LiquiMAX α-Amylase-SLR

($CNPG_3$ METHOD)

ORDERING INFORMATION.

Ref. No.	Pack Size
AVAMY - 10	2 x 5 ml
AVAMY - 20	4 x 5 ml
AVAMY - 50	5 x 10 ml

INTENDED USE:

LiquiMAX Amylase is an in-vitro diagnostic kit is use for the quantitative determination of α-amylase in human Serum.

CAPITAL:

1. Single Liquid Reagent.
2. Uses CNPG3 as a Direct Chromogenic Substrate.
3. Achives 98% hydrolysis.
4. Reagent system does not require auxiliary enzymes like Galactosidase and Glucoamylase.
5. One step kinetic assay. Kinetic reaction time 2 minutes.
6. Linearity 2000 IU/L.
7. Kinetic factor 3178 at 405 nm at 37° C.
8. Can be used on any discrete semi automated and automated analyzers.

CLINICAL SIGNIFICANCE:

The α-amylases (1,4-α-D-glucanohydrolases, EC 3.2.1.1) catalyze the hydrolytic degradation of polymeric carbohydrates such as amylase, amylopectin and glycogen by cleaving 1,4-α-glycosidic bonds. In polysaccharides and oligosaccharides, several glycosidic bonds are hydrolyzed simultaneously. Maltotriose, the smallest such unit, is converted into maltose and glucose. Two types of α-amylases can be distinguished, the pancreatic type (P type) and the salivary type (S-type). Whereas the P-type can be attributed almost exclusively to the pancreas and is therefore organ specific,

The S-type can originate from a number of sites. Apart from appearing in the salivary glands it can also be found in tears, sweat, human milk, amniotic fluid, the lungs, testes and the epithelium of the fallopian tube. Because of the sparsity 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), tumors of the lungs or ovaries, pulmonary inflammation, diseases of the salivary gland, diabetic ketoacidosis, cerebral trauma, surgical interventions or in the case of macroamylasemia. To confirm pancreatic specificity, it is recommended that an additional pancreas-specific enzyme - lipase or pancreatic-á-amylase- also be determined. Numerous methods have been described for the determination of α-amylase. These either determine the decrease in the amount of substrate viscometrically, turbidimetrically, nephelometrically and amyloclastically or measure the formation of degradation products saccharogenically or kinetically with the aid of enzyme-catalyzed subsequent reactions.The kinetic method described here is based on the cleavage of 2-chloro-4-nitrophenyl-α-D-maltotrioside (CNP-G3) by α-amylase.

PRINCIPLE:

The Direct Amylase assay involves the use of a chromogenic **substrate $CNPG_3$ (2-choloro-4-nitrophenyl linked with Galactomaltoside)** which acts upon α-Amylase to release more than 98% of 2-choloro-4-nitrophenyl (CNP), and forms 2-choloro-4-nitrophenyl-α-D-maltoside ($CNPG_2$), maltotriose (G_3) and Glucose (G).

The rate of formation of 2-choloro-4-nitrophenyl is proportional to the α-Amylase activity in the sample, which can be monitored by kinetic assay at 405 nm.

$$CNPG_3 \xrightarrow{\alpha\text{-amylase}} CNP + CNPG_2 + G_3 + G$$

STORAGE & STABILITY:

The Reagent should be stored at 2-8°C and is stable till the expiry date indicated on the label.

DO NOT FREEZE THE REAGENT. Contamination of the reagent should be strictly avoided.

It is recommended that when the reagent is not in use for prolonged periods of the reagent be capped and stored at 2-8°C.

KIT COMPONENTS

1. Amylase Reagent

COMPOSITION

MES Buffer, pH 6.0	:	100 mMol/L
$CNPG_3$	:	7 mMol/L
KSCN	:	200 mMol/L
Azide	:	0.1 %

REAGENT RECONSTITUTION & STABILITY

Reagent are liquid stable no need for reconstitution.

When the reagent is stored properly at 2-8°C & the contamination avoided, it is stable up to the expiry date mention on the label & kit box.

MATERIAL REQUIRED BUT NOT PROVIDED

Laboratory Instrumentation, Spectrophotometer UV/VIS with thermostatic cuvette holder or clinical chemistry analyzer: semi auto, calibrated micropipettes, glass or high quality polystyrene cuvettes, test tube/rack, heating bath controls, saline.

REAGENT DETERIORATION

Discard any turbid reagent or reagent absorbance exceeds 1.4 at 405 nm against distilled water.

WARNING & PRECAUTIONS

- Reagent may contain some non reactive and preservative components. It is recommended to handle carefully, avoiding contact with skin and ingestion.
- Specimen should be considered infectious and handled appropriately.
- Contamination by soap or glycerol will affect this assay.
- Perform the test according to the general " Good Laboratory Practice" GLP.

SPECIMEN

1. Unhemolysed serum is the specimen of choice.
2. E.D.T.A, Oxalate or Citrate inhibit Amylase activity hence cannot be used.
3. 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.

SYSTEM PARAMETERS:

Reaction Type	:	Kinetic
Reaction Direction	:	Increasing
Wavelength	:	405 nm
Flow cell temp	:	37°C
Zero setting with	:	Distilled Water
Delay Time	:	60 seconds
Measuring Time	:	60 seconds
Sample Volume	:	25 µl
Reagent Volume	:	1.0 ml
Factor	:	3178
Linearity	:	2000
Units	:	IU/L

TEST PROCEDURE :

Reagent	Test
Amylase Reagent (Ready to Use)	1.0 ml
Sample	25 µl

Mix thoroughly and transfer the assay mixture immediately to the thermostated cuvette maintained at 37°C and record the first reading after 1 minute and subsequently two more readings with 30 seconds interval at 405 nm.

CALCULATION:

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

Activity of Amylase in IU/L = ΔAbs/min X 3178 (Kinetic Factor)

Note: Kinetic Factor 3178 is derived based on the Absorbance coefficient of 2-chloro-p-nitrophenol at 405 nm.

EXPECTED VALUES:

Serum : 25-110 IU/L

Since the expected values are affected by age, sex, diet and geographical location, each laboratory should strongly urge to establish its own reference range for this procedure.

As Amylase is generally measured by the hydrolysis of different non natural substrates, values obtained can change widely for each Substrate. Therefore reference values mentioned for other Substrates should not be compared or correlated with the reference values mentioned for $CNPG_3$ Substrate.

QUALITY CONTROL & CALIBRATION

It is recommend to perform internal quality control with assayed normal (BioNorm) and assayed abnormal (BioPath), to confirm the validity of the test and assure the accuracy of patient result.

Using the recommended calibrator (Avecon) or the standard included, calibrate the assay:

a) When using a new reagent or lot.

b) When QC values are out of range.

PERFORMANCE CHARACTERISTICS

1. Linearity

Linearity : 2000 IU/L

2. Sensitivity/ Limit of Detection (LOD)

The lower limit of detection is 7 IU/L

3. Interferences

No significant interference was observed from Bilirubin upto 20mg/dl (Both conjugated and unconjugated Bilirubin), Lipema as Triglycerides upto 2000mg/dl,Ascorbic acid upto 30mg/dl.

4. PRECISION

Intra-assay precision	mean	SD	CV
N=20	(IU/L)	(IU/L)	(%)
Sample 1	187	1.48	0.79
Sample 2	446	2.1	0.47
Sample 3	507	2.64	0.52

Intra-assay precision	mean	SD	CV
N=20	(IU/L)	(IU/L)	(%)
Sample 1	196	2.83	1.44
Sample 2	474	6.25	1.32
Sample 3	542	6.73	1.24

Reproducibility was determined using human samples and controls between day (n = 20). The following results were obtained:

Method comparison:

A comparison of the LiquiMAX Amylase - SLR (y) with a commercial obtainable assay (x) gave with 36 samples the following results

y = 0.972x + 1.282; r = 0.999

LIMITATION

Measuring range: 7-2000 IU/L. Determine samples having higher concentrations manually dilute with 0.9% NaCl or distilled/deionized water (e.g. 1 + 1). Multiply the result by the appropriate dilution factor (e.g. 2).

WASTE DISPOSAL

Reagents must be disposed off in accordance with local regulations.

NOTE:

1. 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.
2. 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.
3. If the amylase activity is above 2000 IU/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.

REFERENCES:

1. Winn-Deen, E.S. David, H. Siglet E, and Chavzer R., Clin,chem., 34/10, 2005-2008 (1988).
2. Junge W.et al., Biochem, 22, 109 (1989).

Symbols Used on Pack

REF	Catalogue Number		Warning/Caution
LOT	Batch No.	IVD	In vitro diagnostic device
	Manufacturing Date		Storage Limit
	Expiry Date		Consult instruction for use
	Manufacturer		Keep away from sunlight
	Keep Dry		Do not use if package is damaged
	Contains sufficient no. of test		

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E-mail : helpdesk@aveconhealthcare.com, Website : www.aveconhealthcare.com

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