Enzymatic Assay of PHOSPHATASE, ACID, PROSTATIC  
(EC 3.1.3.2)

PRINCIPLE:

\[ \text{Prostatic Acid Phosphatase} \]
\[ p\text{-Nitrophenyl Phosphate} + H_2O \rightarrow p\text{-Nitrophenol} + P_i \]

Abbreviation used:

\( P_i \) = Inorganic phosphate

CONDITIONS:  \( T = 37^\circ\text{C}, \quad pH = 4.8, \quad A_{410nm}, \text{ Light path } = 1 \text{ cm} \)

METHOD:  Spectrophotometric Stop Rate Determination

REAGENTS:

A.  90 mM Citrate Buffer Solution, pH 4.8 at 37\(^\circ\)C  
(Use Citrate Buffer Solution, Sigma Stock No. 104-4.)

B.  40 mM Tartrate Buffer, with 90 mM Citrate, pH 4.8 at 37\(^\circ\)C.  
(Use Tartrate Acid Buffer Solution, Sigma Stock No. 104-12.)

C.  11.3 mM \( p\)-Nitrophenyl Phosphate (PNPP)  
(Prepare 5 ml in deionized water using \( p\)-Nitrophenyl Phosphate Sigma 104 Phosphatase Substrate, Sigma Stock No. 104-0.)

D.  100 mM Sodium Hydroxide Solution (NaOH)  
(Prepare 50 ml in deionized water using Sodium Hydroxide, Anhydrous Sigma Prod. No. S-5881.)

E.  Prostatic Acid Phosphatase Enzyme Solution  
(Immediately before use, prepare a solution containing 0.015 - 0.02 unit/ml of Acid Phosphatase in cold deionized water.)
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PROCEDURE:

Pipette (in milliliters) the following reagents into suitable containers:

<table>
<thead>
<tr>
<th>Test1</th>
<th>Test2</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Citrate Buffer)</td>
<td>0.50</td>
<td>------</td>
</tr>
<tr>
<td>Reagent B (Tartrate Buffer)</td>
<td>------</td>
<td>0.50</td>
</tr>
<tr>
<td>Reagent C (PNPP)</td>
<td>0.50</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Mix and equilibrate to 37°C. Then add:

<table>
<thead>
<tr>
<th>Test1</th>
<th>Test2</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent E (Enzyme Solution)</td>
<td>0.20</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Immediately mix and incubate at 37°C for exactly 30 minutes. Then add:

| Reagent D (NaOH) | 3.80  | 3.80  |
| Reagent E (Enzyme Solution) | ------ | ------ | 0.20 |

Mix and record the $A_{410nm}$ for both the Tests and Blank in a suitable spectrophotometer.

CALCULATIONS:

Total Acid Phosphatase Activity:

$$\text{Units/ml Total enzyme} = \frac{(\Delta A_{410nm/min \ Test 1} - \Delta A_{410nm/min \ Blank})(5.0)(df)}{(30)(18.3)(0.2)}$$

Nonprostatic Acid Phosphatase Activity:

$$\text{Units/ml Nonprostatic enzyme} = \frac{(\Delta A_{410nm/min \ Test 2} - \Delta A_{410nm/min \ Blank})(5.0)(df)}{(30)(18.3)(0.20)}$$

Prostatic Acid Phosphatase Activity:

$$\text{Units/ml Prostatic Enzyme} = \text{Units/ml Total Enzyme} - \text{Units/ml Nonprostatic Enzyme}$$
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(E C 3.1.3.2)

CALCULATIONS: (continued)

5.0 = Total volume (in milliliters) of solution
30 = Time of assay (in minutes)
18.3 = Millimolar extinction coefficient of p-Nitrophenol at 410 nm at an Alkaline pH
0.2 = Volume (in milliliter) of enzyme used
df = Dilution factor

\[
\text{units/ml Prostatic Enzyme} = \frac{\text{Units/mg solid}}{\text{mg solid/ml Total Enzyme}}
\]

\[
\text{units/ml Prostatic Enzyme} = \frac{\text{Units/mg protein}}{\text{mg protein/ml Total Enzyme}}
\]

UNIT DEFINITION:

One unit will hydrolyze 1.0 \( \mu \)mole of p-nitrophenyl phosphate per minute at pH 4.8 at 37°C. Prostatic acid phosphatase activity is the difference between the total acid phosphatase activity and the acid phosphatase activity in the presence of 20 mM tartrate.

FINAL ASSAY CONCENTRATION:

In a 1.20 ml reaction mix, the final concentrations are 38 mM citric acid, 4.7 mM p-nitrophenyl phosphate and 0.004 unit prostatic acid phosphatase.

NOTES:

1. Tartrate Buffer negates roughly 95% of the prostatic acid phosphatase.

2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

This procedure is for informational purposes. For a current copy of Sigma’s quality control procedure contact our Technical Service Department.