Enzymatic Assay of APYRASE
(EC 3.6.1.5)
AMP as Substrate

PRINCIPLE:

AMP + H₂O $\underset{\text{Apyrase}}{\rightarrow}$ Adenosine + Pᵢ

Abbreviations:
AMP = Adenosine 5'-Monophosphate
Pᵢ = Inorganic Phosphate

CONDITIONS:  T = 30°C, pH 6.5, A₆₆₀nm, Light path = 1 cm

METHOD:  Colorimetric

REAGENTS:

A.  40 mM Succinate Buffer with 4 mM Calcium Chloride, pH 6.5 at 30°C
   (Prepare 100 ml in deionized water using Succinic Acid, Free Acid, and Calcium Chloride, Dihydrate. Adjust to pH 6.5 at 30°C with 1 M KOH.)

B.  2 mM Adenosine 5'-Monophosphate Solution (AMP)
   (Prepare 15 ml in Reagent A using Adenosine 5'-Monophosphate, Sodium Salt. Adjust to pH 6.5 at 25°C using 10 mM NaOH.)

C.  0.1% Albumin Solution (BSA)
   (Prepare 50 ml in deionized water using Albumin Bovine Serum.)

D.  Apyrase Enzyme Solution
   (Immediately before use, prepare a solution containing 5.0 - 10 AMPase units/ml of Apyrase in cold Reagent C.)

E.  Phosphorus Standard Solution
   (Use Phosphorus Standard Solution. The concentration is, 20 µg/ml, 0.645 µmoles/ml.)
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REAGENTS: (continued)

F. 10% (w/v) Ammonium Molybdate Solution (Amm. Moly.)
(Prepare 25 ml in 5 M Sulfuric Acid using Molybdic Acid, Ammonium Tetrahydrate Salt.)

G. Taussky-Shorr Reagent (TSCR)
(Prepare by adding 10 ml Reagent F to 70 ml of deionized water. Then add 5 g Ferrous Sulfate, Heptahydrate, and mix until dissolved. Add enough deionized water to 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 (AMP)</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>Reagent C (BSA)</td>
<td>------</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent D (Enzyme Solution)</td>
<td>0.10</td>
<td>------</td>
</tr>
</tbody>
</table>

Immediately mix 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>Deionized Water</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Reagent G (TSCR)</td>
<td>5.00</td>
<td>5.00</td>
</tr>
</tbody>
</table>

Mix and incubate at 25°C for 5 minutes.

Transfer to suitable cuvettes and record the A_{660nm} for both Test and Blank using a suitable spectrophotometer.
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COLORIMETRIC ASSAY:

Standard Curve:

A standard curve is made 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>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized Water</td>
<td>4.80</td>
<td>4.60</td>
<td>4.40</td>
<td>4.20</td>
<td>4.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Reagent E (Phosphorus Std)</td>
<td>0.20</td>
<td>0.40</td>
<td>0.60</td>
<td>0.80</td>
<td>1.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Reagent G (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>

Transfer to suitable cuvettes and record the A$_{660nm}$ for Standards and Blank.

CALCULATIONS:

Find the slope from the plot of the A$_{660nm}$ of the Standards vs Phosphorus concentration. Use the slope (M) to determine the concentration of the test mixture.

$$\text{Units/mg enzyme} = \frac{A_{660nm} \text{ Test} - A_{660nm} \text{ Blank}}{10 \times (M) \times (\text{mg enzyme/RM})}$$

10 = Time of Assay (Unit Definition)
RM = Reaction Mix (volume = 2.00 ml)

UNIT DEFINITION:

One unit will liberate 1.0 µmole of inorganic phosphate from adenosine 5'-monophosphate per minute at pH 6.5 at 30°C.

FINAL ASSAY CONCENTRATION:

In a 2 ml reaction mix, the final concentrations are 38 mM sodium succinate, 3.8 mM calcium chloride, 1.9 mM AMP, 0.005% BSA, and 0.5 - 1.0 units apyrase.

REFERENCES:

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NOTES:

1. All product and stock numbers, unless otherwise indicated, are OUR product and stock numbers.

This procedure is for informational purposes. For a current copy of our quality control procedure contact our Technical Service Department.