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

PRINCIPLE:

\[ \text{p-Nitrophenyl Phosphate + H}_2\text{O} \xrightarrow{\text{Alkaline Phosphatase}} \text{p-Nitrophenol + P}_i \]

Abbreviation:
\( P_i = \text{Inorganic Phosphate} \)

CONDITIONS: \( T = 37\,^\circ\text{C}, \, \text{pH} = 8.8, \, A_{410\text{nm}}, \, \text{Light path} = 1 \, \text{cm} \)

METHOD: Stopped Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Glycine Buffer with 1.0 mM Magnesium Chloride, pH 8.8 at 37°C

B. 15.2 mM p-Nitrophenyl Phosphate Solution (PNPP)
   (Prepare 5 ml in deionized water using Sigma 104 Phosphatase Substrate, Sigma Stock No. 104-0. \textit{PREPARE FRESH}.)

C. 20 mM Sodium Hydroxide Solution (NaOH)
   (Prepare 100 ml in deionized water using Sodium Hydroxide, Anhydrous, Sigma Stock No. 505-8.)

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

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>
<tr>
<td>Deionized Water</td>
<td>------</td>
<td>0.10</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>10.00</td>
<td>10.00</td>
</tr>
</tbody>
</table>

Mix by swirling and transfer the solutions to suitable cuvettes and record the $A_{410nm}$ for both Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(A_{410nm} \text{ Test} - A_{410nm} \text{ Blank})(11.1)(df)}{(18.3)(0.1)(10)}$$

11.1 = Volume (in milliliters) of assay
df = Dilution factor
18.3 = Millimolar extinction coefficient of p-nitrophenol at 410 nm
0.1 = Volume (in milliliters) of enzyme used
10 = Time of assay (in minutes) as per the Unit Definition

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

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

One unit will hydrolyze 1.0 \( \mu \)mole of p-nitrophenyl phosphate per minute at pH 8.8 at 37\( ^\circ \)C.

FINAL ASSAY CONCENTRATIONS:

In a 1.10 ml reaction mix, the final concentrations are 45 mM glycine, 0.45 mM magnesium chloride, 6.9 mM p-nitrophenyl phosphate and 0.01 - 0.02 unit alkaline phosphatase.

REFERENCES:


NOTES:

1. This assay is based on the cited reference.

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.