Enzymatic Assay of PHOSPHATASE, ALKALINE
(EC 3.1.3.1)
Diethanolamine Assay

PRINCIPLE:

\[ \text{p-Nitrophenyl Phosphate} + H_2O \xrightarrow{\text{Alkaline Phosphatase}} \text{p-Nitrophenol} + P_i \]

Abbreviation used:
\[ P_i = \text{Inorganic Phosphate} \]

CONDITIONS: \( T = 37^\circ C, \ \text{pH} = 9.8, \ A_{405\text{nm}}, \ \text{Light path} = 1 \ cm \)

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 1.0 M Diethanolamine Buffer with 0.50 mM Magnesium Chloride, pH 9.8 at 37°C
   (Prepare 50 ml using Diethanolamine, Sigma Prod. No. D-8885, and Magnesium Chloride,
   Hexahydrate, Sigma Prod. No. M-0250. Dissolve the Magnesium Chloride complete in
   deionized water before adding the Diethanolamine. Adjust to pH 9.8 at 37°C with 5 M HCl.
   PREPARE FRESH.)

B. 150 mM p-Nitrophenyl Phosphate Solution (PNPP)
   (Prepare 2 ml in deionized water using Sigma 104 Phosphatase Substrate, Sigma Stock No.
   104-0. PREPARE FRESH.)

C. Phosphatase, Alkaline Enzyme Solution
   (Immediately before use, prepare a solution containing 0.1 - 0.2 unit/ml of Alkaline
   Phosphatase in cold Reagent A.)

PROCEDURE:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
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</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>2.70</td>
<td>2.80</td>
</tr>
<tr>
<td>Reagent B (PNPP)</td>
<td>0.30</td>
<td>0.30</td>
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</tbody>
</table>
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PROCEDURE: (continued)

Mix by inversion and equilibrate to 37°C. Monitor the $A_{405nm}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
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</thead>
<tbody>
<tr>
<td>Reagent C (Enzyme Solution)</td>
<td>0.10</td>
<td>------</td>
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Immediately mix by inversion and record the increase in $A_{405nm}$ for approximately 5 minutes. Obtain the $\Delta A_{405nm}$/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{405nm}/\text{min Test} - \Delta A_{405nm}/\text{min Blank})(3.1)(\text{df})}{(18.5)(0.1)}$$

3.1 = Volume (in milliliters) of assay  
$\text{df} =$ Dilution factor  
18.5 = Millimolar extinction coefficient of p-Nitrophenol at 405 nm  
0.1 = Volume (in milliliters) 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 $\mu$ mole of p-nitrophenyl phosphate per minute at pH 9.8 at 37°C.

FINAL ASSAY CONCENTRATIONS:

In a 3.10 ml reaction mix, the final concentrations are 903 mM diethanolamine, 0.45 mM magnesium chloride, 14 mM p-nitrophenyl phosphate and 0.01 - 0.02 unit alkaline phosphatase.
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REFERENCES:


NOTES:

1. This enzyme assay is not to be used to assay Phosphatase, Alkaline, in which the specific activity is cited only in glycine units.

2. This assay is based on the cited reference.

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

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