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

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, Sigma Prod. No. T-1503. 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, Sigma Prod. No. N-0505.})
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REAGENTS: (continued)

C. 1 M Magnesium Acetate Solution (MgOAC)
(Prepare 1 ml in deionized water using Magnesium Acetate, Tetrahydrate, Sigma Prod. No. M-9147.)

D. 1 M D-Glucose Solution (Glucose)
(Prepare 2 ml in deionized water using D-(+)-Glucose, Anhydrous, Sigma Prod. No. G-8270.)

E. 20 mM Adenosine 5'-Phosphosulfate (APS)
(Immediately before use, prepare 1 ml in deionized water using Adenosine 5'-Phosphosulfate, Sodium Salt, Sigma Prod. No. A-5508.)

F. 100 mM Pyrophosphate Solution, pH 8.0 at 30°C (PPi)
(Prepare 5 ml in deionized water using Tetrasodium Pyrophosphate, Decahydrate, Sigma Prod. No. P-9146. 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, Sigma Prod. No. H-8629 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.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>34.00</td>
</tr>
<tr>
<td>B-NADP</td>
<td>0.34</td>
</tr>
<tr>
<td>MgOAC</td>
<td>0.28</td>
</tr>
<tr>
<td>Water</td>
<td>65.38</td>
</tr>
</tbody>
</table>

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:

| Reagent F (PP$_i$) | 0.10 | 0.10 |

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

CALCULATIONS:

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

2.95 = Total volume (in milliliters) of assay
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.

4. 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.