PHOSPHOTRANSCETYLASE (PTA)

[EC 2. 3. 1. 8]

from *Bacillus stearothermophilus*

Acetyl-CoA + Pi ↔ Acetylphosphate + CoA

**SPECIFICATION**

<table>
<thead>
<tr>
<th>State</th>
<th>Lyophilized</th>
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<tbody>
<tr>
<td>Specific activity</td>
<td>more than 5,000 U/mg protein</td>
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<tr>
<td>Contaminants</td>
<td>(as PTA activity = 100 %)</td>
</tr>
<tr>
<td></td>
<td>Acetate kinase</td>
</tr>
<tr>
<td></td>
<td>Adenylate kinase</td>
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<tr>
<td></td>
<td>Lactate dehydrogenase</td>
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</tbody>
</table>

**PROPERTIES**

| Molecular weight       | ca. 70,000          |
| Subunit molecular weight | ca. 35,000          |
| Optimum pH             | 7.5                 |
| pH stability           | 7.0 - 11.0          |
| Isoelectric point      | 4.5                 |
| Thermal stability      | No detectable decrease in activity up to 50 °C. |
| Michaelis constants    | (87mM Tris-HCl buffer, pH 7.5, at 30 °C) |
|                        | Coenzyme A          | 0.4 mM |
|                        | Acetyl Phosphate    | 1.1 mM |

**STORAGE**

Stable at -20 °C for at least one year

**APPLICATION**

The enzyme is useful for determination of CoA or acetate.
ASSAY

**Principle**
The change in absorbance is measured at 233 nm according to the following reaction.

\[
\text{Acetylphosphate} + \text{CoA} \xrightarrow{\text{PTA}} \text{Acetyl-CoA} + \text{Pi}
\]

**Unit Definition**
One unit of activity is defined as the amount of PTA that forms 1 μmol of acetyl-CoA per minute at 30 °C.

**Solutions**
I. Buffer solution; 100 mM Tris-HCl, pH 7.5
II. CoA solution; 6.4 mM (50 mg CoA trilithium salt/10 mL distilled water)
III. Acetylphosphate solution; 217 mM (0.400 g acetylphosphate potassium lithium salt/10 mL distilled water)
IV. Ammonium sulfate (AmS) solution; 1 M (13.2 g AmS/100 mL distilled water)

**Preparation of Enzyme Solution**
Dissolve the lyophilized enzyme with distilled water and dilute to 5 to 20 U/mL with 50 mM Tris-HCl buffer, pH 8.0.

**Procedure**
1. Prepare the following reaction mixture and pipette 3.00 mL of reaction mixture into a cuvette.
   - Solution I: 26.0mL
   - Solution II: 2.0mL
   - Solution III: 1.0mL
   - Solution IV: 1.0mL
2. Incubate at 30 °C for about 3 minutes.
3. Add 0.01 mL of enzyme solution into the cuvette and mix.
4. Read absorbance change at 233 nm per minute (ΔAbs\textsubscript{233}) in the linear portion of curve.

**Calculation**
\[
\text{Volume activity (U/mL)} = \frac{(\Delta\text{Abs}_{233}) \times (3.00 + 0.01)}{4.44 \times 0.01} \times \text{d.f.}
\]

\[
\text{Specific activity (U/mg protein)} = \frac{\text{Volume activity (U/mL)}}{\text{Protein concentration (mg/mL)}}
\]

\text{d.f.} \text{ ; dilution factor}
\text{4.44 ; differential millimolar extinction coefficient between acetyl-CoA and CoA (cm}^2/\mu\text{mol)}
\text{*Protein concentration ; determined by Bradford's method}
**Fig. 1** pH profile

- ● Tris-HCl

**Fig. 2** pH stability

- △ acetate,
- ○ phosphate,
- ● Tris-HCl,
- ■ Gly-KOH

**Fig. 3** Thermal stability

- treated for 15 min in 50 mM Tris-HCl buffer, pH 8.0

**Fig. 4** Thermal stability

- treated in 50 mM Tris-HCl buffer, pH 8.0
- ○ 50 °C, □ 60 °C, ● 65 °C