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:

	Test	Blank
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

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 (^{14}C-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:

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

1000 = Conversion factor from µmoles to nmoles
0.030 = Aliquot volume for ^{14}C-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.