etc. Application sheets for use on specific semiautomatic / batch analysers are available on request.

Input parameters for semiauto / auto analyzers are given below:

INPUT PARAMETERS	VALUES
Type of reaction	Kinetic
Slope of reaction	Increasing
Wavelength	405 nm
Factor	3178
Incubation time	60 sec.
Interval time	60 sec.
Interval No.	2
Units	IU/l
Temperature	37°C
Upper Normal value	140 IU/l
Lower Normal value	25 IU/l
Linearity	2000 IU/l
Enzyme Reagent volume	1.0 ml
Sample volume	25 µl

#### QUALITY CONTROL:

For accuracy, it is necessary to run known serum controls with each assay.

#### REFERENCES:

1. Young DS, Effects of drugs on clinical laboratory tests, 4th ed AACC press. Washington DC ; 3-43 to 3-47 1995.
2. Junge W, Troge B, Klein G et al. Evaluation of a New Assay for Pancreatic Amylase: Performance Characteristics and Estimation of Reference Intervals. Clin Biochem 1989;22:109- 114.
3. Kurrle-Jarres JD, Hafknscheid JCM, Hohenwallner W et al. Evaluation of a New -Amylase Assay Using 4.6-Ethylidene-(G7)-1-4-nitrophenyl-(G1)-a-D-maltohe-ptaoside as Substrate. J Clin Chem Clin Biochem 1989;27:103-113.
4. Salt WB II, Schenker S. Amylase-its clinical significance: a review of the literature [Review]. Medicine 1976: 55:269-281.
5. Steinberg WM, Goldstein SS, Davies ND et al. Diagnostic assays in acute Pancreatitis [Review]. Ann Intern Med 1985; 102:576 - 580.