

Amilasa 405

AA

Kinetic method at 405 nm for the determination of amylase in serum, plasma or urine. CNPG3 substrate



SUMMARY

Amylase, mainly produced in the exocrine fraction of the pancreas and salivary glands, splits α -1-4 glycosidic bonds of polysaccharides (starch and glycogen).

Serum amylase levels increase in patients with acute pancreatitis, reaching its highest values between 24 and 30 hours after onset, then returning to normal levels during the 24 to 48 following hours. In this case the urinary output of the enzyme is also increased, hyperamylasuria lasting 3 to 5 days, once serum activity has reached normal levels.

It is also possible to find increased values in cases of "acute abdomen" or surgical operation surrounding the pancreas. Both bacterial parotiditis and mumps, which obstruct salivary amylase secretion, are also related to increases of serum amylase levels.

PRINCIPLE

α -amylase hydrolyzes the 2-chloro-p-nitrophenyl- α -D-maltotrioxide (CNP-G3) definite substrate to release 2-chloro-p-nitrophenol (CNP), resulting in 2-chloro-nitrophenyl- α -D-maltotrioxide (CNP-G2), maltotriose (G3) and glucose. CNP absorbs at 405 nm and color development is directly proportional to enzyme activity.

The use of a definite substrate, a substance of known structure and molecular weight, enables expression of results in U/l and does not require additional enzymes.

PROVIDED REAGENTS

A. Reagent A: solution containing 2.25 mmol/l CNP-G3, 5 mmol/l calcium chloride, 70 mmol/l sodium chloride, 900 mmol/l potassium thiocyanate and MES buffer pH 6, 100 mmol/l.

INSTRUCTIONS FOR USE

Reagent A: ready to use.

WARNINGS

The Reagent A is for "in vitro" diagnostic use.

H315+H320: Causes skin and eye irritation. P262 Do not get in eyes, on skin, or on clothing. P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P302 + P352 IF ON SKIN: Wash with plenty of soap and water. P280 Wear protective gloves/protective clothing/eye protection/face protection. Use the reagents according to the working procedures for clinical laboratories.

The reagents and samples should be discarded according to the local regulations in force.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagent: stable in refrigerator (2-10°C) until the expiration date shown on the box.

INSTABILITY OR DETERIORATION OF REAGENTS

The Reagent A may develop a yellowish coloration that does not affect its performance. Suspect deterioration of Reagent A if absorbance readings are higher than 0.500 O.D. (at 405 nm) after setting instrument to zero with distilled water.

SAMPLE

Serum, heparinized plasma or urine

a) Collection: if serum is used, collect in the usual way. Separate serum from clot as soon as possible. In case plasma is used, it should be heparinized. If urine is used, assay can be performed on an occasional urine sample.

b) Additives: in case plasma is used, use heparin for collection. If urine is used, refer to d).

c) Known interfering substances: no interferences are observed by: bilirubin up to 22 mg/dl (220 mg/l), hemoglobin up to 180 mg/dl, triglycerides up to 1400 mg/dl (14 g/l), nor heparin up to 50 U/ml.

Do not add hydrochloric acid as preservative for urine.

See Young, D.S. in References for effect of drugs on the present method.

d) Stability and storage instructions: serum amylase is stable for one week at room temperature (provided bacterial contamination is avoided) or for several months in refrigerator.

If urine amylase sample is not assayed on the same day, it is advisable to set pH at about 7 (with sodium hydroxide) since acid pH irreversibly inactivates the enzyme. With a pH 7, sample could be kept for at least 10 days in refrigerator without any loss of activity, provided there is no bacterial contamination.

REQUIRED MATERIAL (non-provided)

- Spectrophotometer
- Micropipettes and pipettes to measure stated volumes
- Spectrophotometric square cuvettes
- Water bath at selected reaction temperature
- Stopwatch

ASSAY CONDITIONS

- Wavelength: 405 nm
- Reaction temperature: 25, 30 or 37°C
- Reaction time: 2 minutes

PROCEDURE

A) 25-30°C

In a cuvette at the selected temperature, place:

Reagent A _____ 2 ml

Preincubate 3-4 minutes. Then add:

Sample _____ 100 ul

Mix at once and read absorbance after 1 and 2 minutes. Determine the difference between second and first readings. Use this value for calculations. Volumes can be proportionally reduced using 1 ml Reagent A and 50 ul Sample.

B) 37°C

Since the activity is greater at this temperature, use 50 ul as sample. Follow the same procedure as indicated in A). Volumes can be reduced using 1 ml Reagent A and 20 ul Sample.

CALCULATIONS

Amylase (U/l) = $\Delta A/\text{min} \times \text{factor}^*$

Temperature	Reagent A	Sample	Factor
25-30°C	2 ml	100 ul	1,628
	1 ml	50 ul	1,628
37°C	2 ml	50 ul	3,178
	1 ml	20 ul	3,953

*the factors are calculated following the formula below:

$$\text{Factor} = \frac{TV}{SV \times b \times \epsilon_{\text{CNP}} \times 10^{-3}}$$

where:

TV: total volume

SV: sample volume

b: light pass

ϵ_{CNP} : CNP milimolar absorption coefficient

10^{-3} : conversion factor (milimolar to micromolar)

QUALITY CONTROL METHOD

Each time the test is performed, analyze two levels of a quality control material (**Standatrol S-E 2 niveles**) with known amylase activity.

REFERENCE VALUES

Temperature	25°C	30°C*	37°C
Serum up to	84 U/l	100 U/l	125 U/l
Occasional urine up to**	455 U/l	540 U/l	680 U/l

*Calculated

**These reference values were obtained from a healthy population (n = 40), of both sexes, aged between 17 and 40 years old, with a normal diet and with no symptoms of apparent disease.

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

SI SYSTEM UNITS CONVERSION

Amylase (U/l) \times 0.017 = Amylase (ukat/l)

PROCEDURE LIMITATIONS

See Known interfering substances under SAMPLE.

Never pipette by mouth.

The contamination of the Reagent A with saliva constitutes cause of erroneous results, since it contains elevated amylase activity. In this case, discard the Reagent.

Avoid the contact with rubber elements (rubber caps, inside covers) which deteriorate the Reagent A.

PERFORMANCE

a) Reproducibility: simultaneously processing replicates of the same sample in the same day, the following values were obtained:

Level	S.D.	C.V.
51 U/l	\pm 0.978 U/l	1.9 %
467 U/l	\pm 2.139 U/l	0.46 %

b) Sensitivity: depends on the photometer used. In spectrophotometer at 405 nm (with 1 cm optical length square cuvettes) for $\Delta A/\text{min}$ of 0.001, the smallest detectable activity change will be of 4 U/l (at 37°C).

c) Linearity: the reaction is linear up to 2000 U/l amylase activity. For higher values use sample diluted with saline solution, repeat the test and multiply the results by the dilution factor.

PARAMETERS FOR AUTOANALYZERS

For programming instructions check the user manual of the autoanalyzer in use.

WIENER LAB PROVIDES

- 3 x 10 ml (Cat. N° 1021404).
- 3 x 10 ml (Cat. N° 1009326).
- 4 x 20 ml (Cat. N° 1009243).
- 4 x 20 ml (Cat. N° 1009603).

REFERENCES

- Rauscher, E. et al - Clin. Chem. 31/1:14, 1985.
- Tietz, N. - Clinical Guide to Laboratory Tests - W.B. Saunders Co., 1983.
- Lorenzo, L.; Demaría, I.; Setta, F.; Taborda, M. - 44th National Meeting, AACC, 19-23 julio, 1992, Chicago, Illinois. Clin. Chem. 38/6:935, Abs. 3, 1992.
- Sociedad Española de Bioquímica Clínica y Patología Molecular - Química Clínica 15/1:51, 1996.
- Young, D.S. - "Effects of Drugs on Clinical Laboratory Tests", AACC Press, 4th ed., 2001.

Symbols

The following symbols are used in the packaging for Wiener lab. diagnostic reagent kits.



This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices



Authorized representative in the European Community



"In vitro" diagnostic medical device



Contains sufficient for <n> tests



Use by



Temperature limitation (store at)



Do not freeze



Biological risks



Volume after reconstitution



Contents



Batch code



Manufactured by:



Harmful



Corrosive / Caustic



Irritant



Consult instructions for use



Calibrator



Control




Positive Control



Negative Control



Catalog number

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