



# $\alpha$ -AMYLASE - L



Kinetic-colorimetric determination of  $\alpha$ -Amylase  
in biological fluids



## ORDER INFORMATION

REF	Kit size
GA4175 00	5x20 ml
KL4175 00	12x20 ml
BK4175 00	2x40 ml

## INDICATION

$\alpha$ -Amylase is an enzyme that is secreted by the salivary and pancreatic glands. It is important for the digestion of starches and is rapidly cleared by the kidneys.

Increased values of Amylase are found in acute pancreatitis, obstruction of the pancreatic ducts, and (mildly) in obstruction of the parotid gland. Decreased values are found in acute or chronic hepatocellular damage.

## METHOD PRINCIPLE

$\alpha$ -Amylase catalyzes hydrolysis of 2-chloro-4-nitrophenyl- $\alpha$ -maltotriose (CNP-G3) to glucose polymers and p-nitrophenyl oligosaccharide at short chain producing 2-chloro-4-nitrophenol (CNP).

The increased extinction can be measured by spectrophotometry at 405 nm and results proportional to the activity of  $\alpha$ -Amylase in the sample .

## COMPOSITION

### REAGENT A:

CNP-G3	2 mmol/l
Sodium chloride	250 mmol/l
Calcium chloride	6 mmol/l
MES buffer	100 mmol/l
Potassium thiocyanate	600 mmol/l
Sodium azide	< 0.1%

### Preparation:

The reagent is liquid ready to use.

### Storage and stability

Store at 2-8 °C. Do not freeze the reagents! The reagents are stable until the expiry date, if contamination and evaporation are avoided, protected from light. The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature.

Saliva and skin contain  $\alpha$ -Amylase: **never pipette by mouth and avoid skin contact with reagents.**

## ANCILLARY EQUIPMENT

- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- Temperature controlled water bath
- NaCl solution 9 g/l

## SAMPLES

Serum, heparin plasma and urine.  
Stable 7 days at 2-8 °C or 30 days at -20 °C.

### Specimen collection / Preanalytical factors

It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

## INTERNAL QUALITY CONTROL

It is recommended to use commercial Quality Control sera with known  $\alpha$ -Amylase activity. Check that the values obtained are within the reference range provided.

## ANALYTICAL PROCEDURE

Working temperature	37 °C
Wavelength	405 nm (400-410 nm)
Optical path	1 cm
Reaction	Kinetic (increase)

Allow the reagents to reach working temperature before using.

Pipette into disposable or well clean cuvettes:

	Reagent Blank	Sample
Reagent A	1000 $\mu$ l	1000 $\mu$ l
Distilled H <sub>2</sub> O	25 $\mu$ l	-
Sample	-	25 $\mu$ l

Mix and incubate for 1 minute at 37 °C.

Read initial absorbance and repeat the absorbance reading after 1, 2, 3 minutes against reagent blank. Calculate  $\Delta A/\text{min}$ .

## CALCULATION OF RESULTS

Activity (U/l) =  $\Delta A/\text{min} \times 3178$

## REFERENCE VALUES

Serum or plasma	up to 90 U/l
Urine	up to 480 U/l

Each laboratory should establish reference ranges for its own patients population.

## ANALYTICAL PERFORMANCES

### Precision

Within-run and between-run coefficients of variation have been calculated on replicates of three samples at different enzymatic activities. The obtained results are reported in the following tables:

Sample	Mean (U/l)	Within Run		Between Run	
		SD	%CV	SD	%CV
Serum 1	72.50	0.92	1.3	1.74	2.4
Serum 2	124.80	1.19	1.0	4.94	4.0
Serum 3	175.32	1.74	1.0	6.13	3.5

### Linearity

The assay is linear up to 1500 U/l.

### Sensitivity

Test sensitivity, in terms of limit of detection, is 3 U/l.

### Correlation

A correlation study comparing the present method an a commercial one gave the following results:

$$y = 1.145x + 1.600 \text{ U/l} \quad r = 0.9967$$

### Interferences

Hemoglobin	> 500 mg/dl
Bilirubin	> 40 mg/dl
Triglycerides	> 1500 mg/dl

**PRECAUTIONS IN USE**

The reagents contain inactive components such as preservatives (Sodium Azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.

The use of laboratory reagents according to good laboratory practice is recommended.

**Waste Management**

Please refer to local legal requirements.

**BIBLIOGRAPHY**

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4. International Federation of Clinical Chemistry and Laboratory Medicine (IFCC): "Approved Recommendation on IFCC Methods for the measurement of catalytic concentration of enzymes – Part 9. IFCC Method for  $\alpha$ -Amylase (1,4- $\alpha$ -D-Glucan 4-Glucanohydrolase, EC 3.2.1.1)" – Clin Chem Lab Med 1998; 36(3):185-203.
5. EU-Dir 1999/11 Commission Directive of 8 March 1999 adapting to technical progress the principles of good laboratory practice as specified in Council Directive 87/18/EEC.
6. NCCLS Document, "Procedures for the collection of arterial blood specimens", Approved Standard, 3rd Ed. (1999).