

**Enzymatic Assay of CHOLINE ACETYLTRANSFERASE
(EC 2.3.1.6)****PRINCIPLE:**

Choline + ^{14}C -Acetyl CoA $\xrightarrow{\text{Choline Acetyltransferase}}$ CoA + ^{14}C -Acetyl Choline

Abbreviations:

CoA = Coenzyme A

^{14}C -Acetyl CoA = ^{14}C -Acetyl Coenzyme A

CONDITIONS: T = 37°C, pH 7.5

METHOD: Radiolabelled Stop Reaction

REAGENTS:

- A. 75 mM Sodium Phosphate Buffer with 0.133 mM Eserine Salicylate, pH 7.5 at 37°C
(Prepare 1 ml in deionized water using Sodium Phosphate Dibasic, Anhydrous, Prod. No. S-0876 and Eserine, Salicylate Salt, Prod. No. E-8500. Adjust to pH 7.5 with 1 M H_3PO_4 at 37°C.)
- B. 10 mM Choline Chloride Solution (Chol Chlor)
(Prepare 1 ml in Reagent A (Buffer) using Choline, Chloride Salt, Prod. No. C-1879.)
- C. 167 nmole/ml ^{14}C -Acetyl Coenzyme A Solution (^{14}C -Acetyl CoA)
(Prepare 0.300 ml in deionized water using 2 - 10 mCi/mmol ^{14}C -Acetyl Coenzyme A.)
- D. 88 mM Tetraphenylboron Solution (TPB)
(Prepare 1 ml in 3-Butenenitrile, Aldrich Stock No. 12,279-3, using Tetraphenylboron, Sodium Salt, Prod. No. T-4125.)
- E. Choline Acetyltransferase Enzyme Solution
(Immediately before use, prepare a solution containing 15 - 30 units/ml of Choline Acetyltransferase in cold deionized water.)

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PROCEDURE: (continued)

- F. Scintillation Cocktail
(Use Sigma-Fluor Universal LSC Cocktail for Aqueous Samples, Prod. No. S-4273.)

PROCEDURE:

Pipette (in milliliters) the following reagents into an Eppendorf tube:

	<u>Test</u>	<u>Blank</u>
Reagent C (¹⁴ C-Acetyl CoA)	0.030	0.030
Reagent B (Chol Chlor)	0.030	0.030

Mix by swirling and equilibrate for 5 minutes at 37°C. Then add:

Reagent E (Enzyme Solution)	0.005	-----
Deionized Water	-----	0.005

Immediately mix by swirling and incubate for exactly 10 minutes at 37°C. Then add:

Reagent D (TPB)	0.050	0.050
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Centrifuge both the Test and Blank solutions. Remove 0.030 ml of the upper phase from the Test and Blank and place in 7.0 ml glass scintillation vials with 5 ml of Reagent F. Mix by swirling and count for 2 minutes with a suitable scintillation counter.

To determine the total potential decays per minute (dpm), pipette 0.03 ml of Reagent C (¹⁴C-Acetyl CoA), 0.030 ml of Reagent B (Chol Chlor) and 0.005 ml of Reagent E (Enzyme Solution) into a 7.0 ml glass scintillation vial with 5 ml of Reagent F. Mix by swirling and count for 2 minutes with a suitable scintillation counter.

CALCULATIONS:

$$\text{nmoles of } ^{14}\text{C-Acetyl Choline} = \frac{(\mu\text{Ci/ml}) (1000) (0.030)}{(\text{mCi/nmole}) (5.0)}$$

1000 = Conversion factor from μ moles to nmoles

0.030 = Aliquot volume for ¹⁴C-Acetyl CoA

5 = Volume of scintillation fluid

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CALCULATIONS: (continued)

$$\text{Potential dpm/nmole} = \frac{\text{dpm of reaction mix}}{\text{total nmoles of } ^{14}\text{C-Acetyl CoA}}$$

$$\text{Units/mg enzyme} = \frac{(\text{dpm Test} - \text{dpm Blank}) (0.065) (0.115)}{(0.030) (\text{potential dpm/nmole}) (\text{mg enzyme/RM}) (10)}$$

0.065 = Total volume of reaction mixture

0.115 = Volume of assay counted

10 = Time of assay (in minutes)

0.030 = Volume of organic layer counted

RM = Reaction Mixture

UNIT DEFINITION:

One unit will catalyze the transfer of 1.0 nanomole of acetate from ^{14}C -acetyl CoA to choline per minute at pH 7.5 at 37°C.

FINAL ASSAY CONCENTRATIONS:

In a 0.065 ml reaction mix, the final concentrations are 5.01 nmoles of ^{14}C -acetyl coenzyme A, 35 mM sodium phosphate, 0.061 mM eserine salicylate, 4.6 mM choline chloride, and 0.075 - 0.15 unit choline acetyltransferase.

REFERENCE:

Shea, P.A. and Aprison, M.H. (1973) *Analytical Biochemistry* **56**, 165.

NOTES:

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

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