Enzymatic Assay of PHOSPHATASE, ACID  
(EC 3.1.3.2)

PRINCIPLE:

\[ \text{p-Nitrophenyl Phosphate} + H_2O \xrightarrow{\text{Phosphatase, Acid}} \text{p-Nitrophenol} + \text{P}_i \]

Abbreviation:

\( \text{P}_i = \text{Inorganic phosphate} \)

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

METHOD:  Spectrophotometric Stop Rate Determination

REAGENTS:

A.  90 mM Citrate Buffer, pH 4.8 at 37°C  
(Prepare 100 ml in deionized water using Citric Acid, Trisodium, Dihydrate, Sigma Prod. No. C-7254, or Citrate Buffer Solution, Sigma Stock No. 104-4. Adjust to pH 4.8 at 37°C with 1 M NaOH or 1 M HCl.)

B.  15.2 mM p-Nitrophenyl Phosphate (PNPP)  
(Prepare 5 ml in deionized water using Sigma 104 Phosphatase Substrate, Sigma Stock No. 104-0.)

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

D.  Acid Phosphatase Enzyme Solution  
(Immediately before use, prepare a solution containing 0.15 - 0.25 unit/ml of Phosphatase, Acid in cold deionized water.)

PROCEDURE:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Reagent B (PNPP)</td>
<td>0.50</td>
<td>0.50</td>
</tr>
</tbody>
</table>
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PROCEDURE: (continued)

Mix by inversion and equilibrate to 37°C. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent D (Enzyme Solution)</td>
<td>0.10</td>
<td>------</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and incubate at 37°C for exactly 10 minutes. Then add:

<p>| | | |</p>
<table>
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<tbody>
<tr>
<td>Reagent C (NaOH)</td>
<td>4.00</td>
<td>4.00</td>
</tr>
<tr>
<td>Reagent D (Enzyme Solution)</td>
<td>------</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Mix by inversion and record the $A_{410\text{nm}}$ for both the Test and Blank in a suitable spectrophotometer.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(A_{410\text{nm Test}} - A_{410\text{nm Blank}})(5.1)(\text{df})}{(10)(18.3)(0.1)}$$

- $5.1 = \text{Total volume (in milliliters) of solution}$
- $\text{df} = \text{Dilution factor}$
- $10 = \text{Time of assay (in minutes) as per the Unit Definition}$
- $18.3 = \text{Millimolar extinction coefficient of p-Nitrophenol at 410 nm}$
- $0.1 = \text{Volume (in milliliter) of enzyme used}$

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will hydrolyze 1.0 µmole of p-nitrophenyl phosphate per minute at pH 4.8 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 1.10 ml reaction mix, the final concentrations are 41 mM citric acid, 6.9 mM p-nitrophenyl phosphate and 0.015 - 0.025 unit phosphatase, acid.
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REFERENCE:


NOTES:

1. This assay is based on the cited reference.

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

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