Fosfatasas Acida

Prostática cinética

Kinetic method at 405 nm for the determination of prostatic acid phosphatase in serum. Substrate: α-naphthyl phosphate

SUMMARY

Acid phosphatases are present in almost all body tissues, being the quantity of these enzymes specially high in prostate, stomach, liver, muscle, spleen, erythrocytes, and platelets.
The various isoenzymes differentiate between each other by their optimal pH, molecular weight, and activators and inhibitors requirements.
The prostatic acid phosphatase (PAcP) constitutes a valuable tool for the early diagnosis of prostate cancer, one of the neoplasm forms with highest morbility.
The kinetic determination of PAcP using a-naphthyl phosphate as substrate, has shown a sensitivity and a specificity comparable to radioimmunological techniques, having similar discriminatory capability and evident practical advantages.

PRINCIPLE

The PAcP (E.C.3.1.3.2.) hydrolyzes the α-naphthyl phosphate at pH 5.2, producing phosphate and α-naphthol. In turn, the naphthol reacts with a diazo reagent present in the 4-chloro-toluene-1,5-diazo α-naphthalenedisulfonato (4-CTD) system, producing a yellow pigment. Therefore, the increase of absorbance, read at 405 nm, is proportional to the phosphatase activity of the sample.

PROVIDED REAGENTS

A. Reagent A: tablets containing 6 umol a-naphthyl phosphate (NP) and 2 umol 4-CTD each.
B. Reagent B: 0.1 mol/l citrate buffer with 14 mmol/l activator (1.5-pentanediol + butanol).

Final concentrations

NP	3 mmol/l
4-CTD	1 mmol/l
Citrate buffer	0.1 mol/l, pH 5.2

INSTRUCTIONS FOR USE

Reagent A; preparation: for each determination dissolve a Reagent A tablet in 2 ml Reagent B, gently shaking until complete dissolution.

WARNING

Reagents are for “in vitro” diagnostic use.
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 (2-10ºC) until the expiration date shown on the box. Long exposures to room temperature may deteriorate the Reagent A.
Reconstituted Reagent A: is stable 24 hours in refrigerator (2-10ºC) or 12 hours at room temperature.

INSTABILITY OR DETERIORATION OF REAGENTS

Readings above 0.250 O.D. of the reconstituted Reagent A at 405 nm, against water, are a sign of the deterioration of the reagents. In that case, discard.

SAMPLE

Serum
a) Collection: obtain serum free from hemolysis. Do not use plasma. Prostatic acid phosphatase is very instable “in vitro” and at serum pH at room temperature there can be a loss of activity up to 50% in a few hours. Therefore, the serum should be separated from the clot within 1 hour from the extraction, keeping it refrigerated until use.
b) Additives: to avoid the inactivation during the storage, the sample can be acidified by adding 20 ul acetate buffer, 5 M, pH 5, per each ml of serum. This preservative is prepared by adding concentrated sodium hydroxide at 29 ml p.a. glacial acetic acid to obtain a pH 5, completing 100 ml with distilled water.
c) Known interfering substances:
- Intensely icteric samples show a low recovery of the enzymatic activity, thus, their use should be avoided.
- Do not use serum with visible hemolysis.
- Anticoagulants interfere with the reaction, so plasma should not be used in the determination.

Refer to Young, D.S. in references for drugs’ effect on the present method.
d) Stability and storage instructions: if the preservative mentioned in b) is used, the sample can be refrigerated (2-10ºC) for several days with no significant loss of activity. If this preservative is not used, the sample must be processed immediately.

REQUIRED MATERIAL (non-provided)
- Spectrophotometer.
- Micropipettes and pipettes for measuring stated volumes.
- Spectrophotometer cuvettes.
- Stopwatch.

ASSAY CONDITIONS
- Wavelength: 405 nm

- Reaction temperature: 25, 30 or 37ºC
- Reaction time: 4-5 minutes
- Sample volume: 200 ul
- Final reaction volume: 2.2 ml

PROCEDURE

In a cuvette kept at the selected temperature, place:

Reconstituted Reagent A	2 ml
Preincubate a couple of minutes. Then add:	
Sample	200 ul

Simultaneously start the stopwatch. At 4 minutes record the O.D. Then, read the absorbance each minute for 3 minutes. Determine the average difference absorbance/minute (ΔA/min) subtracting each reading from the previous one and averaging the values. Use this mean for the calculations.

CALCULATIONS

Prostatic acid phosphatase (U/l) = ΔA/min x 853
(405 nm; sample/substrate ratio 1:10)

EXPECTED VALUES

The PAcP activity is scarce in a healthy individual and almost null in women, thus, the expected values are below the instrumental error limit or very close to the system's detection limit.

Temperature	25ºC	30ºC	37ºC
PAcP (U/l)	0 - 2.6	0 - 3.0	0 - 3.5

When values are 60% above the normal superior value, the presence of prostate cancer must be suspected.

PROCEDURE LIMITATIONS

- It is known that enzymes are sensitive to the action of certain contaminants and enzymatic poisons (heavy metals, cyanides, surfactants, etc.). Therefore, it is recommended to extreme precautions in the cleaning of the material used in the samples extraction and in the determination.
- Tablets (Reagent A) may show a pink coloration, or small spots on their surface, which do not alter their reactivity.
- Iatrogenic alterations: some medicines may affect the PAcP plasma values, as well as massage, catheterization, and other prostate manipulations. Therefore, it is convenient to ask the patient or the physician about the medication, treatment or diagnosis procedure to which the patient is subjected.

PERFORMANCE

a) Reproducibility: simultaneously processing replicates of one sample, the following results were obtained:

Level	S.D.	C.V.
10 U/l	± 0.51 U/l	5.1 %
52 U/l	± 0.71 U/l	1.3 %

b) Linearity: the reaction is lineal up to 128 U/l (Δ/min = 0.150 O.D.). For higher values, repeat the determination diluting the sample 1:2 or 1:5 with saline solution, correcting the results accordingly.

c) Detection limit: depends on the wavelength and the photometer used. According to the required sensitivity, in spectrophotometers (with 1 cm optical length square cuvettes, ± 2 nm reproducibility, ≥ 0.5% stray light and ≥ 8 nm pathlength) for a ΔA/min of 0.001 the minimum detectable change of activity will be 0.8 U/l.

WIENER LAB. PROVIDES

Kit for 20 tests (Cat. 1351401).

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Symbols

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

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