

**Enzymatic Assay of INORGANIC PYROPHOSPHATASE  
(EC 3.6.1.1)  
From Bacillus stearothermophilus**

**PRINCIPLE:**

Pyrophosphate + H<sub>2</sub>O  $\xrightarrow{\text{Inorganic Pyrophosphatase}}$  2 Orthophosphate

**CONDITIONS:** T = 50°C, pH 9.0, A<sub>660nm</sub>, Light path = 1 cm

**METHOD:** Colorimetric

**REAGENTS:**

- A. 50 mM Glycine Buffer, pH 9.0 at 50°C  
(Prepare 100 ml in deionized water using Glycine, Free Base, Sigma Prod. No. G-7126. Adjust to pH 9.0 at 50°C with 1 M NaOH.)
- B. 50 mM Tris HCl, pH 8.0 at 25°C (Enzyme Diluent)  
(Prepare 50 ml in deionized water using Trizma, Hydrochloride Sigma Prod. No. T-3253. Adjust to pH 8.0 at 25°C with 1 M KOH.)
- C. 10 mM Sodium Pyrophosphate Solution (Pyrophosphate)  
(Prepare 25 ml in deionized water using Pyrophosphate, Tetrasodium, Decahydrate, Sigma Prod. No. P-9146.)
- D. 10 mM Magnesium Chloride Solution (MgCl<sub>2</sub>)  
(Prepare 50 ml in deionized water using Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250.)
- E. 10% (w/v) Ammonium Molybdate Solution  
(Prepare 25 ml in 10 N H<sub>2</sub>SO<sub>4</sub> using Molybdic Acid, Ammonium, Tetrahydrate Salt, Sigma Prod. No. M-0878.)
- F. Taussky-Shorr Color Reagent (TSCR)  
(Prepare by adding 10 ml of Reagent E to 70 ml of deionized water. Add 5 g of Ferrous Sulfate, Heptahydrate, Sigma Prod. No. F-0131. Bring the volume to 100 ml with deionized water.)

**Enzymatic Assay of INORGANIC PYROPHOSPHATASE  
(EC 3.6.1.1)  
From Bacillus stearothermophilus**

**REAGENTS:** (continued)

- G. Phosphorus Standard  
(Use Phosphorus Standard Solution, Sigma Stock No. 661-9. The phosphorus concentration is 20 µg/ml, 0.645 µmoles/ml.)
- H. Inorganic Pyrophosphatase Enzyme Solution  
(Immediately before use, prepare a solution containing 6 - 12 units/ml of Inorganic Pyrophosphatase in cold Reagent B.)

**PROCEDURE:**

Pipette (in milliliters) the following reagents into suitable containers:

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	4.00	4.00
Reagent B (Enzyme Diluent)	-----	0.10
Reagent D (MgCl <sub>2</sub> )	1.00	1.00
Reagent H (Enzyme Solution)	0.10	-----

Mix by swirling and equilibrate to 50°C. Then add:

Reagent C (Pyrophosphate)	1.00	-----
Deionized Water	-----	1.00

Immediately mix by swirling and incubate at 50°C for exactly 10 minutes. Pipette (in milliliters) the following reagents into suitable containers:

	<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 5</u>	<u>Std Blank</u>
Reagent F (TSCR)	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Test Mixture	1.00	----	----	----	----	----	----	----
Test Blank Mixture	----	1.00	----	----	----	----	----	----
Reagent G (Standard)	----	----	0.50	0.70	1.00	1.20	1.50	----
Deionized Water	4.00	4.00	4.50	4.30	4.00	3.80	3.50	5.00

Mix by swirling and incubate at 25°C for 10 minutes. Transfer to suitable cuvettes and record the A<sub>660nm</sub> for Test, Test Blank, Standards, and Standard Blank.

**Enzymatic Assay of INORGANIC PYROPHOSPHATASE  
(EC 3.6.1.1)  
From Bacillus stearothermophilus**

**CALCULATIONS:**

Standard curve:

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

Prepare a standard curve by plotting the  $r \quad A_{660\text{nm}} \text{ Standard}$  vs micromoles of Phosphate.

Sample Determination:

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

Determine the micromoles of Phosphate liberated using the standard curve.

$$\text{Units/ml enzyme} = \frac{(\mu\text{moles of Phosphate released})(6.1)(\text{df})}{(1)(10)(0.1)}$$

6.1 = Total volume (in milliliters) of assay

df = Dilution factor

10 = Time of assay (in minutes) as per the Unit Definition

1 = Volume (in milliliter) of Test Mixture used in Colorimetric Determination

0.1 = Volume (in milliliter) of enzyme used

0.2

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

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

**UNIT DEFINITION:**

One unit will liberate 1.0  $\mu\text{mole}$  of inorganic orthophosphate per minute at pH 9.0 at 50°C.

**FINAL ASSAY CONCENTRATION:**

In a 6.10 ml reaction mix, the final concentrations are 33 mM glycine, 1.6 mM sodium pyrophosphate, 1.6 mM magnesium chloride, 0.82 mM Tris and 0.6 - 1.2 unit inorganic pyrophosphatase.

**Enzymatic Assay of INORGANIC PYROPHOSPHATASE  
(EC 3.6.1.1)  
From *Bacillus stearothermophilus***

**REFERENCE:**

Taussky, H.H. and Shorr, E. (1953) *Journal of Biological Chemistry* **202**, 675-685

**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.**