AMYLASE MR CE

CONTENTS			
REF	1107005	α-Amylase	5 x 20 mL
For *in vitro* diagnostic use only			

α-AMYLASE MR

Colorimetric enzymatic method

KINETIC

PRINCIPLE

In this direct method[1,2] α-amylase catalyzes the hydrolysis of 2-chloro-p-nitrophenyl-α-D-maltotrioside (CNP-G3) substrate at pH 6.0 forming 2-chloro-p-nitrophenol (CNP) and free glycosides.
The reaction is monitored kinetically at 405 nm by the rate of formation of the colored CNP produced, proportional to the activity of the α-amylase in the sample.

$$10\ \text{CNP-G3} \xrightarrow[\text{pH 6.0}]{\alpha\text{-AMYLASE}} 9\ \text{CNP} + 1\text{CNP-G2} + \text{G3} + \text{G}$$

CNP-G2 = 2- Chloro-nitrophenyl-α-D-maltoside
G3 = Maltotriose
G = Glucose

REAGENT COMPOSITION

R1 **Monoreagent**. MES buffer 50 mmol/L pH 6.0, calcium acetate 5 mmol/L, sodium chloride 300 mmol/L, sodium thiocyanate 450 mmol/L, CNP-G3 2.25 mmol/L.

STORAGE AND STABILITY

Store at 2-8ºC.
The Monoreagent is stable until the expiry date stated on the label. Avoid contamination and recap the vials immediately after use (See Notes).

REAGENT PREPARATION

The Monoreagent is ready-to-use.

SAMPLES

Serum, heparinized plasma and urine.
Serum and plasma α-amylase is stable for 30 days at 2-8ºC.
Random urine samples should be clear and precipitate free for testing. Check the pH. Urines with a pH < 5 may reduce the enzyme stability. Stable for 10 days at 2-8ºC.

INTERFERENCES

- Common anticoagulants, as citrate, oxalate or EDTA inhibit the enzyme.
- Bilirubin (> 20 mg/dL) and lipids (triglycerides > 10 g/L) do not interfere.
- Other drugs and substances may interfere[3].

MATERIALS REQUIRED

- Photometer or spectrophotometer with a thermostatted cell compartment set at 37ºC, capable of reading at 405 nm.
- Stopwatch, strip-chart recorder or printer.
- Cuvettes with 1-cm pathlength.
- Pipettes to measure reagent and samples.

PROCEDURE

1. Preincubate working reagent, samples and controls to reaction temperature.
2. Set the photometer to 0 absorbance with distilled water.
3. Pipette into a cuvette:

Reaction temperature	37ºC	
Monoreagent	1.0 mL	1.0 mL
Serum/plasma	20 µL	–
Urine	–	10 µL

4. Mix gently by inversion. Insert cuvette into the cell holder and start stopwatch.
   Incubate for 1 minute and record initial absorbance reading.
5. Repeat the absorbance readings exactly after 1, 2 and 3
6. minutes.
7. Calculate the difference between absorbances.
8. Calculate the mean of the results to obtain the average change in absorbance per minute (ΔA/min).

CALCULATIONS

Serum, plasma

U/L = ΔA/min x 3591

Urine

U/L = ΔA/min x 7113

ε_{405}^{CNP} = 14.2 (Toyobo Biochemicals)

Samples with ΔA/min exceeding 0.500 at 405 nm should be diluted 1:10 with saline and assayed again. Multiply the results by 10.

If results are to be expressed as SI units apply:
U/L x 0.01667 = µkat/L

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REFERENCE VALUES[4]

Serum, plasma

< 86 U/L (1.43 μkat/L)

Urine

< 470 U/L (7.83 μkat/L)

It is recommended that each laboratory establishes its own reference range.

QUALITY CONTROL

To ensure adequate quality control (QC), each run should include a set of controls (normal and abnormal) with assayed values handled as unknowns.

REF **1980005** HUMAN MULTISERA NORMAL
Borderline level of α-amylase. Assayed.

REF **1985005** HUMAN MULTISERA ABNORMAL
Elevated level of α-amylase . Assayed.

CLINICAL SIGNIFICANCE

Amylase activity tests in serum and urine are mainly used in the diagnosis of diseases of the pancreas and in the investigation of pancreatic function.
Amylase is found chiefly in the saliva and in pancreatic tissue. Normally, small amounts of amylase are present in the blood, but with various forms of pancreatic disturbance large amounts of amylase are secreted into the blood by the pancreas.

The activity of the amylase in serum may fluctuate rapidily rising acutely during an attack and subsiding to normal levels shortly afterward.

Increased levels are found associated with acute pancreatitis, pancreatic duct obstrution, intra-abdominal diseases, mumps and bacterial parotitis.
A significant amount of the serum amylase is excreted in the urine, and as a result elevation of serum activity is reflected in the rise of urinary amylase activity. Urine amylase appears to be more frecuently elevated, reaches higher levels, and persists for longer periods.[4]

ANALYTICAL PERFORMANCE

- ***Linearity.*** Up to 1000 U/L
- ***Precision***

U/L	Within-run			Between-run		
Mean	50.3	134.1	306	50.3	129	320
SD	0.78	2.31	2.79	1.16	2.6	3.1
CV%	1.55	1.72	0.65	2.32	2.01	0.96
N	10	10	10	10	10	10

Replicates: 10 for each level. Replicates: 10 for each level
Instrument: CECIL CE 2001 for 8 days.

- ***Sensitivity*****.** Using this reagent and method an ΔA/min of 0.001 read at 405 nm is equivalent to 5 U/L of amylase activity.
- ***Correlation*****.** This assay (y) was compared with a similar commercial method (x). The results were:
N = 30 r = 0.998 y = 0.9241x + 5.237

NOTES

- Do not pipette by mounth, use gloves and avoid contact with skin. They both contain amylase.

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