

**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°C, pH = 8.1, A<sub>310nm</sub>, Light path = 1 cm

**METHOD:** Continuous Spectrophotometric Rate Determination

**REAGENTS:**

- A. 67 mM Tris H<sub>2</sub>SO<sub>4</sub> 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, Sigma Prod. No. T-1503, Succinic Acid, Disodium Salt, Hexahydrate, Sigma Prod. No. S-2378, and Magnesium Sulfate, Heptahydrate, Sigma Prod. No. M-1880. Adjust to pH 8.1 at 25°C with 1 M H<sub>2</sub>SO<sub>4</sub>.)
- B. 3.0 mM Acetoacetyl CoA Solution (Acet CoA)  
(Prepare 0.1 ml in deionized water using Acetoacetyl Coenzyme A, Sodium Salt, Sigma Prod. No. A-1625.)
- C. 67 mM Tris H<sub>2</sub>SO<sub>4</sub> Buffer, pH 8.1 at 25°C (Enz Dil)  
(Prepare 25 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 8.1 at 25°C with 1 M H<sub>2</sub>SO<sub>4</sub>.)
- 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:

	<u>Test</u>	<u>Blank</u>
Reagent A (Succ)	0.94	0.94
Reagent B (Acet CoA)	0.01	0.01

Mix by inversion and equilibrate to 25°C. Monitor the  $A_{310\text{nm}}$  until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent D (Enzyme Solution)	0.05	-----
Reagent C (Enz Dil)	-----	0.05

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

**CALCULATIONS:**

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

1 = Total volume (in milliliter) of assay

df = Dilution factor

9.25 = Millimolar extinction coefficient<sup>1</sup> of acetoacetyl coenzyme A at 310 nm

0.05 = Volume (in milliliter) of enzyme used

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

**UNIT DEFINITION:**

One unit will form 1.0  $\mu\text{mole}$  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:**

White, H., Solomon, F., and Jencks, W.P. (1976) *Journal of Biological Chemistry* **251**, 1700-1707

White, H. and Jencks, W.P. (1976) *Journal of Biological Chemistry* **251**, 1688-1699

**NOTES:**

1. The millimolar extinction coefficient of acetoacetyl coenzyme A is described in White, H., Solomon, F., and Jencks, W.P. (1976).
2. This assay is based on the cited references.
3. 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.**