Enzymatic Assay of POLYNUCLEOTIDE PHOSPHORYLASE
(EC 2.7.7.8)

PRINCIPLE:

\[
\text{ADP} + \text{ADP} \xrightarrow{\text{Polynucleotide Phosphorylase}} \text{ppApA} + \text{P}_i
\]

Abbreviations:
ADP = Adenosine 5'-Diphosphate
ppApA = Adenylyl(3'-5')Adenosine 5'-Diphosphate
\( \text{P}_i = \text{Inorganic Phosphate} \)

CONDITIONS: \( T = 37^\circ\text{C}, \ pH = 9.0, \ A_{660nm}, \ \text{Light path} = 1\ \text{cm} \)

METHOD: Colorimetric

REAGENTS:

A. 1 M Tris HCl Buffer, pH 9.0 at 37°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 9.0 at 37°C with 1 M HCl.)

B. 125 mM Adenosine 5'-Diphosphate Solution (ADP)
(Prepare 20 ml in deionized water using Adenosine 5'-Diphosphate, Sodium Salt, Sigma Prod. No. A-2754. PREPARE FRESH.)

C. 62.5 mM Magnesium Chloride Solution (MgCl\(_2\))
(Prepare 100 ml in deionized water using Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250.)

D. 4 mM Ethylenediaminetetraacetic Acid Solution (EDTA)
(Prepare 100 ml in deionized water using Ethylenediaminetetraacetic Acid, Tetrasodium Salt, Hydrate, Sigma Stock No. ED4SS. Adjust to pH 7.0 at 25°C with 100 mM HCl.)

E. 0.2\% (w/v) Bovine Serum Albumin Solution (BSA)
(Prepare 10 ml in deionized water using Albumin, Bovine, Sigma Prod. No. A-7030.)
Enzymatic Assay of POLYNUCLEOTIDE PHOSPHORYLASE
(EC 2.7.7.8)

REAGENTS: (continued)

F. 2.5% (v/v) Perchloric Acid (PCA)
(Prepare 500 ml in deionized water using Perchloric Acid, Aldrich Stock No. 24,425-2.)

G. Acid Molybdate Solution (Moly)
(Use Acid Molybdate Solution, Sigma Stock No. 661-11.)

H. Fiske & Subbarow Reducer (FS)
(Use Fiske & Subbarow Reducer Sigma Stock No. 661-8. Prepare as per instructions on bottle.)

I. Phosphorus Standard Solution (Std)
(Use Phosphorus Standard Solution, Sigma Prod. No. 661-9. The concentration is 20 µg/ml.)

J. 0.1% (w/v) Bovine Serum Albumin Solution (BSA)
(Prepare 10 ml in deionized water using Albumin, Bovine, Sigma Prod. No. A-7030.)

K. Polynucleotide Phosphorylase Enzyme Solution
(Immediately before use, prepare a solution containing 5 - 15 units/ml in cold Reagent J.)

PROCEDURE:

Prepare a stock reaction cocktail by pipetting (in milliliters) the following reagents into a suitable container:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized Water</td>
<td>1.50</td>
</tr>
<tr>
<td>Reagent A (Buffer)</td>
<td>2.50</td>
</tr>
<tr>
<td>Reagent B (ADP)</td>
<td>4.00</td>
</tr>
<tr>
<td>Reagent C (MgCl₂)</td>
<td>2.00</td>
</tr>
<tr>
<td>Reagent D (EDTA)</td>
<td>2.50</td>
</tr>
</tbody>
</table>

Mix by swirling and adjust to pH 9.0 at 37°C with either 100 mM HCl or 100 mM NaOH, if necessary.
Enzymatic Assay of POLYNUCLEOTIDE PHOSPHORYLASE (EC 2.7.7.8)

PROCEDURE: (continued)

Prepare a reaction cocktail by pipetting (in milliliters) the following reagents into a suitable container:

Deionized Water  0.80  
Stock Reaction Cocktail  2.00  
Reagent E (BSA)  0.40

Mix by swirling and equilibrate to 37°C.

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>Reaction Cocktail</td>
<td>0.08</td>
<td>0.08</td>
</tr>
<tr>
<td>Reagent K (Enzyme Solution)</td>
<td>0.02</td>
<td>---</td>
</tr>
</tbody>
</table>

Mix by swirling and incubate at 37°C for exactly 15 minutes. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent F (PCA)</td>
<td>0.90</td>
<td>0.90</td>
</tr>
<tr>
<td>Reagent K (Enzyme Solution)</td>
<td>------</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Mix by swirling. Incubate in an ice bath for 10 minutes. Centrifuge both Test and Blank.

COLORIMETRIC DETERMINATION:

Pipette (in milliliters) the following into suitable tubes (Mix by swirling after each addition):

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Test</th>
<th>Std 1</th>
<th>Std 2</th>
<th>Std 3</th>
<th>Std 4</th>
<th>Std</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized Water</td>
<td>----</td>
<td>----</td>
<td>0.70</td>
<td>0.60</td>
<td>0.40</td>
<td>0.20</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td>Test Supernatant</td>
<td>0.80</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td></td>
</tr>
<tr>
<td>Test Blank</td>
<td>----</td>
<td>0.80</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td></td>
</tr>
<tr>
<td>Reagent I (Std)</td>
<td>----</td>
<td>----</td>
<td>0.10</td>
<td>0.20</td>
<td>0.40</td>
<td>0.60</td>
<td>----</td>
<td></td>
</tr>
<tr>
<td>Reagent G (Moly)</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
<td></td>
</tr>
</tbody>
</table>

Mix by swirling and incubate at 25°C for 5 minutes. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Test</th>
<th>Std 1</th>
<th>Std 2</th>
<th>Std 3</th>
<th>Std 4</th>
<th>Std</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent H (FS)</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td></td>
</tr>
</tbody>
</table>

Mix by swirling.
Enzymatic Assay of POLYNUCLEOTIDE PHOSPHORYLASE
(EC 2.7.7.8)

PROCEDURE: (continued)

Incubate at 25°C for 10 minutes. Record the absorbance at 660 nm for Test, Blank and Standards using a suitable spectrophotometer.

CALCULATIONS:

Standard Curve:
\[ r_{A_{660nm} \text{ Standard}} = A_{660nm} \text{ Standard} - A_{660nm} \text{ Standard blank} \]

Plot the \( r_{A_{660nm} \text{ Standard}} \) vs µmoles of phosphorus.

Sample Determination:
\[ r_{A_{660nm} \text{ Sample}} = A_{660nm} \text{ Test} - A_{660nm} \text{ Test Blank} \]

Determine the µmoles of phosphorus liberated using the Standard curve.

\[
\text{Units/ml enzyme} = \frac{\text{(µmoles of phosphorus liberated)} \times (df)}{(0.8) \times (0.02)}
\]

\( df \) = Dilution factor
0.8 = Volume of assay (in milliliters) used in Colorimetric Determination
0.02 = Volume (in milliliters) of enzyme assay

UNIT DEFINITION:

One unit will polymerize 1.0 µmole of ADP releasing 1.0 µmole of inorganic phosphate in 15 minutes at pH 9.0 at 37°C.

FINAL ASSAY CONCENTRATIONS:

In a 0.10 ml reaction mix, the final concentrations are 100 mM Tris, 20 mM adenosine 5'-diphosphate, 5 mM magnesium chloride, 0.4 mM ethylenediaminetetraacetic acid, 0.04% (w/v) bovine serum albumin, and 0.1 - 0.3 unit polynucleotide phosphorylase.

REFERENCES:


Enzymatic Assay of POLYNUCLEOTIDE PHOSPHORYLASE
(EC 2.7.7.8)

NOTES:

1. This assay is based on the cited references.

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.