Enzymatic Assay of POLYNUCLEOTIDE PHOSPHORYLASE
(EC 2.7.7.8)

PRINCIPLE:

$$ADP + ADP \xrightarrow{\text{Polynucleotide Phosphorylase}} ppApA + P_i$$

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 ($MgCl_2$)
(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:

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.

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:

	Test	Blank
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):

	Test	Test Blank	Std 1	Std 2	Std 3	Std 4	Std Blank
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

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:

ΔA_{660nm} Standard = A_{660nm} Standard - A_{660nm} Standard blank

Plot the ΔA_{660nm} Standard vs µmoles of phosphorus.

Sample Determination:

ΔA_{660nm} Sample = A_{660nm} Test - A_{660nm} 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.

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.