Enzymatic Assay of ADENOSINE 5'-TRIPHOSPHATE SULFURYLASE
(EC 2.7.7.4)

PRINCIPLE:

\[
\text{APS} + \text{PP}_i \xrightarrow{\text{ATPS}} \text{ATP} + \text{SO}_4^{2-} \\
\text{ATP} + \text{d-Glucose} \xrightarrow{\text{HK}} \text{d-Glucose 6-Phosphate} + \text{ADP} \\
\text{d-Glucose 6-Phosphate} + \beta\text{-NADP} \xrightarrow{\text{G-6-PDH}} \text{6-PG} + \beta\text{-NADPH}
\]

Abbreviations used:

- APS = Adenosine 5'-Phosphosulfate
- PP\_i = Inorganic Pyrophosphate
- ATPS = Adenosine 5'-Triphosphate Sulfurylase
- ATP = Adenosine 5'-Triphosphate
- HK = Hexokinase
- ADP = Adenosine 5'-Diphosphate
- \beta\text{-NADP} = \beta\text{-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form}
- G-6-PDH = Glucose 6-Phosphate Dehydrogenase
- 6-PG = 6-Phospho-d-Gluconate
- \beta\text{-NADPH} = \beta\text{-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form}

CONDITIONS:  \( T = 30^\circ C, \ \text{pH} \ 8.0, \ \text{A}_{340nm}, \ \text{Light path} = 1 \ \text{cm} \)

METHOD:  Continuous Spectrophotometric Rate Determination

REAGENTS:

A.  400 mM Tris Buffer, pH 8.0 at 30°C
(Prepare 50 ml in deionized water using Trizma Base, Adjust to pH 8.0 at 30°C with 1 M Acetic Acid.)

B.  200 mM \beta\text{-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form, Solution (\beta\text{-NADP})}  
(Prepare 1 ml in deionized water using \beta\text{-Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt.})
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REAGENTS: (continued)

C. 1 M Magnesium Acetate Solution (MgOAC)
   (Prepare 1 ml in deionized water using Magnesium Acetate, Tetrahydrate.)

D. 1 M $\text{d}$-Glucose Solution (Glucose)
   (Prepare 2 ml in deionized water using $\text{d}$-(+)Glucose, Anhydrous.)

E. 20 mM Adenosine 5'-Phosphosulfate (APS)
   (Immediately before use, prepare 1 ml in deionized water using Adenosine 5'-Phosphosulfate, Sodium Salt.)

F. 100 mM Pyrophosphate Solution, pH 8.0 at 30°C (PP$i$)
   (Prepare 5 ml in deionized water using Tetrasodium Pyrophosphate, Decahydrate.
   Adjust to pH 8.0 at 30°C with 1 M HCl.)

G. Hexokinase and Glucose 6-Phosphate Dehydrogenase Enzyme Solution (HK/G-6-PDH)
   (Immediately before use, prepare a solution containing approximately 20 units/ml of Glucose 6-Phosphate Dehydrogenase using Hexokinase and Glucose 6-Phosphate Dehydrogenase in cold deionized water.)

H. Adenosine 5'-Triphosphate Sulfurylase Enzyme Solution (ATPS)
   (Immediately before use, prepare a solution containing 0.2 - 0.6 unit/ml of Adenosine 5'-Triphosphate Sulfurylase in cold deionized water.)

PROCEDURE:

Prepare a reaction cocktail by pipetting (in milliliters) the following reagents into a suitable container.

| Reagent A (Buffer) | 34.00 |
| Reagent B (NADP)  | 0.34  |
| Reagent C (MgOAC) | 0.28  |
| Deionized Water   | 65.38 |

Mix by swirling. Adjust to pH 8.0 at 30°C if necessary with either 1 M HCl or 1 M NaOH.
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PROCEDURE: (continued)

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction Cocktail</td>
<td>2.50</td>
<td>2.50</td>
</tr>
<tr>
<td>Reagent D (Glucose)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent G (HK/G-6-PDH)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent E (APS)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Reagent H (ATPS)</td>
<td>0.10</td>
<td>------</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>------</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 30°C. Monitor the $A_{340nm}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent F (PP$_i$)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the increase in $A_{340nm}$ for approximately 5 minutes. Obtain the $rA_{340nm}$/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(rA_{340nm}/\text{min Test} - rA_{340nm}/\text{min Blank})(2.95)(\text{df})}{(6.22)(0.1)}$$

2.95 = Total volume (in milliliters) of assay

$\text{df}$ = Dilution factor

6.22 = Millimolar extinction coefficient of β-NADPH at 340 nm

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 produce 1.0 µmole of ATP from APS and inorganic pyrophosphate per minute at pH 8.0 at 30°C.
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FINAL ASSAY CONCENTRATION:

In a 2.95 ml reaction mix, the final concentrations are 115 mM Tris, 0.58 mM ß-nicotinamide adenine dinucleotide phosphate, 2.4 mM magnesium acetate, 34 mM D-glucose, 4 units hexokinase, 2 units glucose 6-phosphate dehydrogenase, 0.3 mM adenosine 5'-phosphosulfate, 0.02 - 0.06 unit adenosine 5'-triphosphate sulfurylase, and 3.4 mM pyrophosphate.

REFERENCES:


NOTES:

1. This assay is based on the cited references.

2. Hexokinase Unit Definition: One unit will phosphorylate 1.0 µmole of D-glucose per minute at pH 7.6 at 25°C.

3. Glucose 6-Phosphate Dehydrogenase Unit Definition: One unit will oxidize 1.0 µmole of D-glucose 6-phosphate to 6-phospho-D-gluconate per minute in the presence of NADP at pH 7.4 at 25°C.