

**Enzymatic Assay of CARNITINE ACETYLTRANSFERASE
(EC 2.3.1.7)**

PRINCIPLE:

Acetyl-L-Carnitine + CoA $\xrightarrow{\text{Carnitine Acetyltransferase}}$ Acetyl-CoA + L-Carnitine

Abbreviations:

CoA = Coenzyme A

Acetyl-CoA = Acetyl Coenzyme A

CONDITIONS: T = 25°C, pH = 8.0, A_{233nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 100 mM Tris Buffer, pH 8.0 at 25°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 8.0 at 25°C with 1 M HCl.)
- B. 11 mM Coenzyme A
(Prepare 2 ml in deionized water using Coenzyme A, Sodium Salt, Sigma Prod. No. C-3144.)
- C. 83.4 mM Acetyl-DL-Carnitine
(Prepare 10 ml in deionized water using Acetyl-DL-Carnitine Hydrochloride, Sigma Prod. No. A-1509.)
- D. Carnitine Acetyltransferase Enzyme Solution
(Immediately before use, prepare a solution containing 0.3 - 0.6 unit/ml of Carnitine Acetyltransferase in cold Reagent A.)

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PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.65	2.75
Reagent B (Coenzyme A)	0.05	0.05
Reagent C (Acetyl-DL-Carnitine)	0.20	0.20

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

Reagent D (Enzyme Solution)	0.10	-----
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Immediately mix by inversion and record the increase in $A_{233\text{nm}}$ for approximately 5 minutes. Obtain the $r A_{233\text{nm}}$ /minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(r A_{233\text{nm}}/\text{min Test} - r A_{233\text{nm}}/\text{min Blank})(3)(\text{df})}{(4.5)(0.1)}$$

3 = Total volume (in milliliters) of assay

df = Dilution factor

4.5 = Millimolar extinction coefficient of Acetyl-CoA at 233 nm

0.1 = 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 convert 1.0 μmole of acetyl-L-carnitine and CoA to L-carnitine and acetyl-CoA per minute at pH 8.0 at 25°C.

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FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 92 mM Tris, 0.18 mM coenzyme A, 5.6 mM acetyl-DL-carnitine and 0.03 - 0.06 unit of carnitine acetyltransferase.

REFERENCE:

Bergmeyer, H.U., Gawehn, K., and Grassl, M. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U. ed.) Volume I, 438, Academic Press, Inc, New York, NY

NOTES:

1. This assay is based on the cited reference.
2. 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.