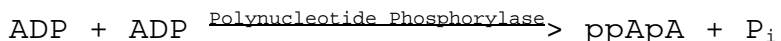


**Enzymatic Assay of POLYNUCLEOTIDE PHOSPHORYLASE
(EC 2.7.7.8)**

PRINCIPLE:



Abbreviations:

ADP = Adenosine 5'-Diphosphate

ppApA = Adenylyl(3'-5')Adenosine 5'-Diphosphate

P_i = Inorganic Phosphate

CONDITIONS: T = 37°C, pH = 9.0, A_{660nm}, Light path = 1 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 (MgCl₂)
(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.)

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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:

Deionized Water	1.50
Reagent A (Buffer)	2.50
Reagent B (ADP)	4.00
Reagent C (MgCl ₂)	2.00
Reagent D (EDTA)	2.50

Mix by swirling and adjust to pH 9.0 at 37°C with either 100 mM HCl or 100 mM NaOH, if necessary.

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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:

	<u>Test</u>	<u>Blank</u>
Reaction Cocktail	0.08	0.08
Reagent K (Enzyme Solution)	0.02	---

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

Reagent F (PCA)	0.90	0.90
Reagent K (Enzyme Solution)	-----	0.02

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):

	<u>Test</u>	<u>Test Blank</u>	<u>Std 1</u>	<u>Std 2</u>	<u>Std 3</u>	<u>Std 4</u>	<u>Std Blank</u>
Deionized Water	----	----	0.70	0.60	0.40	0.20	0.80
Test Supernatant	0.80	----	----	----	----	----	----
Test Blank	----	0.80	----	----	----	----	---
Reagent I (Std)	----	----	0.10	0.20	0.40	0.60	----
Reagent G (Moly)	1.50	1.50	1.50	1.50	1.50	1.50	1.50

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

Reagent H (FS)	0.20	0.20	0.20	0.20	0.20	0.20	0.20
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Mix by swirling.

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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:

$$r A_{660\text{nm}} \text{ Standard} = A_{660\text{nm}} \text{ Standard} - A_{660\text{nm}} \text{ Standard blank}$$

Plot the $r A_{660\text{nm}}$ Standard vs μmoles of phosphorus.

Sample Determination:

$$r A_{660\text{nm}} \text{ Sample} = A_{660\text{nm}} \text{ Test} - A_{660\text{nm}} \text{ Test Blank}$$

Determine the μmoles of phosphorus liberated using the Standard curve.

$$\text{Units/ml enzyme} = \frac{(\mu\text{moles of phosphorus liberated}) (df)}{(0.8) (0.02)}$$

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:

Littauer, U.Z. and Kornberg, A. (1957) *J. Biol. Chem.* **226**, 1077-1092.

Fiske, C.H. and Subbarow, Y. (1925) *J. Biol. Chem.* **66**, 375-400.

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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.