Amilasa 405

cinética unitest/AA

Kinetic method at 405 nm for the determination of amylase in biological fluids with definite substrate

SUMMARY

Amylase, mainly produced in the exocrine fraction of 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 48 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 operations 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-maltotrioside (CNP-G3) definite substrate to release 2-chloro-p-nitrophenol (CNP), resulting in 2-chloro-nitrophenyl-α-D-maltoside (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: vials containing CNP-G3.
B. Reagent B: MES buffer pH 6; 100 mmol/l.

Final concentrations

CNP-G3	2.25 mmol/l
Calcium acetate	6 mmol/l
Sodium chloride	70 mmol/l
MES	100 mmol/l
Potassium tiocyanate	900 mmol/l

INSTRUCTIONS FOR USE

Reagent B: ready to use.
Reagent A; preparation: reconstitute each vial with the Reagent B volume indicated on the label. Cap and shake until complete dissolution.

WARNINGS

Reagents are for "in vitro" diagnostic use.

Reagent B contains potassium thiocyanate. 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.

To reduce possible reagent contamination with salivary amylase, neither Reagent B nor reconstituted Reagent A should be pipetted with the mouth.

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 Reagents: stable in refrigerator (2-10°C) until the expiration date shown on the box.
Reconstituted Reagent A: stable for 60 days refrigerated (2-10°C) and 15 days at room temperature.

INSTABILITY OR DETERIORATION OF REAGENTS

Suspect deterioration of reconstituted 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, 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 the case plasma is used, use heparin for collection. If urine is used, refer to d).

c) Known interfering substances: hemolysis, anticoagulants (citrate, oxalate and EDTA). Do not add hydrochloric acid as preservative for urine.

No interferences are observed from bilirubin up to 200 mg/l, triglycerides up to 13 g/l, hemoglobin up to 0.5 g/dl.

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

d) Stability and storage instructions: serum amylase is stable one week at room temperature (provided bacterial contamination is avoided) or 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 can be kept at least 10 days in refrigerator without 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:

Reconstituted Reagent A	2 ml

Pre-incubate 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 reconstituted Reagent A and 50 ul Sample.

B) 37°C

Since the activity is higher at this temperature, use 50 ul as sample. Follow the same procedure as indicated in A).

Volumes can be reduced using 1 ml reconstituted Reagent A and 20 ul Sample.

CALCULATIONS

Amylase (U/l) = ΔA/min x factor*

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

*the factors are calculated following the formula below:

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

where:
TV: total volume
SV: sample volume
b: light pass
ε_{CNP}: CNP milimolar absorption oefficient
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 box 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) x 0.017 = Amylase (ukat/l)

PROCEDURE LIMITATIONS

See Known interfering substances under SAMPLE.
Never pipette using the mouth.
The contamination of the Reagent 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.
50.6 U/l	± 1.76 U/l	3.48 %
417.3 U/l	± 6.29 U/l	1.51 %

Performing the same assay on different days, the following results were obtained:

Level	S.D.	C.V.
48.0 U/l	± 2.65 U/l	5.53 %
389.9 U/l	± 7.62 U/l	1.95 %

b) Detection limit: depends on the photometer used. According to the required sensitivity, in spectrophotometer at 405 nm (with 1 cm optical length square cuvettes, ± 2 nm reproducibility, ≤ 0.5% stray light, ≤ 8 nm pathlength) for ΔA/min of 0.001, the smallest detectable activity change will be of 1.6 U/l (at 25°C).

c) Dynamic range: the reading range is extended up to 0.250 O.D. For higher values use sample diluted with saline solution (for urine dilute 1/10) and correct results accordingly.

PARAMETERS FOR AUTOANALYZERS

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

WIENER LAB PROVIDES

Amilasa 405 cinética unitest:
- 20 x 2 ml (40 ml Reagent B) (Cód. 1021402).

Amilasa 405 cinética AA:
- 3 x 10 ml (40 ml Reagent B) (Cód. 1021403).

REFERENCES

- Rauscher, E. et al - Clin. Chem. 31/1:14 (1985).
- Tietz, N. - Fundamentals of Clinical Chemistry - W.B. Saunders Co. (1970).
- 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 reagents kits.

CE	This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices
EC REP	Authorized representative in the European Community
IVD	"In vitro" diagnostic medical device
Σ	Contains sufficient for <n> tests
	Use by
	Temperature limitation (store at)
	Do not freeze
	Biological risks
⟶	Volume after reconstitution
Cont.	Contents
LOT	Batch code
	Manufactured by:
	Harmful
	Corrosive / Caustic
!	Irritant
	Consult instructions for use
Calibr.	Calibrator
CONTROL	Control
CONTROL +	Positive Control
CONTROL −	Negative Control
REF	Catalog number

Wiener Laboratorios S.A.I.C.
Riobamba 2944
2000 - Rosario - Argentina
http://www.wiener-lab.com.ar
Dir. Téc.: Viviana E. Cétola
Biochemist

A.N.M.A.T. Registered product
Amilasa 405 cinética unitest:
disp. Nº: 2525/95 - 334/00
Amilasa 405 cinética AA:
disp. Nº: 5234/98 - 967/02

Wiener lab.

2000 Rosario - Argentina