**Enzymatic Assay of SUCCINYL COENZYME A TRANSFERASE**  
(EC 2.8.3.5)

**PRINCIPLE:**
Acetoacetyl CoA + Succinate $\xrightarrow{\text{SCT}}$ Succinyl CoA + Acetoacetate

Abbreviations used:
SCT = Succinyl Coenzyme A Transferase  
Acetoacetyl CoA = Acetoacetyl Coenzyme A  
Succinyl CoA = Succinyl Coenzyme A

**CONDITIONS:**  
$T = 25^\circ C$, $\text{pH} = 8.1$, $A_{310\text{nm}}$, Light path = 1 cm

**METHOD:**  
Continuous Spectrophotometric Rate Determination

**REAGENTS:**

A. 67 mM Tris $\text{H}_2\text{SO}_4$ Buffer with 10 mM Succinate and 5.0 mM Magnesium Sulfate, pH 8.1 at 25°C (Succ)  
(Prepare 10 ml in deionized water using Trizma Base, Succinic Acid, Disodium Salt, Hexahydrate and Magnesium Sulfate, Heptahydrate. Adjust to pH 8.1 at 25°C with 1 M H$_2$SO$_4$.)

B. 3.0 mM Acetoacetyl CoA Solution (Acet CoA)  
(Prepare 0.1 ml in deionized water using Acetoacetyl Coenzyme A, Sodium Salt.)

C. 67 mM Tris $\text{H}_2\text{SO}_4$ Buffer, pH 8.1 at 25°C (Enz Dil)  
(Prepare 25 ml in deionized water using Trizma Base. Adjust to pH 8.1 at 25°C with 1 M H$_2$SO$_4$.)

D. Succinyl Coenzyme A Transferase Enzyme Solution  
(Immediately before use, prepare a solution containing 0.04 - 0.1 unit/ml of Succinyl Coenzyme A Transferase in cold Reagent C.)
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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>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Succ)</td>
<td>0.94</td>
<td>0.94</td>
</tr>
<tr>
<td>Reagent B (Acet CoA)</td>
<td>0.01</td>
<td>0.01</td>
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</tbody>
</table>

Mix by inversion and equilibrate to 25°C. Monitor the A\text{310nm} 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 D (Enzyme Solution)</td>
<td>0.05</td>
<td>------</td>
</tr>
<tr>
<td>Reagent C (Enz Dil)</td>
<td>------</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the decrease in A\text{310nm} for approximately 5 minutes. Obtain the r A\text{310nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

\[
\text{Units/ml enzyme} = \frac{(r \ A_{310nm}/\text{min Test} - r \ A_{310nm}/\text{min Blank})(1)(df)}{(9.25)(0.05)}
\]

1 = Total volume (in milliliter) of assay
df = Dilution factor
9.25 = Millimolar extinction coefficient\(^1\) of acetoacetyl coenzyme A at 310 nm
0.05 = Volume (in milliliter) of enzyme used

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

UNIT DEFINITION:

One unit will form 1.0 µmole of succinyl CoA from acetoacetyl CoA and succinic acid per minute at pH 8.1 at 25°C.

FINAL ASSAY CONCENTRATIONS:

In a 1.00 ml reaction mix, the final concentrations are 66 mM Tris, 9.4 mM succinic acid, 4.7 mM magnesium sulfate, 0.03 mM acetoacetyl coenzyme A, and 0.002 – 0.005
unit succinyl coenzyme A transferase.

REFERENCE:


NOTES:


2. This assay is based on the cited references.

3. 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.