

LiquiMAX Pancreatic - Amylase

(EPS / IFCC Method)

Ordering information

Ref./Cat.	Pack Size	Presentation
AVPAMY-10	2 x 5 ml	2 x 4 ml (R1) + 2 ml (R2)
AVPAMY-20	2 x 10 ml	2 x 8 ml (R1) + 2 x 2 ml (R2)

Intended Use:

Kit for the determination of Pancreatic Amylase in Human Serum and Heparinized Plasma

Product Features

- Liquid Stable, Ready to use two reagents (R1 Substrate+ R2 Antibody) Incorporates EPS G7 substrate and Monoclonal Antibodies to inhibit Salivary Amylase
- 3 Minutes increasing kinetic reaction (60 Sec Delay+120 Sec Measuring).
- Linearity 1200 IU/L.
- Measuring Wavelength 405 nm.
- Kinetic factor 1666 at 37° C.
- Serum / Heparinized Plasma as Specimens.
- Available as multipurpose reagents and dedicated system packs.

Measurements of amylase are used 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. Amylase determinations are useful in the diagnosis of diseases of the pancreas and parotids. Elevated serum levels are associated with acute pancreatitis and other pancreatic disorders as well as mumps and bacterial parotitis. The salivary isoenzyme is inhibited by monoclonal antibodies.

Amylases are hydrolytic enzymes which break down starch into maltose. In the human body amylases originate from various organs: the pancreatic amylase is produced by the pancreas and released into the intestinal tract, the salivary amylase is synthesized in the salivary glands and secreted into saliva. As the pancreatic and the salivary amylase show a structural homology of 97%, the only method to distinguish both sufficiently is to use an assay based on monoclonal antibodies to inhibit the salivary enzyme. The amylase present in the blood is eliminated through the kidney and excreted into the urine. Therefore, an elevation of serum activity is reflected in the rise of urinary amylase activity. Measurement of amylase in serum and urine is mainly applied for the diagnosis of pancreatic disorders as well as for detecting the development of complications. In acute pancreatitis the blood amylase activity increases within few hours after onset of abdominal pain, peaks after approx. 12 hours and returns to values within the reference range at the latest after 5 days. Although the pancreatic amylase is much more specific for detection of pancreatic disorders than the total amylase, for confirmation of an acute pancreatitis an additional measurement of lipase is recommended.

Enzymatic photometric test, in which the substrate 4,6- ethylidene-(G7)-p-nitrophenyl-(G1)- D-maltoheptaoside (EPS-G7) is cleaved by amylases into various fragments. These are further hydrolyzed in a second step by -glucosidase producing glucose and p-nitrophenol. As the salivary isoenzyme is inhibited selectively by a combination of two monoclonal antibodies during the pre incubation phase, the increase in absorbance represents the pancreatic amylase activity in the sample

Principle

Determination of the pancreatic-amylase after inhibition of salivary amylase using monoclonal antibodies. The procedure utilizes a different auxiliary enzyme a-glucosidase, which cleaves all primary degradation products and leads to a 100% chromophore release from the substrate.

5-Ethylidene-G7-pNP+5 H₂O $\xrightarrow{\text{pancreatic-amylase}}$

2 Ethylidene-G5 + 2 G2-pNP+ 2 Ethylidene-G4 + 2 G3- pNP+Ethylidene-G3 + G4-pNP

2G2-pNP+2G3-pNP+G4-pNP+14 H₂O $\xrightarrow{\text{A-glucosidase}}$ 5pNP+14G G:
Glucose, pNP: p-nitrophenol

Reagents:

1. Substrate Reagent (R1)

HEPES buffer, pH 7.15	50 mmol/l
NaCl	70 mmol/l
MgCl ₂	10 mmol/l
A-glucosidase	≥4 kU/l

2. Antibody Reagent (R2)

4,6-Ethylidene-G7-pNP(EPS)	3 mmol/l
HEPES buffer, pH 7.15	50 mmol/l
Monoclonal antibodies	

Reference Range :

	Women	Men
Serum/ Heparinized Plasma	< 60 U/L	< 60 U/L

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

SPECIMEN COLLECTION AND HANDLING:

- Unhemolysed serum and Heparinized Plasma are the specimens
- E.D.T.A, Oxalate or Citrate inhibit Pancreatic Amylase activity hence cannot be used.
- Pancreatic 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
Kinetic Interval	: 60 seconds
Number of readings	: 3
Blank Absorbance limit	: < 0.800 Abs.
Sample Volume	: 25 µl
Reagent Volume	: 1.0 ml
Kinetic Factor	: 1666
Linearity	: 1200
Units	: IU/L

ASSAY PROCEDURE :

Pipette the reagents in to glass tube separately and proceed for testing.

Pipette in to tubes	Test
Substrate Reagent	800 µl
Antibody	200 µl
Sample	25 µl

Mix well and after 60 seconds incubation, measure the change of optical density per 60 seconds during next 120 seconds against distilled water at 405 nm as follows.

- A₀ - Exactly after 60 seconds (Delay)
A₁, A₂ - Exactly after every 60 seconds for 120 seconds.
(Measuring)



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CALCULATION:

Calculate the average change in absorbance per minute (Δ Abs/minute)
Activity of Pancreatic Amylase in IU/L = Δ Abs/min X1666 (Kinetic Factor)

Linearity

The method was found linear up to 1200 U/l

Quality Control

A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

Sensitivity

It is recommended that each laboratory establishes its own range of sensitivity as this is limited by the sensitivity of the spectrophotometer used. Under manual conditions however, a change of 0.002 Abs units/min is equivalent to 3.33 U/l pancreatic-amylase activity at 405 nm.

Accuracy

Obtained values of control serum samples with known amount of pancreatic-amylase activity fall within +/- 10%.

Precision

	Repeatability CV%
Serum	<5

Specificity















Bilirubin 1026 mmol/l (60 mg/dl), lipid 1000 mg/dl, glucose 111 mmol/l (2000 mg/dl) and ascorbic acid 5.68 mmol/l (100 mg/dl) don't interfere with the assay up to the given levels.

Literature

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Symbols Used on Avecon's Labels

	Catalog Number		Warning/ Caution
	Batch No.		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
	Do Not Reuse		Contains sufficient no. of test



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Plot No.: # 338, Sector-2, Industrial Growth Centre, Saha (Haryana) India-133104.
E-mail : helpdesk@aveconhealthcare.com, Website : www.aveconhealthcare.com
Customer Care No. : +91 93065 12576



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