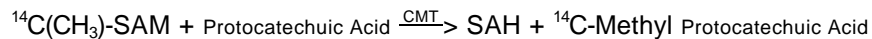


Enzymatic Assay of CATECHOL-O-METHYL TRANSFERASE (EC 2.1.1.6)

PRINCIPLE:



Abbreviations used:

$^{14}\text{C}(\text{CH}_3)\text{-SAM}$ = S-Adenosyl-L(Methyl- ^{14}C)-Methionine

CMT = Catechol-O-Methyl Transferase

SAH = S-Adenosyl-Homocysteine

CONDITIONS: T = 37°C, pH 7.9

METHOD: Radiolabelled Stop Reaction

REAGENTS:

Prepare all reagents in deionized water which has been boiled for 10 minutes. Purge all reagents with nitrogen gas.

- A. 500 mM Tris HCl Buffer, pH 7.9 at 37°C
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. Adjust to pH 7.9 at 37°C with 1 M HCl.)
- B. 100 mM Magnesium Chloride Solution (MgCl_2)
(Prepare 10 ml in deionized water using Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250.)
- C. 100 mM DL-Dithiothreitol Solution (DTT)
(Prepare 10 ml in deionized water using DL-Dithiothreitol, Sigma Prod. No. D-0632.)
- D. 10 mM Ethylenediaminetetraacetic Acid Solution (EDTA)
(Prepare 10 ml in deionized water using Ethylenediaminetetraacetic Acid, Tetrasodium Salt, Hydrate, Sigma Stock No. ED4SS.)
- E. 10 mM Protocatechuic Acid Solution (PCA)
(Prepare 20 ml in deionized water using Protocatechuic Acid, Sigma Prod. No. P-5630.)

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REAGENTS: (continued)

- F. S-Adenosyl-L-(Methyl-¹⁴C)-Methionine Solution (Hot SAM)
(Use 60 mCi/mmol, 25 μ Ci/ml)
- G. 0.2% (w/v) S-Adenosyl-L-Methionine (Cold SAM)
(Immediately, prior to use, prepare 5 ml in Reagent A using S-Adenosyl-L-Methionine, p-Toluenesulfonate Salt, Sigma Prod. No. A-2408.¹ **PREPARE FRESH.**)
- H. 0.1% (w/v) Bovine Serum Albumin Solution with 11 mM DL-Dithiothreitol (Enz Dil)
(Prepare 10 ml in deionized water using Albumin Bovine, Sigma Prod. No. A-4503, and DL-Dithiothreitol, Sigma Prod. No. D-0632.)
- I. Catechol-O-Methyl Transferase Enzyme Solution
(Immediately before use, prepare a solution containing at least 1300-2000 units/ml of Catechol-O-Methyl Transferase in cold Reagent H.)
- J. Scintillation Cocktail (Scint)
(Use Sigma-Fluor Universal LSC Cocktail for Aqueous Samples, Sigma Prod. No. S-4273.)
- K. Ethyl Acetate Solution (ETOAC)
(Use Ethyl Acetate, Sigma Stock No. 27,052-0.)

PROCEDURE:

Prepare a reaction cocktail by pipetting (in milliliters) the following reagents into a suitable container:

Reagent A (Buffer)	1.00
Reagent F (Hot SAM)	0.120
Reagent G (Cold SAM)	0.80
Reagent E (PCA)	1.00
Reagent B (MgCl ₂)	0.10
Reagent C (DTT)	0.05
Reagent D (EDTA)	0.10
Deionized Water	6.83

Mix by swirling and equilibrate 100 μ l of the reaction cocktail to 37°C. Keep in a N₂ environment.

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PROCEDURE: (continued)

Inject (in milliliters) the following reagents into serum vials (which have been flushed with nitrogen and sealed with serum caps):

	<u>Test</u>	<u>Blank</u>
Reaction Cocktail	0.10	0.10
Deionized Water	-----	0.01
Reagent I (Enzyme Solution)	0.01	-----

Mix by swirling and purge with nitrogen gas. Incubate at 37°C for 15 minutes. Then add:

1 M HCl	1.00	1.00
Reagent K (ETOAC)	5.00	5.00

Shake well for several minutes and centrifuge to separate the two phases. Withdraw 2 ml of the ethyl acetate layer (upper layer) from each vial and place into a suitable scintillation vial.

To a separate scintillation vial, add 0.05 ml of the reaction cocktail and 2 ml of Reagent K (ETOAC). This is the total potential counts. Then to each vial add:

Reagent J (Scint)	5.00	5.00
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Mix thoroughly and count in a suitable scintillation counter.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\text{dpm Test} - \text{dpm Blank})(5)(4)(\text{df})}{(\text{SA of SAM})(2)(0.70)(0.01)}$$

5 = Volume (in milliliters) of ethyl acetate added

4 = Conversion factor from minutes to hours (takes reaction time into account)

df = Dilution factor

SA = Specific activity in dpm/nmole from total potential count vials.

2 = Volume (in milliliters) of organic phase which was counted

0.70 = Efficiency of the extraction of the methylated product

0.01 = Volume (in milliliter) of enzyme used

Enzymatic Assay of CATECHOL-O-METHYL TRANSFERASE (EC 2.1.1.6)

UNIT DEFINITION:

One unit will catalyze the methylation of 1.0 nanomole of protocatechuic acid per hour at pH 7.9 at 37°C using S-adenosyl-L(methyl ¹⁴C)-methionine as the methyl donor.

FINAL ASSAY CONCENTRATION:

In a 0.11 ml reaction mix, the final concentrations are 82 mM Tris, 0.9 mM magnesium chloride, 1.5 mM DL-dithiothreitol, 0.1 mM ethylenediaminetetraacetic acid, 0.9 mM protocatechuic acid, 0.4 mM S-adenosyl-L-methionine, 0.01% (w/v) bovine serum albumin, and 13-20 units catechol-o-methyl transferase.

REFERENCES:

Nikodejevic, B., Senoh, S., Daly, J.W., and Creveling, C.R. (1970) *The Journal of Pharmacology and Experimental Therapeutics* **174**, 83-93

NOTES:

1. The concentration of 0.2% (w/v) is based upon the pure S-adenosyl-L-methionine. This does not take into account salts, solvent, water, etc. that may be present.
2. This assay is based on the cited reference.
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.