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

PRINCIPLE:

Pyrophosphate + H₂O → 2 Orthophosphate

CONDITIONS:  T = 25°C, pH = 7.2, A₆₆₀nm, 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:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>4.00</td>
<td>4.00</td>
</tr>
<tr>
<td>Reagent B (Enz Dil)</td>
<td>------</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent C (Pyrophosphate)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Reagent D (MgCl₂)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

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

Reagent H (Enzyme Solution) | 0.10 | -----

Immediately mix by swirling and incubate at 25°C for exactly 10 minutes.

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
<th>Std1</th>
<th>Std2</th>
<th>Std3</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent F (TSCR)</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Test Mixture</td>
<td>1.00</td>
<td>-----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>Test Blank Mixture</td>
<td>----</td>
<td>1.00</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>Reagent G (Standard)</td>
<td>----</td>
<td>----</td>
<td>0.50</td>
<td>1.00</td>
<td>1.50</td>
<td>----</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>4.00</td>
<td>4.00</td>
<td>4.50</td>
<td>4.00</td>
<td>3.50</td>
<td>5.00</td>
</tr>
</tbody>
</table>

Mix by swirling and incubate at 25°C for exactly 5 minutes. Transfer to suitable cuvettes and record the A₆₆₀nm 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 \( r \ A_{660}\text{nm} \text{ Standard} \) vs \( \mu \)moles 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 \( \mu \)moles of Phosphate liberated using the standard curve.

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

\[ 6.1 = \text{Total volume (in milliliters) of assay} \]
\[ \text{df} = \text{Dilution factor} \]
\[ 10 = \text{Time of assay (in minutes) as per the Unit Definition} \]
\[ 1 = \text{Volume (in milliliter) of Test Mixture used in Colorimetric Determination} \]
\[ 0.1 = \text{Volume (in milliliter) of enzyme used} \]

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

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

UNIT DEFINITION:

One unit will liberate 1.0 \( \mu \)mole 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:


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.