AMYLASE

(LIQUID)

3 x 60 ml

RE – ORDER AML1030

INTENDED USE

This reagent is intended for the quantitative determination of a-Amylase activity in serum.

METHOD AND HISTORY

The classic method of determining a-Amylase activity is enzymatic hydrolysis of a starch substrate followed by product analysis. The saccharogenic analysis method measures the quantity of reducing sugars formed. There are difficulties associated with this method such as inconsistent results due to variations in substrate preparation and treatment.

Other methods of analysis include the dye-starch substrate methods and the amyloclastics methods. Each has drawbacks. Modified saccharogenic methods have been introduced recently which utilize a defined oligosaccharide substrate. These substrates produce colorimetric products when coupled with p-nitrophenyl. Wallenfels et all introduced p-nitrophenylglycosides as defined substrates for a-amylase determination in a procedure that eliminates interference from endogenous glucose and pyruvate.

TEST PRINCIPLE

The present method is based on the use of a chromogenic substrate, 2-chloro-p-nitrophenol linked with maltotriose. The reaction of amylase with this substrate results in the formation of 2-chloro-p-nitrophenol, which can be measured spectrophotometrically at 405nm. This reaction proceeds very rapidly, no coupling enzymes are required, and the reaction is not readily inhibited by endogenous factors.

$$10\ CNPG3 \xrightarrow{\text{a-amylase}} 9CNP + CNPG2 + 9G3 + G$$

a-Amylase hydrolyzes the 2-chloro-p-nitrophenyl-a-D-malthoheptaoside (CNPG3) to release 2-chloro-nitrophenol and form 2-chloro-p-nitrophenyl-a-D-maltoside (CNPG2), maltotriose (G3) and glucose (G). The rate of increase in absorbance is measured at 405nm and is proportional to the a-amylase activity in the sample.

CLINICAL SIGNIFICANCE

Assay for the a-Amylase activity are of interest for the evaluation of pancreatic function of the diagnosis of pancreatic disease. The greatest elevation in serum a-Amylase activity is seen in acute pancreatitis and obstruction of pancreatic duct.

PATIENT PREPARATION

No special patient preparation is required.

SPECIMEN COLLECTION.

Unhemolyzed serum is the specimen of choice. Specimens should be colleted as per NCCLS document H4-A3.

Anticoagulants, such as Citrate and EDTA, bind calcium which is needed for amylase activity. Therefore, plasma with these anticoagulants should not be used.

SPECIMEN STORAGE

Amylase in serum is reported stable for one week at room temperature (18-26°C) and for two months when stored refrigerated at 2-8°C.

It is recommended that testing be done as soon as possible after sample collection and preparation. If testing cannot occur immediately, store the sample properly using the guidelines above.

MATERIALS

Reagents necessary for the determination of a-Amylase are included in the kit.

REAGENT

a-Amylase reagent contains:

MES Buffer	ph 6.0 ± 0.1
2-Chloro-p-nitrophenyl-a-D-maltotrioside	1.8 mM
sodium chloride	350 mM
calcium acetatee	6 mM
Potassium thiocyanate	900 mM
sodium azide as preservative	0.01%

WARNINGS AND PRECAUTIONS

This reagent kit is intended for in vitro diagnostic use only.

This reagent contains potassium thiocyanate. POISON. Do not ingest.

This reagent contains sodium azide (0.01%) as a preservative. Do not ingest. May react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with a large volume of water to prevent azide build up.

All specimens and controls should be handled as potentially infectious. using safe laboratory procedures. (NCCLS M29-T2).

REAGENT PREPARATION

The reagent is provided as a ready-to-use liquid. No preparation is required.

REAGENT STORAGE AND STABILITY

Store reagent at 2°-8°C.

The reagent is stable until the expiration date if stored as directed. When stored at 2°-8°C unopened reagents are stable until the expiration date printed on the label.

Do not use if the absorbance of the reagent is greater than 0.600 when measured at 405nm against water in a cuvette with a 1cm path length or if the reagent fails to meet stated parameters of performance.

Do not use if the reagent is turbid or displays other evidence of bacterial contamination.

ADDITIONAL MATERIALS REQUIRED

A spectrophotometer or colorimeter capable of reading absorbance accurately at 405 nm.

1 cm cuvettes or a flow cell capable of transmitting light at 405 nm.

Test tubes capable of holding 2 ml.

Pipettes capable of delivering 1 ml and 25 µl.

Timer with 30 second increments.

Constant temperature heat source which can be adjusted to 37° C.

King normal and abnormal controls for quality control.

TEST PROCEDURE

The following is a general procedure for use on a manual instrument.

PROCEDURE CONDITIONS

Wavelength	405 nm
Temperature	37° C
Pathlength	1 cm
Mode	Kinetic
Reaction Time	2 min.
Sample Volume	25 µl
Reagent volume	1.0 ml
Total Volume	1.025 ml
Sample to reagent ratio	1/40

INSTRUMENT

Any instrument capable of reading absorbance accurately with a sensitivity of 0.001 absorbance at 405 nm may be used. The band width should be 10 nm or less, stray light 0.5% or less, and the wavelength accuracy within 2 nm.

CALIBRATION

No reagent calibration is necessary as this method is standardized by means of the molar absorptivity of 2-chloro-p-nitrophenyl taken as 12.9 at 405nm under the test conditions described.

PROCEDURE

Pipette 1.0ml of reagent into tubes labelled "control", "patient", etc. DO NOT PIPETTE BY MOUTH.

Pre-incubate all tubes at 37°C for at least five minutes.

Zero spectrophotometer with water at 405nm.

Add 0.025ml (25µl) of sample and read after 30 seconds.

Record the absorbance at 30 second intervals for 2 minutes.

CALCULATION AND RESULTS

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Amylase U/L =

$$\frac{\Delta A/min \times assay\ volume\ (ml) \times 1000}{12.9 \times light\ path\ (cm) \times sample\ volume\ (ml)} = \Delta A/min \times 3178$$

ΔA/min = change in absorbance per minute
assay volume = 1.25 (ml)
1000 = converts U/ml to U/L
12.9 = absorbance coefficient of 2-chloro-p-nitrophenyl at 405 nm
lightpath = 1 (cm)
sample volume = 0.025 (ml)
3178 = factor derived from constants in the equation
Example:

$$\text{Amylase U/L} = \frac{0.019 \times 1.02 \times 1000}{12.9 \times 1 \times 0.02} = 0.019 \times 3178 = 60\ U/L$$

EXPECTED VALUES

The range of expected values is: 25 - 125 U/L (37 degrees C)
These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located.

MEDICAL ALERT VALUES

Each laboratory should establish low and high values beyond which the patient would require immediate attention by a physician. If a "medical alert value" is reached, always repeat the test to confirm the result and notify a physician if the result is confirmed.

LIMITATIONS OF PROCEDURE

Young gives a list of drugs and other substances that interfere with the determination of amylase activity.

QUALITY CONTROL

Standard practice for quality control should be applied to this system. Commercially available lyophilized controls can be used to monitor the daily acceptable variations. Normal and abnormal controls should be assayed at the beginning of each run of patient samples, whenever a new reagent or a different lot number is being used, and following any system maintenance.
A satisfactory level of performance is achieved when the analyte values obtained are within the "acceptable range" established by the laboratory.

CALIBRATION PROCEDURES

No routine reagent calibration is necessary as this method is standardized by means of the molar absorptivity of 2-chloro-p-nitrophenyl taken as 12.9 at 405nm under the test conditions described.
The results obtained when measuring the activity of a kinetic reaction are based on the change in absorbance per minute. In order to accurately monitor and report this reaction rate, the operating parameters of the spectrophotometer (wavelength, temperature of the reaction and timing of the test) must be known and controlled.

PERFORMANCE CHARACTERISTICS

PRECISION

The estimates of precision shown below were performed following the guidelines contained in NCCLS document EP5-T2.

Within-Run

Mean (U/L)	SD (U/L)	CV (%)
14	± 0.5	3.6
106	± 0.7	0.7
418	± 1.1	0.3
1392	± 7.8	0.6

Between-Run

Mean (U/L)	SD (U/L)	CV (%)
15	± 0.7	4.7
109	± 2.3	2.1
421	± 5.6	1.3
1413	± 16.6	1.2

CORRELATION

A correlation study was done comparing this method (y) and a similar comparative method (x). The samples (n=125) ranged from 32 to 2112 U/L. The study yielded a regression curve of x = 0.98x + 5.4 with a correlation of 0.999.
Note: CNPG3 values greater than 2000 U/L were run after dilution with an equial volume of saline.

LINEARITY

Linearity: This procedure is linear through 2000 U/L beyond which the specimen should be diluted with an equal volume of saline. Reassay the specimen and multiply the results by 2.

SENSITIVITY

A change in absorbance of 0.003 ΔA/min at 405nm at 37° C corresponds to 9.53 U/L.

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