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

PRINCIPLE:

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

CONDITIONS:  \( T = 37^\circ\text{C}, \) \( pH = 10.4, \) \( A_{410nm}, \) Light path = 1 cm

METHOD:  Spectrophotometric Stop Rate Determination

REAGENTS:

A.  100 mM Glycine Buffer with 1 mM Magnesium Chloride, pH 10.4 at 37\(^\circ\)C  
(Prepare 50 ml in deionized water using Glycine, Prod. No. G-7126, and Magnesium Chloride Hexahydrate, Prod. No. M-0250. Adjust to pH 10.4 at 37\(^\circ\)C with 1 M NaOH. PREPARE FRESH.)

B.  15.2 mM p-Nitrophenyl Phosphate Solution (PNPP)  
(Prepare 2 ml in deionized water using Sigma Phosphatase Substrate, Stock No. 104-0. PREPARE FRESH.)

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

D.  20 mM Sodium Hydroxide Solution (NaOH)  
(Prepare 100 ml in deionized water using Sodium Hydroxide, Stock No. 505-8.)
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PROCEDURE:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized Water</td>
<td>----</td>
<td>0.10</td>
</tr>
<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>

Mix by inversion and equilibrate to 37°C. Monitor the $A_{410nm}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent C (Enzyme Solution) 0.10 ----

Immediately mix by inversion and incubate for exactly 10 minutes. Then add:

Reagent D 10.00 10.00

Record the $A_{410nm}$ for both the test and blank.

CALCULATIONS:

\[
\text{Units/mg protein} = \frac{(\Delta A_{410nm} \text{ Test} - \Delta A_{410nm} \text{ Blank}) (11.1)}{(10) (18.3) (mg \text{ protein/RM})}
\]

11.1 = total volume  
10 = Time of Assay (Unit Definition)  
18.3 = Millimolar extinction coefficient for p-nitrophenol  
RM = Reaction Mix

UNIT DEFINITION:

One unit will hydrolyze 1.0 µmole of p-nitrophenyl phosphate per minute at pH 10.4 at 37°C.

FINAL ASSAY CONCENTRATIONS:

In a 1.1 ml reaction mix, the final concentrations are 45 mM glycine, 0.45 mM magnesium chloride, 6.9 mM p-nitrophenyl phosphate and 0.01 to 0.02 units alkaline phosphatase.
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NOTES:

1. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

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