Enzymatic Assay of APYRASE
(EC 3.6.1.5)
ATP as Substrate

PRINCIPLE:

\[
\text{ATP} + \text{H}_2\text{O} \xrightarrow{\text{Apyrase}} \text{ADP} + \text{P}_i
\]

Abbreviations:
ATP = Adenosine 5’-Triphosphate
ADP = Adenosine 5’-Diphosphate
P_i = Inorganic Phosphate

CONDITIONS: \( T = 30^\circ\text{C}, \text{pH } 6.5, A_{660\text{nm}}, \text{Light path } = 1 \text{ cm} \)

METHOD: Colorimetric

REAGENTS:

A. 40 mM Succinate Buffer with 4 mM Calcium Chloride, pH 6.6 at 30°C
(Prepare 100 ml in deionized water using Succinic Acid, Free Acid, Sigma Prod. No. S-7501, and Calcium Chloride, Dihydrate, Sigma Prod. No. C-3881. Adjust to pH 6.6 at 30°C with 5 M KOH.)

B. 2.0 mM Adenosine 5’-Triphosphate Solution (ATP)
(Prepare 15 ml in Reagent A using Adenosine 5’-Triphosphate, Disodium Salt, Sigma Prod. No. A-3377. Adjust to pH 6.5 at 30°C using 1 M NaOH.)

C. Apyrase Enzyme Solution
(Immediately before use, prepare a solution containing 0.50 - 1.5 ATPase units/ml of Apyrase in cold deionized water.)

D. Phosphorus Standard Solution
(Use Phosphorus Standard Solution, Sigma Stock No. 661-9. The concentration is 20 \( \mu \text{g/ml}, 0.645 \mu \text{moles/ml} \).)

E. 10% (w/v) Ammonium Molybdate Solution (Amm. Moly.)
(Prepare 25 ml in 10 N \( \text{H}_2\text{SO}_4 \) using Molybdic Acid, Ammonium Tetrahydrate Salt, Sigma Prod. No. M-0878.)
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REAGENTS:  (continued)

F.  Taussky-Shorr Reagent (TSCR)  
(Prepare by adding 10 ml of Reagent E to 70 ml of deionized water.  Then add a 5 g vial of  
Ferrous Sulfate Heptahydrate, Sigma Prod. No. F-0131, and mix until dissolved.  Add enough  
deionized water for a final volume of 100 ml.)

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 B (ATP)</td>
<td>1.90</td>
<td>1.90</td>
</tr>
</tbody>
</table>

Equilibrate to 30°C.  Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized Water</td>
<td>-----</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent C (Enzyme Solution)</td>
<td>0.10</td>
<td>-----</td>
</tr>
</tbody>
</table>

Immediately mix by swirling and incubate at 30°C for exactly 10 minutes.  Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent F (TSCR)</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>3.00</td>
<td>3.00</td>
</tr>
</tbody>
</table>

Mix by swirling and immediately (within 1 - 2 minutes) transfer to suitable cuvettes and record the  
$A_{660nm}$ for both the Test and Blank using a suitable spectrophotometer.

COLORIMETRIC ASSAY:

Standard Curve:

Prepare a standard curve by pipetting (in milliliters) the following reagents into suitable containers:

<table>
<thead>
<tr>
<th></th>
<th>Std 1</th>
<th>Std 2</th>
<th>Std 3</th>
<th>Std 4</th>
<th>Std 5</th>
<th>Std Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized Water</td>
<td>4.50</td>
<td>4.00</td>
<td>3.50</td>
<td>3.00</td>
<td>2.50</td>
<td>5.00</td>
</tr>
<tr>
<td>Reagent D (Phosphorus Std)</td>
<td>0.50</td>
<td>1.00</td>
<td>1.50</td>
<td>2.00</td>
<td>2.50</td>
<td>0.00</td>
</tr>
<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>
</tbody>
</table>

Mix by swirling and immediately (within 1-2 minutes) transfer to suitable cuvettes and record the  
$A_{660nm}$ for the Standards and 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 \( \Delta A_{660\text{nm}} \text{ Standard} \) versus \( \mu \text{moles of Phosphate} \).

Sample Determination:

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

Determine the micromoles of phosphate liberated using the Standard curve.

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

\( df \) = Dilution factor
\( 10 \) = Time (in minutes) of assay as per the Unit Definition
\( 0.1 \) = 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 \( \mu \text{mole} \) of inorganic phosphate from adenosine 5'-triphosphate per minute at pH 6.5 at 30°C.

FINAL ASSAY CONCENTRATION:

In a 2.00 ml reaction mix, the final concentrations are 38 mM sodium succinate, 3.8 mM calcium chloride, 1.9 mM adenosine 5'-triphosphate, and 0.05 - 0.15 unit apyrase.
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REFERENCES:


NOTES:

1. This assay is based on the cited references.

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.