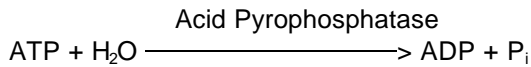


Enzymatic Assay of PYROPHOSPHATASE, ACID

PRINCIPLE:



Abbreviations:

ATP = Adenosine 5'-Triphosphate

ADP = Adenosine 5'-Diphosphate

P_i = Inorganic Phosphate

CONDITIONS: T = 37°C, pH 5.0, A_{660nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

- A. 50 mM Sodium Acetate Buffer with 10 mM 2-Mercaptoethanol, 1 mM Ethylenediaminetetraacetic Acid, pH 5.0 at 37°C
(Prepare 25 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625, 2-Mercaptoethanol, Sigma Prod. No. M-6250, and Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate, Sigma Stock No. ED2SS. Adjust to pH 5.0 at 37°C with 1 M HCl.)
- B. 2 mM Adenosine 5'-Triphosphate Solution, pH 5.0 (Substrate Solution)
(Prepare 10 ml in cold Reagent A using Adenosine 5'-Triphosphate, Disodium Salt, Dihydrate, Sigma Prod. No. A-5394.)
- C. 10 N Sulfuric Acid Solution (H₂SO₄)
(Prepare 50 ml in deionized water using Sulfuric Acid, Sigma Prod. No. S-1526.)
- D. Taussky-Shorr Reagent (TSCR)
(Prepare by adding 10 ml of 10% (w/v) Ammonium Molybdate, Tetrahydrate, Sigma Prod. No. A-7302, in 10 N H₂SO₄, to 70 ml deionized water. Add 5 g of Ferrous Sulfate, Heptahydrate, Sigma Prod. No. F-0131. Bring the volume to 100 ml with deionized water.)

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REAGENTS: (continued)

- E. 0.2 mM Phosphorus Standard (Std)
(Prepare 5 ml in deionized water using Phosphorus Standard Solution, Sigma Stock No. 661-9. The Phosphorus concentration is 20 $\mu\text{g/ml}$, 0.645 $\mu\text{moles/ml}$.)
- F. Acid Pyrophosphatase Enzyme Solution
(The enzyme solution should be used undiluted and should be approximately 2000 - 4000 units/ml.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable microcentrifuge tubes:¹

	<u>Test</u>	<u>Blank</u>
Reagent B (Substrate Solution)	0.045	0.050

Equilibrate to 37°C. Then add:

Reagent F (Enzyme Solution)	0.005	-----
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Immediately mix by swirling and incubate at 37°C for exactly 30 minutes. Then add:

Reagent D (TSCR)	0.900	0.900
Deionized Water	0.050	0.050

Mix by swirling and incubate at 25°C for 10 minutes. Transfer to suitable cuvettes and record the $A_{660\text{nm}}$ for both Test and Blank in a suitable spectrophotometer.

Standard Preparation:

Prepare standards by pipetting (in milliliters) the following reagents into suitable microcentrifuge tubes:

	<u>Std1</u>	<u>Std2</u>	<u>Std3</u>	<u>Std4</u>	<u>Std5</u>	<u>Std</u> <u>Blank</u>
Deionized Water	0.095	0.090	0.080	0.050	-----	0.100
Reagent E (Std)	0.005	0.010	0.020	0.050	0.100	-----
Reagent D (TSCR)	0.900	0.900	0.900	0.900	0.900	0.900

Mix and incubate at 25°C for 10 minutes. Transfer to suitable cuvettes and record the $A_{660\text{nm}}$ for Standards and Standard Blank.

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CALCULATIONS:

Standard Curve:

$$\Delta A_{660\text{nm}} \text{ Standard} = A_{660\text{nm}} \text{ Standard} - A_{660\text{nm}} \text{ Standard Blank}$$

Prepare a standard curve by plotting the $\Delta A_{660\text{nm}}$ Standard vs nanomoles of Phosphorus.

Sample Determination:

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

Determine the nanomoles of Phosphate liberated using the Standard Curve.

$$\text{Units/ml enzyme} = \frac{\text{(nanomoles of Phosphate released)}}{(0.005)(30)}$$

0.005 = Volume (in milliliters) of enzyme used

30 = Time (in minutes) as per the Unit Definition

UNIT DEFINITION:

One unit will release 1.0 nanomole of inorganic phosphorus from ATP in 30 minutes at pH 5.0 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 0.050 milliliter reaction mix, the final concentrations are 45 mM sodium acetate, 9 mM 2-mercaptoethanol, 1.8 mM adenosine 5'-triphosphate, 0.9 mM ethylenediaminetetraacetic acid, and 10 - 20 units acid pyrophosphatase.

REFERENCE:

Shinshi, H., Miwa, M., Kato, K., Noguchi, M., Matsushima, T., and Sugimura, T. (1976) *Biochemistry* **15**, 2185-2190.

NOTES:

1. Since very small volumes are utilized, accurate pipetting of solutions is critical.
2. The time period between the addition of Taussky-Shorr reagent and measuring the absorbance in a spectrophotometer must be consistent for all standards and samples.
3. This assay is based on the cited reference.
4. 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.