ALPHA AMYLASE

($CNPG_3$ Kinetic Method)

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INTENDED FOR USE:

The Alpha amylase is an *in vitro* assay for the quantitative determination of Amylase in serum, plasma and urine.

CLINICAL SIGNIFICANCE:

Alpha – Amylase originates from pancreas and partial glands. The α-1, 4 glucosidic linkages of starch and other related polysaccharides are catalyzed by α-Amylase to produce maltose and other related polysaccharides. Alpha –Amylase is increased due to inflammation of salivary glands, inflammation of pancreas or cancer of pancreas, blockage of, or severe damage to the intestine (bowel infarction), a stomach ulcer, gallstones, cystic fibrosis, pregnancy and diabetic ketoacidosis and a ruptured ectopic pregnancy. Alpha-Amylase is decreased due to an uncommon condition called macroamylasemia, a severe liver disease. Some factors which may effect the test are medication, including warfarin, aspirin, birth control pills, diseases like hepatitis, Chronic kidney disease which may cause high level, when the kidneys are no longer able to remove amylase from the blood.

PRINCIPLE:

The direct amylase assay involves the use of a chromogenic substrate $CNPG_3$ (2-chloro-4-nitrophenyl linked with Galactomaltoside) which acts upon α-Amylase to release more than 90% of 2 - chloro – 4 -nitro phenol (CNP), and form 2- chloro - 4 – nitrophenyl – α – D - maltoside ($CNPG_2$), maltotriose (G_3) and Glucose (G).The rate of formation of 2-chloro-4-nitrophenol is proportional to the Alpha amylase activity in the sample, which can be monitored by the kinetic assay at 405 nm.

$$10\ CNPG_3 \xrightarrow[pH\ 6.0]{\alpha\text{ - amylase}} 9CNP + 1CNPG2 + G_3 + G$$

REAGENT COMPOSITION:

Alpha Amylase Reagent

MES Buffer	100 mmol /L
$CNPG_3$	3.1 mmol /L
Sodium Chloride	300 mmol/L
Calcium Acetate	6 mmol/L
Potassium thiocyanate	100 mmol/L

REAGENT STORAGE:

All reagents are stable at 2-8°C until the expiry date mentioned on the label. Reagents are ready to use. Before the assay bring all the reagents to room temperature. Avoid contamination of the reagents during the assay process and should be protected from direct light.

SAMPLE COLLECTION:

Unhemolyzed Serum, Urine. E.D.T.A, Oxalate or citrate inhibit amylase activity and hence cannot be used. Amylase in serum is reported to be stable for one week at room temperature and for 2 months when stored at 2-8^{o}C.

PRECAUTIONS & WARNING:

Avoid pipette with mouth. The preparation, according to current regulation, is classified as not dangerous. The total concentration of non active components (preservatives, detergents, stabilizers) is below the minimum required for citation. Anyway handle with care, avoid ingestion, avoid contact with eyes, skin and mucous membranes**.** The samples must be handle as potentially infected from HIV or Hepatitis.

SYSTEM PARAMETERS:

Reaction Type	:	Kinetic Method
Wave Length	:	405 nm
Optical path length	:	1 cm
Abs max	:	≤ 0.5
Flow cell Temperature	:	37^{0}C
Blank	:	Distilled Water
Delay Time	:	60 Sec
Read Time	:	30 Sec
Number of readings	:	3
Working Reagent	:	1000 µL
Sample Volume	:	25 µL
Factor	:	4640
Reaction Slope	:	Increasing
Low Normal	:	25
High Normal	:	140
Linearity	:	2000
Units	:	IU/L

Note:

1. Do not leave the unused reagent at room temperature when not in use. Take only the required amount of the reagent and keep the reagent back immediately at 2-8^{o}C.
2. Saliva and sweat contain α-Amylase. To avoid possible contamination do not pipette by mouth and avoid contact of the reagent and pipette tips with the skin.
3. The expected values of amylase are dependent on the substrate used in the formulation. Results cannot be compared with the kits based on formulations using other substrates.
4. Reagent should not be used if its absorbance exceeds 0.800 at 405 nm, against distilled water.
5. Sample having a very high activity show a very initial absorbance. If this is suspected then dilute the sample and repeat the assay. Adherence to the reaction time should be meticulously followed.

ASSAY PROCEDURE:

Pipette in to test tubes labeled Test (T) as follows**:**

Addition Sequence	Test (µL)
Alpha Amylase Reagent	1000
Sample	25

Assay temperature 37°C mix well and read absorbance against distilled water at 405 nm as Follows:
Read absorbance after 60 seconds. Repeat the reading twice after every 30 seconds i.e. up to 90 seconds.
Determine the average change in absorbance per minute (ΔA/min).

CALCULATION:

Alpha Amylase activity in IU/L = ΔA/min X 4640

SENSITIVITY / LIMIT OF DETECTION:

The method will accurately measure Alpha amylase level is 8.3 IU/L.

LINEARITY:

The procedure is linearity up to 2000 IU/L. If values exceed this limit dilute the sample with normal saline and repeat the assay. Calculate the value using the proper dilution factor.

QUALITY CONTROL:

To ensure adequate quality control, the use of commercial reference control serum is recommended with each assay batch. Use of quality control material checks both the instrument and reagent function

REFERENCE RANGE:

Serum/Plasma
Serum **:** 25 - 140 IU/L
Urine **:** 1 - 17 IU/24hrs
Note: It is recommended that each laboratory establish its own normal range representing its patient population.

INTERFERENCE:

No interference was observed by up to glucose 500 mg/dL, bilirubin 40 mg/dL, hemoglobin 500 mg/dL and triglycerides 1500 mg/dL.

PRECISION:

Precision studies were performed with two controls using NCCLS protocol EP5 –A. The results of the precision studies are shown below:

Sample	Within - Run		Between - Run		Total	
	Mean	CV%	Mean	CV%	Mean	CV%
Control-1	62	1.20	64	1.50	126	2.70
Control-2	402	1.50	410	1.80	812	3.30

REFERENCES:

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SYMBOLS USED ON THE LABELS:

MONOZYME
Reagents & Equipments
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