



SIGMA QUALITY CONTROL TEST PROCEDURE

Product Information

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

PRINCIPLE:



CONDITIONS: T = 50°C, pH 9.0, A_{660nm}, 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 (MgCl₂)
(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 H₂SO₄ 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.)

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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 ₂)	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_{660nm} for Test, Test Blank, 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 micromoles of Phosphate.

Sample Determination:

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

Determine the micromoles of Phosphate liberated using the standard curve.

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

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 μmole 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.

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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.

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