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

PRINCIPLE:

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

CONDITIONS:  \( T = 37^\circ \text{C}, \) \( \text{pH} = 10.4, \) \( A_{410\text{nm}}, \) 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°C
   (Prepare 50 ml in deionized water using Glycine, and Magnesium Chloride Hexahydrate, Adjust to pH 10.4 at 37°C with 1 M NaOH. PREPARE FRESH.)

B.  15.2 mM p-Nitrophenyl Phosphate Solution (PNPP)
   (Prepare 2 ml in deionized water using Phosphatase Substrate. 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.)
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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>
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</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>
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</tbody>
</table>

Mix by inversion and equilibrate to 37°C. Monitor the $A_{410\text{nm}}$ 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 $A_{410\text{nm}}$ for both the test and blank.

CALCULATIONS:

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

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.