Determination of the Concentration and Molecular Weight of COENZYME A using PHOSPHOTRANSACETYLASE

PRINCIPLE:

Acetyl Phosphate + CoA $\rightarrow$ Acetyl-CoA + Pi

Abbreviations used:

CoA = Coenzyme A
Acetyl-CoA = Acetyl Coenzyme A
Pi = Inorganic Phosphate

CONDITIONS:  $T = 25^\circ C$, $pH = 7.6$, $A_{233nm}$, Light path = 1 cm

METHODS:  Spectrophotometric

REAGENTS:

A.  100 mM Tris Buffer, pH 7.6 at 25°C
   (Prepare 100 ml in deionized water using Trizma Base, Prod. No. T-1503. Adjust to pH 7.6 at 25°C with 1 M HCl.)

B.  100 mM Acetyl Phosphate Solution
   (Prepare 10 ml in deionized water using Acetyl Phosphate, Lithium Potassium Salt, Prod. No. A-0262. PREPARE FRESH.)

C.  Coenzyme A Solution (CoA)
   (Weigh two samples accurately, approximately 3.0 mg and dissolve each in 10 ml of deionized water which has been purged with nitrogen.)

D.  Phosphotransacetylase Enzyme Solution
   (Prepare a solution containing 100 units/ml of Phosphotransacetylase, from Clostridium kluyveri, Prod. No. P-5907, in Reagent A.)
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PROCEDURE:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
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</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>1.90</td>
<td>1.90</td>
</tr>
<tr>
<td>Reagent B (Acetyl Phosphate)</td>
<td>0.10</td>
<td>------</td>
</tr>
<tr>
<td>Reagent C (CoA)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>------</td>
<td>0.10</td>
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</tbody>
</table>

Mix by inversion and equilibrate to 25°C. Monitor the \( A_{233\text{nm}} \) until constant, using a suitably thermostatted spectrophotometer. Then add:

<table>
<thead>
<tr>
<th>Reagent D (Enzyme Solution)</th>
<th>Test</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0.02</td>
<td>0.02</td>
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Mix by inversion and allow the reaction to proceed for 10 minutes. Upon completion of the reaction record the \( A_{233\text{nm}} \) and calculate the ?\( A_{233\text{nm}} \) and the apparent molecular weight.

CALCULATIONS:

\[ r_A = A_i - A_f \]

\( A_i = \) Initial absorbance

\( A_f = \) Final absorbance

\[ \text{Micromoles CoA/weighed sample} = \frac{r_A \times 3.02 \times 10}{4.44} \]

3.02 = Volume of assay

10 = Sample dilution factor

4.44 = Extinction coefficient of acetyl coenzyme A at 233 nm

\[ \text{mg sample weighed x 1000} \]

\[ \text{apparent mw} = \frac{\text{\mu moles CoA/weighed sample}}{\text{\mu moles CoA/weighed sample}} \]

FINAL CONCENTRATION:

In a 3.02 ml reaction mix, the final concentrations are 63.6 mM tris HCl, 3.3 mM acetyl phosphate, and 2.0 unit phosphotransacetylase.
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REFERENCES:


NOTES:

1. All products and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

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