Enzymatic Assay of ADENOSINE 5'-TRIPHOSPHATASE  
(EC 3.6.1.3)

PRINCIPLE:

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

Abbreviations used:
ATPase = Adenosine 5'-Triphosphatase  
ATP = Adenosine 5'-Triphosphate  
ADP = Adenosine 5'-Diphosphate  
\(P_i\) = Inorganic Phosphate

CONDITIONS: \(T = 37^\circ\text{C}, \text{pH 7.8}, A_{660\text{nm}}, \text{Light path} = 1 \text{ cm}\)

METHOD: Colorimetric

REAGENTS:

A. 24 mM Tris HCl Buffer with 0.68 mM Ethylenediaminetetraacetic Acid and 6.0 mM Magnesium Chloride, pH 7.8 at 37°C  
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503, Ethylenediaminetetraacetic Acid, Free Acid, Sigma Stock No. ED, and Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250. Adjust to pH 7.8 at 37°C with 1 M HCl.)

B. 15 mM Ouabain Solution (Ouabain)  
(Prepare 10 ml in Reagent A using Ouabain Octahydrate, Sigma Prod. No. O-3125.)

C. 2 M Sodium Chloride Solution (NaCl)  
(Prepare 10 ml in deionized water using Sodium Chloride, Sigma Prod. No. S-9625.)

D. 45 mM Potassium Chloride and 2 M Sodium Chloride Solution (KCl/NaCl)  
(Prepare 10 ml in Reagent C, using Potassium Chloride, Sigma Prod. No. P-4504.)
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REAGENTS: (continued)

E.  80 mM Adenosine 5'Triphosphate Solution (ATP)
(Prepare 10 ml in deionized water using Adenosine 5'Triphosphate, Tris Salt, Sigma Prod. No. A-9062. Adjust to pH 7.8 at 37°C with 1 M Tris.)

F. Taussky-Shorr Reagent
(Prepare by adding 10 ml of 10% Ammonium Molybdate, Tetrahydrate Sigma Prod. No. M-0878, in 10 N H₂SO₄, to 70 ml deionized water; then add a 5 g vial of Ferrous Sulfate, Heptahydrate, Sigma Prod. No. F-0131. Bring the volume to 100 ml with deionized water. Store in an amber container.)

G. 20% (w/v) Trichloroacetic Acid (TCA)
(Prepare 100 ml in deionized water using Trichloroacetic Acid Solution, approximately 100% (w/v) 6.1 N, Sigma Stock No. 490-10.)

H. Phosphorus Standard (P Std)
(Use Phosphorus Standard Solution, Sigma Stock No. 661-9. The concentration is 20 µg/ml, 0.645 µmole/ml.)

I. Adenosine 5'Triphosphatase Enzyme Solution
(Immediately before use, prepare a solution in cold deionized water containing 0.3 - 0.6 unit/ml.)

PROCEDURE:

Step 1:

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

<table>
<thead>
<tr>
<th></th>
<th>Test 1</th>
<th>Blank 1</th>
<th>Test 2</th>
<th>Blank 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>1.25</td>
<td>1.25</td>
<td>1.15</td>
<td>1.15</td>
</tr>
<tr>
<td>Reagent B (Ouabain)</td>
<td>----</td>
<td>----</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent C (NaCl)</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>Reagent D (KCl/NaCl)</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent I (Enzyme Solution)</td>
<td>0.10</td>
<td>----</td>
<td>0.10</td>
<td>----</td>
</tr>
</tbody>
</table>

Mix and equilibrate for several minutes at 37°C. Then add:

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent E (ATP)</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>
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PROCEDURE:  (continued)

Mix and incubate at 37°C for exactly 15 minutes. Then add:

<table>
<thead>
<tr>
<th>Test 1</th>
<th>Blank 1</th>
<th>Test 2</th>
<th>Blank 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
</tr>
</tbody>
</table>

Reagent G (TCA)

Immediately mix by inversion. Then add:

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

Mix by inversion and then centrifuge for 3 minutes to clarify.

Step 2:

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

<table>
<thead>
<tr>
<th>Reagent F (Taussky-Shorr)</th>
<th>2.00</th>
<th>2.00</th>
<th>2.00</th>
<th>2.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Supernatant</td>
<td>1.00</td>
<td>----</td>
<td>1.00</td>
<td>----</td>
</tr>
<tr>
<td>Blank Supernatant</td>
<td>----</td>
<td>1.00</td>
<td>----</td>
<td>1.00</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Mix and incubate at 25°C for 5 minutes. Read the \( A_{660nm} \) for both Tests and Blanks.

Standard Curve:

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

<table>
<thead>
<tr>
<th>Std 1</th>
<th>Std 2</th>
<th>Std 3</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent F (Taussky-Shorr)</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Reagent H (P Std)</td>
<td>0.25</td>
<td>0.50</td>
<td>1.00</td>
</tr>
<tr>
<td>Reagent G (TCA)</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>1.25</td>
<td>1.00</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Mix and incubate at 25°C for 10 minutes. Read the \( A_{660nm} \) for the Standard and Standard Blank using a suitable spectrophotometer.
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CALCULATIONS:

Standard Curve:

\[ ?A_{660\text{nm}} \text{ Standard} = A_{660\text{nm}} \text{ Standard} - A_{660\text{nm}} \text{ Standard Blank} \]

Prepare a standard curve by plotting the \( ?A_{660\text{nm}} \) of the Standard vs µmoles of Phosphate.

Sample Determination:

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

Determine the micromoles of Phosphate liberated using the standard curve.

\[
\text{Units/ml Protein} = \frac{\text{(µmoles of Phosphate released)} \times (3.0) \times (\text{df})}{(15)(0.1)(1.0)}
\]

3.0 = Total volume (in milliliters) of Step 1
\( \text{df} \) = Dilution factor
15 = Time (in minutes) of assay as per the Unit Definition
1.0 = Aliquot of Test Supernatant used in Step 2
0.1 = Volume (in milliliter) of enzyme used

Test 1 = ATPase, activated (Na, K, Mg)
Test 2 = ATPase, not Ouabain sensitive
Ouabain sensitive = Test 1 - Test 2

Units/ml enzyme

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

Units/ml enzyme

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

UNIT DEFINITION:

One unit will liberate 1.0 µmole of inorganic phosphorus from ATP per minute at pH 7.8 at 37°C in the presence of Na⁺, K⁺, and Mg²⁺.
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FINAL ASSAY CONCENTRATION:

In a 1.50 ml reaction mix, the final concentrations are 20 mM Tris, 0.57 mM ethylenediaminetetraacetic acid, 5 mM magnesium chloride, 3 mM adenosine 5'-triphosphate, 133 mM sodium chloride and 3 mM potassium chloride, 0.03 - 0.06 unit adenosine 5'-triphosphatase and 1 mM ouabain (when present).

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.