NOVIN BIO KIT

Alpha-Amylase

Colorimetric - Kinetic method CE

IVD *For In-Vitro diagnostic and professional use only*

Store at 2-8□C

INTRODUCTION

Atlas Alpha-amylase is a type of enzyme (biological catalyst) which reduces the activation energy required in the hydrolysis of starch which thus speeds up the reaction rate. Measurements of α-Amylase are used to primarily in the diagnosis and treatment of the diseases of the pancreas ,Amylase is found primarily in the pancreas and salivary glands. When released in the digestive tract , the enzyme hydrolyzes starch.

PRINCIPLE

Atlas Alpha amylase catalyze the hydrolysis of 2-chloro-4-nitrophenyl-1-galactopyranosyl-malioside(GALG2-NP) to glucose polymers and p-nitrophenyl oligosaccaride at short chain producing 2-chloro-4-nitrophenol (CNP).
The increased extension can be measured by spectrophotometry at 405nm and results proportional at the activity of alpha amylase present in the sample.

$$\text{GALG2-CNP} \xrightarrow{\alpha\text{-Amylase}} \text{GALG2} + \text{CNP}$$

REAGENT COMPOSITION:

Sodium Citric acid buffer	100 mmol/L (pH 6.0)
Sodium chloride	300 mmol/L
GALG2-CNP	2.65 mmol/L
EDTA	0.2 mmol/L
Stabilizers and detergents	<0.1%

MATERIALS REQUIRED BUT NOT PROVIDED

- Photometer or spectrophotometer with a thermostatted cell compartment set at 37ºC, capable of reading at 405 nm.
- Stopwatch, strip-chart recorder or printer.
- Cuvettes with 1-cm path length.
- Pipettes to measure reagent and samples.

STORAGE AND STABILITY

- Store at 2-8□C.
- All the kit compounds are stable until the expiry date stated on the label. Do not use reagents over the expiration date.
- Store the vials tightly closed, protected from light and prevented contamination during the use. Avoid contamination and recap the vials immediately after use.

Discard If signs of deterioration appear:

- Presence of particles and turbidity.
- Blank absorbance (A) at 405 nm > 0.5 in 1 cm cuvette.

REAGENT PREPARATION

The Monoreagent is ready-to-use.

SAMPLES

- Serum or plasma and Urine.
- Serum and plasma α-amylase is stable for 30 days at 2-8□C.

INTERFERENCES

no interference up to:

ascorbic acid	500 mg/dL
Free bilirubin	20 mg/dL
hemoglobin	500 mg/dL
Bilirubin Conjugated	20 mg/dL
NaF	500 mg/dL
Glucose	5.0 g/dL
Maltose	5.0 g/dL

PROCEDURE 1 (Kinetic Method)

Wavelength =405 nm. Light path = 1 cm.
Temperature = 37ºC.
Measurement : against reagent blank (Blank:not necessary).

Reaction: Kinetic Increase.

1. Preincubate working reagent, samples and controls to reaction temperature.
2. Set the photometer to 0 absorbance with distilled water.

3. Pipette into a cuvette:

Reaction temperature	37º C	
	Blank	Sample
R1.Monoreagent	1.0 mL	1.0 mL
Dist. Water or saline	40 µl	…. …
Sample	…..…	40 µl

4. Mix gently by inversion. Insert cuvette into the cell holder and start stopwatch.
   Incubate at 37ºC for 1 minute and record initial absorbance reading.
5. Read the absorbance (at 405nm) exactly after 1, 2 and 3 minutes.
6. Calculate the difference between absorbances.
7. Calculate the mean of the results to obtain the average change in absorbance per minute (ΔA/min).

CALCULATIONS

Alpha Amylase (U/L) = ΔA/min X 3178

PROCEDURE 2 (Fixed rate colorimetric method)

Wavelength =405 nm. Light
path = 1 cm.
Temperature = 37ºC.
Measurement : against reagent blank.
Reaction: Fixed Increase.

1. Preincubate working reagent, samples and controls to reaction temperature.
2. Set the photometer to 0 absorbance with distilled water.
3. Pipette into a cuvette:

	Sample
Reagent	1.0 ml
Sample	40 µl

4. Mix gently by inversion. Insert cuvette into the cell holder and start stopwatch.
   Incubate at 37ºC for 1 minute and record initial absorbance reading (A1).
5. Read the absorbance 2 (A2) exactly after 4 minutes.

CALCULATIONS

ΔA = A2- A1

Alpha Amylase (U/L) = ΔA x 794

REFERENCE VALUES

	25ºC	30ºC	37ºC
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
24hours Urine	Up to 205 U/24h	Up to 295 U/24h	Up to 410 U/24h

It is recommended that each laboratory establishes its own reference range.

QUALITY CONTROL

To ensure adequate quality control (QC), each run should include a set of controls (normal and abnormal) with assayed values handled as unknowns.

Elevated level of α-amylase . Assayed.

If the values are found outside of the defined range, check the instrument, reagents and procedure. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

CLINICAL SIGNIFICANCE

Amylase activity tests in serum and urine are mainly used in the diagnosis of diseases of the pancreas and in the investigation of pancreatic function.

Amylase is found chiefly in the saliva and in pancreatic tissue.

Normally, small amounts of amylase are present in the blood, but with various forms of pancreatic disturbance large amounts of amylase are secreted into the blood by the pancreas.

The activity of the amylase in serum may fluctuate rapidly rising acutely during an attack and subsiding to normal levels shortly afterward.

Increased levels are found associated with acute pancreatitis, pancreatic duct obstrution, intra- abdominal diseases, mumps and bacterial parotitis.

A significant amount of the serum amylase is excreted in the urine, and as a result elevation of serum activity is reflected in the rise of urinary amylase activity. Urine amylase

appears to be more frequently elevated, reaches higher levels, and persists for longer periods.

ANALYTICAL PERFORMANCE

Linearity : 1500 U/L

If a sample exceeds 1500 U/L, it should be diluted 1:1 With normal saline and re-assayed. Multiply the result by 2.

Sensitivity: 2 U/L.

Measured Range: 2-1500 U/L.

Precision:

A) Within-run reproducibility

Within series n=20	**Mean (U/L)**	**SD (U/L)**	**CV (%)**
Sample 1	18.989	0.370	1.948
Sample 2	143.010	0.863	0.603
Sample 3	78.735	0.643	0.817

B) Between-run reproducibility

Day to day n=20	**Mean (U/L)**	**SD (U/L)**	**CV (%)**
Sample 1	18.909	0.711	3.758
Sample 2	141.421	1.630	1.153
Sample 3	78.973	1.164	1.474

Correlation:

A comparison between α-Amylase (y) and a Commercial obtainable assay (x) using 50 samples (28

–304 U/L) gave following results:

y = 1.116X - 4.946U/L; R^2 = 0.997.

WARNINGS AND PRECAUTIONS

- For In Vitro Diagnostic Use.
- Take the necessary precautions for the use of laboratory reagents.
- Avoid contamination of the reagent with salivary α-amylase. Do not pipette by mouth, and ensure that the reagent does not come into contact with the skin. (Saliva and sweat contain α-amylase)
- Xn: Harmful

R22: Harmful if swallowed

S2: Keep out of the reach of children.

S13:Keep away from food, drink and animal feedingstuffs.

S36/37: Wear suibable protective clothing and gloves.

S46: If swallowed, seek medical advice immediately and show this container or label.

- Caution: Contains Potassium Thiocyanate. Potassium thiocyanate is not compatible with strong acids.
- Caution: Contains sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azide. On disposal, flush drain with a large volume of water to prevent build up.

REFERENCES

1. Ranson, JHC, Curr, Prob. Surg., 16:1 (1979).
2. Salt, WB II and Schenker, S., Medicine, 55:269 (1976).
3. Stefanini, P., Ermini, M., and Carboni, M., J.Am.Surg., 119:866 (1965).
4. Henry, RJ, and Chiamori, N., Clin Chem., 6:434 (1960).
5. Kaufman, RA and Tietz, NW, Clin Chem., 26:846 (1980).

Temperature limit

REF	Catalogue Nu		
IVD	*In Vitro* diagnostic medical device		Caution
Σ	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry