Enzymatic Assay of ACETATE KINASE
(EC 2.7.2.1)
from Bacillus stearothermophilus

PRINCIPLE:

\[
\text{Acetate Kinase} \\
\text{Acetate + ATP} \rightarrow \text{Acetyl phosphate + ADP}
\]

\[
\text{Pyruvate Kinase} \\
\text{ADP + PEP} \rightarrow \text{ATP + Pyruvate}
\]

\[
\text{Lactic Dehydrogenase} \\
\text{Pyruvate + β-NADH} \rightarrow \text{Lactate + β-NAD}
\]

Abbreviations used:
ATP = Adenosine 5’-Triphosphate
ADP = Adenosine 5’-Diphosphate
PEP = Phospho(enol)pyruvate
β-NADH = β-Nicotinamide Adenine Dinucleotide, Reduced Form
β-NAD = β-Nicotinamide Adenine Dinucleotide, Oxidized Form

CONDITIONS:  T = 30°C, pH = 7.2, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Imidazole Solution
(Prepare 50 ml in deionized water using Imidazole.)

B. Acetic Acid, Glacial (HOAc)
(Use Acetic Acid, Glacial.)

C. 102 mM Phospho(enol)pyruvate Solution (PEP)
(Prepare 5 ml in deionized water using Phospho(enol)pyruvate, Monopotassium Salt.
PREPARE FRESH.)

D. 300 mM Adenosine 5’-Triphosphate Solution (ATP)
(Prepare 3 ml in deionized water using Adenosine 5’-Triphosphate, Disodium Salt.
PREPARE FRESH.)
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REAGENTS: (continued)

E.  7.8 mM \( \beta \)-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (\( \beta \)-NADH)
(Dissolve the contents of one 10 mg vial of \( \beta \)-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, in the appropriate volume of deionized water or dissolve \( \beta \)-Nicotinamide Adenine Dinucleotide, Reduced Form, in the appropriate volume of water.)

F.  300 mM Magnesium Chloride Solution (MgCl\(_2\))
(Prepare 10 ml in deionized water using Magnesium Chloride, Sterile filtered Solution.)

G.  PK/LDH Enzymes Solution
(Use PK/LDH Enzymes Solution in 50% Glycerol.)

H.  Acetate Kinase Enzyme Solution
(Immediately before use, prepare a solution containing 0.50 - 0.75 unit/ml of Acetate Kinase 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>Reagent A (Imidazole)</td>
<td>18.20</td>
</tr>
<tr>
<td>Reagent B (HOAc)</td>
<td>0.74</td>
</tr>
<tr>
<td>Reagent C (PEP)</td>
<td>1.00</td>
</tr>
<tr>
<td>Reagent D (ATP)</td>
<td>1.00</td>
</tr>
<tr>
<td>Reagent F (MgCl(_2))</td>
<td>2.00</td>
</tr>
</tbody>
</table>

Mix by swirling and adjust to pH 7.2 at 30\(^\circ\)C with 1 M KOH. Then add:

| Reagent E (\( \beta \)-NADH) | 1.00 |

Mix by swirling. Then add deionized water to make a final volume of 30 ml. Mix by stirring.

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

<table>
<thead>
<tr>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction Cocktail</td>
<td>2.80</td>
</tr>
<tr>
<td>Reagent G (PK/LDH)</td>
<td>0.10</td>
</tr>
</tbody>
</table>
PROCEDURE: (continued)

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

<table>
<thead>
<tr>
<th>Solution</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized Water</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent H (Enzyme Solution)</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the decrease in $A_{340\text{nm}}$ for approximately 10 minutes. Obtain the $\Delta A_{340\text{nm}}$/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340\text{nm}}/\text{min Test} - \Delta A_{340\text{nm}}/\text{min Blank})(3)(df)}{(6.22)(0.1)}$$

$3 = \text{Total volume (in milliliters) of assay}$
$df = \text{Dilution factor}$
$6.22 = \text{Millimolar extinction coefficient of } \beta\text{-NADH at 340 nm}$
$0.1 = \text{Volume (in milliliter) of enzyme used}$

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$
$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will phosphorylate 1.0 µmole of acetate to acetyl phosphate per minute at pH 7.2 at 30°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 57 mM imidazole, 400 mM acetic acid, 3.2 mM phospho(enol)-pyruvate, 9.3 mM adenosine 5'-triphosphate, 19 mM magnesium chloride, 0.24 mM β-nicotinamide adenine dinucleotide, 70 units pyruvate kinase, 100 units lactic dehydrogenase, and 0.05 - 0.075 unit acetate kinase.
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REFERENCE:


NOTES:

1. Contains approximately than 700 units/ml of Pyruvate Kinase and 1000 units/ml of Lactic Dehydrogenase.

2. Pyruvate Kinase Unit Definition: One unit will convert 1.0 µmole of phospho(enol)pyruvate to pyruvate per minute at pH 7.6 at 37°C.

3. Lactic Dehydrogenase Unit Definition: One unit will reduce 1.0 µmole of pyruvate to L-lactate per minute at pH 7.5 at 37°C.

4. This assay is based on the cited reference.

5. Where our Product or Stock numbers are specified, equivalent reagents may be substituted.

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