α-AMYLASE

Diagnostic reagent for determination of Alpha Amylase concentration.
Liquid. Monoreagent. Store at +2/+8°C. For in Vitro Diagnostic Use (IVD). Do not freeze.

Ref No	Pack
MH-022	120 mL
MH-023	40 mL

Changes made in the instructions for use are marked as grey.

INTENDED USE

The test is applied for the quantitative determination of Zinc in serum, plasma and urine.

GENERAL INFORMATION

α-Amylases (EC 3.2.1.1; 1,4-α-D glucan glucanohydrolase; AMY) are enzymes that catalyze the hydrolysis of 1,4-α-glucosidic bonds in polysaccharides. Both straight-chain (linear) polyglucans (amylose) and branched polyglucans (amylopectin and glycogen) are hydrolyzed at different rates. In amylose hydrolysis, the enzyme cleaves the chains at alternating α-1,4-hemiacetal (-C-O-C-) linkages to form maltose and some residual glucose; hydrolysis of branched-chain polyglucans produces maltose, glucose and a residue of limit dextrins. The enzyme does not act against α-1,6 bonds at the branching points. Amylases are calcium metalloenzymes in which calcium is essential for functional integrity. However, full activity is only displayed in the presence of various anions such as chloride, bromide, nitrate, cholate or monohydrogen phosphate; chloride and bromide are the most effective activators. Amylases in human serum show optimum activity between pH 6.9 and 7.0.[1]

Amylases in human plasma are relatively small molecules with molecular weights ranging from 54 to 62 kDa. The enzyme is therefore small enough to pass through the glomeruli of the kidneys and is the only plasma enzyme normally found in urine. Amylases are found in many organs and tissues. S-type amylase, found in the salivary gland, initiates the hydrolysis of starches when food is in the mouth and esophagus and is the most abundant amylase. Formerly called pityalin, its activity is terminated by exposure to acid pH in the stomach. In the pancreas, the enzyme (type P) is synthesized by acinar cells and then secreted into the intestinal tract via the pancreatic duct system.

The activity of both amylase isoenzyme types (S and P type) is also present in smaller amounts in other parts of the body such as the ileum, jejunum, duodenum, colon, lungs, fallopian tubes, thyroid gland, stomach and ovaries. The enzyme found in serum and urine is predominantly of pancreatic (P-amylase) and salivary gland (S-amylase) origin. These isoenzymes are products of two closely linked loci on chromosome 1. In healthy adults, P-amylase represents approximately 40 to 50% of the total amylase activity in serum. Amylase isoenzymes undergo posttranslational modifications such as deamidation, glycosylation and deglycosylation to form a number of isoforms.[1]

Amylase activity in the blood is physiologically low and is greatly increased in acute pancreatitis and salivary gland inflammation. In acute pancreatitis, serum amylase activity shows an increase within 5 to 8 hours of symptom onset and usually returns to baseline by the third or fourth day. Increases up to 4 to 6 times the upper reference limit (URL) are seen, reaching maximum activity between 12 and 36 hours. The magnitude of the increase in serum enzyme activity does not correlate with the severity of pancreatic involvement; however, the greater the increase, the greater the likelihood of acute pancreatitis. However, the specificity of amylase for the diagnosis of acute pancreatitis is low because elevated values are also found in some acute intra-abdominal diseases and various non-pancreatic conditions. Clearance of amylase from the circulation occurs partly in the urine via renal excretion, and increased serum activity leads to increased urinary amylase activity. Compared to serum amylase, urinary amylase reaches higher concentrations and persists at elevated levels for longer periods.[1]

The low specificity of total amylase measurements has led to the development of other tests (e.g. lipase and pancreatic specific amylase) instead of total amylase activity for the diagnosis of acute abdominal pain.

Studies have shown that total amylase measurement is inferior to other markers for the diagnosis of pancreatitis.[2-5] Measurement of P-amylase activity has higher diagnostic sensitivity and specificity than total amylase activity in the diagnosis of acute pancreatitis.[6] If acute pancreatitis is suspected, serum amylase should be monitored together with lipase or P-amylase. Serial measurements are generally more useful than single determinations to help establish the correct diagnosis.[7]

Various intra-abdominal extrapancreatic conditions such as biliary tract disease can lead to a significant increase

in serum P-amylase activity. Pancreatic hyperamylasemia can also be seen in conditions such as intestinal obstruction, perforated duodenal ulcer, mesenteric infarction and acute appendicitis, which cause disruption of intestinal integrity. Furthermore, in renal failure, serum P-amylase activity increases in proportion to the extent of renal failure (usually no more than five times the URL).

Many non-pancreatic abdominal diseases such as appendicitis and peritonitis can cause hyperamylasemia. Salivary gland lesions caused by infection, irradiation, obstruction, surgery and tumor can increase S-type amylase as well as total amylase levels. In addition, many drugs have been reported to cause an increase in serum amylase measurements.[1,8] Hyperamylasemia also occurs in neoplastic diseases. Lung tumors and serous and mixed (serous and mucinous) carcinomas of the ovary can cause hyperamylasemia (with S-type isoenzyme mobility) with increases up to 50 times the URL. The amylase isoenzyme in cases of ruptured ectopic pregnancy is not well characterized. In severe cases presenting late, the increased isoenzyme may be P-amylase due to pancreatic involvement due to peritonitis, although S-amylase is present in the fallopian tubes.[1]

In 1% of the population macroamylases are present in serum and can cause hyperamylasemia; these are amylase (usually type S) and IgG or IgA complexes. These macroamylases, due to their large size (>200 kDa molecular weight), cannot be filtered through the renal glomeruli and are therefore retained in plasma. Their presence there can increase amylase activity by about two to eight times that of URL. This condition is not accompanied by any clinical symptoms, but some cases have been detected during investigation of abdominal pain.[1,8]

TEST PRINCIPLE

Enzymatic colorimetric method

Conforms to the IFCC reference method.[9] The oligosaccharide 4,6-ethylidene-(G^7)p-nitrophenyl-(G_1)-α,D-maltoheptaoside (ethylidene-G^7PNP) is hydrolyzed into G_2PNP, G_3PNP and G_4PNP fragments by a reaction catalyzed by α-amylase.

These fragments are then hydrolyzed in a second reaction catalyzed by α-glucosidase to form p-nitrophenol and glucose with chromophore properties. The color intensity of the p-nitrophenol formed is directly proportional to α-amylase activity and is detected by an increase in absorbance at 405 nm.

5 ethylidene-G^7PNP[a)] + 5 H_2O $\xrightarrow{\alpha-amylase}$ 2 ethylidene-G_5 + 2 G_2PNP + 2 ethylidene-G_4 + 2 G_3PNP + ethylidene-G_3 + G_4PNP

2 G_2PNP + 2 G_3PNP + G_4PNP + 14 H_2O $\xrightarrow{\alpha-glucosidase}$ 5 PNP + 14 G[b)]

a) PNP: p-nitrophenol
b) G: glucose

REAGENT COMPONENTS

Reagent 1:

Bicarbonate Buffer	≤ 400 mmol/L
5-Br-PAPS	≤ 0.08 mmol/L
Sodium Citrate	≤ 245 mmol/L
Detergent	%1

REAGENT PREPARATION

Reagent is ready for use.

REAGENT STABILITY AND STORAGE

Reagents are stable at +2/+8°C till the expiration date stated on the label which is only for closed vials.

Once opened vials are stable for 25 days at +2/+8°C in optimum conditions. On board stability is strongly related to auto analyzers' cooling specification and carry-over values.

Reagent stability and storage data have been verified by using Clinical and Laboratory Standards Institute (CLSI) EP25-A protocol.[10]

SAMPLE REQUIREMENTS

Serum, plasma and urine can be used. Serum is collected according to the standard procedures. For plasma, lithium heparinized sample tubes should be used. Sample collection tubes with EDTA must not be preferred for plasma. Hemolyzed samples must not be used. For 24 hour or random urine samples, acid-free non-preservative plastic or glass containers shoul be used. Multiple sample freezing and thawing should be avoided. The sample should be homogenized before testing.

Amylase activity stability in serum and plasma[24]:

7 days at +15/+25ºC
1 month at +2/+8ºC
1 year at -20°C

Amylase activity stability in urine[25]:

2 days at +15/+25ºC
10 days at +2/+8ºC

Note 1: Since amylase has an absolute requirement for calcium ions, chelating anticoagulants such as citrate, oxalate and EDTA are not preferred as plasma samples.[7]

Note 2: It is a standard practice to analyze urine samples within 12 hours at room temperature or within 5 days at 5°C and not to freeze the sample.[7,11]

Unit Conversion:
U/L x 0,017 = µkat/L

CALIBRATION AND QUALITY CONTROL

Calibration: The assay requires the use of an Arcal Auto Calibrator. We recommend:

Arcal Auto Calibrator
Ref.No: VT-003

Calibration stability depends on the application characteristics and cooling capacity of the autoanalyzer used. Calibration stability is 7 days.

Control: Commercially available control material with established values determined by this method can be used. We recommend:

Arcon N (Level I Control) Lyophilized
Ref.No: VT-001

Arcon P (Level II Control) Lyophilized
Ref.No: VT-002

At least two level controls must be run once in every 24 hours. Each laboratory should determine its own quality control scheme and procedures. If quality control results are not within acceptable limits, calibration is required.

REFERENCE INTERVALS / MEDICAL DECISION LEVELS[23]

Serum/Plasma

Men / Women	: 28 – 100 U/L

Urine

Men	: 16 - 491 U/L
Women	: 21 - 447 U/L

Note 1: The reference range of the method recommended by IFCC, with which our method is compatible, is 31 to 107 U/L at 37°C.[7]

Note 2: The blood amylase activity of newborns is approximately 18% that of adults. Mean serum amylase activity increases from the neonatal period until 3 to 4 years of age, when it reaches adult levels. There is no significant difference in serum amylase activity between men and women.[12]

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary, determine its own reference range.

Reference interval has been verified by using CLSI EP28-A3c protocol.[13]

PERFORMANCE CHARACTERISTICS

Measuring Interval

According to CLSI EP34-ED1:2018, "Measuring Interval" refers to the interval where the analyte concentration is measured with intended accuracy in terms of medical and laboratory requirements without dilution, concentrating or any kind of pre-treatment that is between the analyte's lower limit of quantitation (LLoQ) and upper limit of quantitation (ULoQ).[14]

The determined analytic measuring interval for Amylase is 3 – 3300 U/L.

Detection Capability

Limit of Detection (LoD): 1,5 U/L

Limit of Quantitation (LoQ): 3 U/L

Note: LoQ values are based on Coefficient of Variation Percentage (CV) ≤ 20%.

LoD and LoQ values have been verified by using CLSI EP17-A2:2012 protocol.[15]

Linearity

This method shows measurement linearity in the activities up to 3300 U/L.

Autoanaylzer's auto-dilution system can be used if the concentrations have higher values. See device manual for further information.

For manual dilution procedure, dilute the sample 10-fold using 0.90% isotonic. After the dilution, multiply the result of rerun sample by the dilution factor. Do not report the sample result after dilution if it is marked as lower than the linear lower limit. Rerun with a suitable dilution.

Linearity Studies data have been verified by using CLSI EP06-A:2003 protocol.[16]

Precision

Running system has been developed according to 20x2x2 "The Single Site" protocol. Repeatibility and Within-Laboratory Precision/Within-Device values have been obtained according to the running results.

According to the protocol in use, 2 separate runs per day have been made for 20 days (no obligation for being consecutive days). This protocol has been applied to each low and high samples separately and 80 results have been obtained for each one. Statistically, the results have been obtained using 2-factor Nested-ANOVA model.[17]

Repeatibility (Within Run) and Repeatibility (Day to Day) SD (standard deviation) and CV% values of Zinc have been given in the table 1 and 2 respectively.

Table 1. Amylase Repeatibility (Within Run) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
62 U/L	0,76	1,22	80
498 U/L	2,52	0,50	80

Note: This working system has been named "Within-Run Precision" in the previous CLSI - EP05-A2 manual.[18]

Table 2. Amylase Repeatibility (Day to Day) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
62 U/L	1,63	2.63	80
498 U/L	15.33	3,08	80

Note: This working system has been named "Total Precision" in the previous CLSI - EP05-A2 manual.[18]

Method Comparison

As a result of the statistical evaluation of the method comparison data:

Passing-Bablock equation:[19]
y= 1,022x – 0,37 U/L
r=0.99

Interference

Endogenous interferant and analyte concentrations that have been used in the Amylase scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.[20,21]

The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from Amylase interference scanning test is appropriate, is determined as ±25%.[22]

In Amylase test results, no significant interaction has been observed in the determined endogenous interferant and analyte concentrations or between interferants and analyte.

Interferant-Concentration	Amylase Target (U/L)	N*	Observed Recovery %
Bilirubin 14.9 mg/dL	60.4	3	91
Lipemia 2773 mg/dL	54.9	3	102

** Total acceptable error rate determined as interference limit and repeatability (within run) pre-detected for the related method were used for the calculations of how many times the control and test samples prepared as a serum pool are going to be run repetitively. In the calculations, the accepted error rate for type 1 (α error) was 5% and for type 2 (β error) was 10% (90% power).*[21]

Note: As reported in the literature, amylase assays are generally not prone to be affected by hemoglobin, bilirubin or triglycerides. Collection of samples in tubes containing oxalate, citrate or EDTA may result in erroneously low values due to chelation of essential amylase cofactors.[7]

It should be noted that endogenous interferants, as well as various medicines and metabolites, anticoagulants (e.g. Heparin, EDTA, citrate, oxalate) and preservatives (e.g. sodium floride, iodoacetate, hydrochloride acide) such as additives, materials that may contact with samples during collection and processing (serum separator devices, sample collection containers and contents, catheters, catheter wash solutions, skin disinfectants, hand cleaners and lotions, glass washing detergents, powder gloves), dietary substances known to affect some specific tests (caffeine, beta-carotene, poppy seeds, etc.), or some substances present in a sample that cause foreign proteins (heterophilic antibodies, etc.), autoimmune response (autoantibodies, etc.), or due to malignancy (for example, interference by paraproteins with phosphate testing and indirect ion selective electrode methods) may show some negative effects that will cause various attempts and some misjudgements.[21]

These performance characteristics have been obtained using an autoanalyzer. Results may vary slightly when using different equipment or manual procedures.

WARNINGS AND PRECAUTIONS

IVD: For in Vitro Diagnostic use only.
Do not use expired reagents.
Reagents with two different lot numbers should not be interchanged.
For professional use.
Follow Good Laboratory Practice (GLP) guidelines.
Contains sodium azide.

CAUTION: Human source samples are processed with this product. All human source samples must be treated as potentially infectious materials and must be handled in accordance with OSHA (Occupational Safety and Health Administration) standards.

Danger

EUH032 :Releases a very toxic gas if contacts with acid.
H317 :May cause allergic skin reaction.

Precaution

P280 :Use protective gloves / clothes / glasses / mask.
P264 :Wash your hands properly after using.
P272 :Contaminated work clothes should not be allowed to be used outside of the workplace.

Intervention

P302+P352	:Wash with plenty of water and soap if it contacts with skin.
P333+P313	:Seek medical help if it irritates your skin or develops rash.
P362+P364	:Remove contaminated clothes and wash properly before using.

Disposal

P501	:Dispose the vials and contents according to the local regulations.

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SYMBOLS

IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
R1	Reagent 1
GTIN	Global Trade Item Number
REF	Reference Number
GLP	Good Laboratory Practices
FOR USE WITH	Identifies Products to Be Used Together
PRODUCT OF TURKEY	Product of Turkey
	Manufacturer
	Expiration Date
	Temperature Limits
	Consult Instructions for Use
	Caution
	Number of Tests