

**Enzymatic Assay of INORGANIC PYROPHOSPHATASE
(EC 3.6.1.1)
from Baker's Yeast**

PRINCIPLE:

Pyrophosphate + H₂O $\xrightarrow{\text{Inorganic Pyrophosphatase}}$ 2 Orthophosphate

CONDITIONS: T = 25°C, pH = 7.2, A_{660nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

- A. 50 mM Tris HCl Buffer, pH 7.2 at 25°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 7.2 at 25°C with 1 M HCl.)
- B. 20 mM Tris HCl Solution, pH 7.2 at 25°C (Enzyme Diluent)
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 7.2 at 25°C with 1 M HCl.)
- C. 10 mM Sodium Pyrophosphate Solution
(Prepare 50 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 5 - 10 units/ml of Inorganic Pyrophosphatase in cold Reagent B.)

PROCEDURE:

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

	Test	Test Blank
Reagent A (Buffer)	4.00	4.00
Reagent B (Enz Dil)	-----	0.10
Reagent C (Pyrophosphate)	1.00	1.00
Reagent D (MgCl ₂)	1.00	1.00

Mix by swirling and equilibrate to 25°C. Then add:

Reagent H (Enzyme Solution)	0.10	-----
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Immediately mix by swirling and incubate at 25°C for exactly 10 minutes.

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

	<u>Test</u>	<u>Blank</u>	<u>Std1</u>	<u>Std2</u>	<u>Std3</u>	<u>Std Blank</u>
Reagent F (TSCR)	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	1.00	1.50	----
Deionized Water	4.00	4.00	4.50	4.00	3.50	5.00

Mix by swirling and incubate at 25°C for exactly 5 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:

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

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

Sample Determination:

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

Determine the μmoles 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

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

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

UNIT DEFINITION:

One unit will liberate 1.0 μmole of inorganic orthophosphate per minute at pH 7.2 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 6.10 ml reaction mix, the final concentrations are 33 mM Tris, 1.6 mM sodium pyrophosphate, 1.6 mM magnesium chloride and 0.5 - 1.0 unit inorganic pyrophosphatase.

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REFERENCE:

Bergmeyer, H.U., Gawehn, K., Grassl, M. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U. ed.) 2nd ed., Volume I, 508, Academic Press, New York, NY

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.