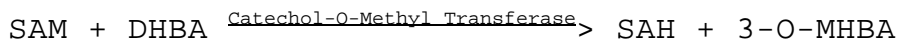


**Enzymatic Assay of CATECHOL-O-METHYL TRANSFERASE
(EC 2.1.1.6)**

PRINCIPLE:



Abbreviations:

SAM = S-Adenosyl-Methionine

SAH = S-Adenosyl-Homocysteine

DHBA = 3,4-Dihydroxybenzoic Acid

3-O-MHBA = 3-O-Methyl,4-hydroxybenzoic Acid

CONDITIONS: T = 37°C, pH = 7.9

METHOD: Radioactive

REAGENTS:

- A. 100 mM Tris buffer, pH 7.9 at 37°C
(Prepare 50 ml in deionized water using Trizma Base, Prod. No. T-1503. Adjust to pH 7.9 at 37°C with 6 M HCl.)
- B. 100 mM Magnesium Chloride (MgCl₂)
(Prepare 10 ml in deionized water using Magnesium Chloride, Prod. No. M-0250.)
- C. 100 mM Dithiothreitol (DTT)
(Prepare 10 ml in deionized water using Dithiothreitol, Prod No. D-0632.)
- D. 10 mM Ethylenediaminetetraacetic Acid (EDTA)
(Prepare 10 ml in deionized water using Ethylenediaminetetraacetic Acid, Tetrasodium Salt, Stock No. ED4SS.)
- E. 100 mM 3,4-Dihydroxybenzoic Acid (DHBA)
(Prepare 20 ml in O₂ free water using 3,4-Dihydroxybenzoic Acid, Prod. No. D-5395. **PREPARE FRESH AND PROTECTED FROM LIGHT.**)

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REAGENTS: (continued)

- F. S-Adenosyl-L-(methyl-¹⁴C)-Methionine (2 µM/ml; 1 µCi/ml)
- G. 0.1% Albumin Buffer (BSA)
(Prepare 25 ml in deionized water using Albumin, Bovine Serum, Prod. No. A-4503.)
- H. Catechol-O-Methyl Transferase Enzyme Solution
(Immediately before use prepare a solution containing 500 units/ml of Catechol-O-Methyl Transferase in cold Reagent G.)
- I. Universal LSC Cocktail for Aqueous Samples
(Use Prod. No. S-4398.)

PROCEDURE:

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

Deionized Water	3.25
Reagent A (Buffer)	5.00
Reagent B (MgCl ₂)	0.10
Reagent C (DTT)	0.05
Reagent D (EDTA)	0.10

Purge material for 5 minutes using N₂. Then add:

Reagent E (DHBA)	1.00
Reagent F ((¹⁴ C-methyl)-SAM)	0.50

Mix by swirling. Equilibrate to 37°C.

Inject into serum vials (flushed with Nitrogen and sealed with serum caps) the following (in milliliters):

	<u>Test</u>	<u>Blank</u>
Reaction Mix	0.50	0.50
Reagent G (Buffer)	----	0.10
Reagent H (Enzyme Solution)	0.10	----

**Enzymatic Assay of CATECHOL-O-METHYL TRANSFERASE
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PROCEDURE: (continued)

Mix by inversion. Incubate at 37°C for 15 minutes. Withdraw 0.1 ml from each vial at time zero, 5 minutes, and 15 minutes, using a syringe and place in suitable dram vials containing 1.0 ml of 0.1 M HCl.

Add (in milliliters) to each dram vial:

Ethyl Acetate	5.00
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Shake well and centrifuge to separate the two phases. Withdraw 4.0 ml of the ethyl acetate layer (upper layer) from each vial and place it in suitable scintillation counting vials.

To a separate scintillation vial add 0.1 ml of the reaction mix. This is the total possible counts/reaction (TCR).

To each scintillation vial add (in milliliters):

Reagent I (LSC Cocktail)	15
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Shake and count in a suitable scintillation counter for.

CALCULATIONS:

CPM = counts per minute of sample - 0 time counts per minute

$$\text{cpm/nMole} = \frac{\text{TCR}}{(0.1) (83.3)}$$

$$\text{Units/mg Protein} = \frac{\text{CPM X 60}}{(\text{cpm/nMole})(\text{mg Protein/reaction})(0.56)}$$

CPM = Net counts per minute of sample
cpm/nMole = Total potential counts of
 S-Adenosyl-L-(methyl-¹⁴C)-Methionine.
TCR = Total possible counts/reaction
0.1 = volume of reaction mix used
83.3 = nMoles of SAM per ml in reaction mix
60 = conversion to hour time frame from minute
0.56 = Efficiency of recovery using ethyl acetate

**Enzymatic Assay of CATECHOL-O-METHYL TRANSFERASE
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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-[methyl¹⁴C]-methionine as the methyl donor.

FINAL ASSAY CONCENTRATIONS:

In a 0.6 ml reaction mix, the final concentrations are 42 mM Tris, 0.83 mM magnesium chloride, 0.42 mM dithiothreitol, 0.083 mM EDTA, 8.3 mM 3,4-dihydroxybenzoic acid, 0.025 µCi S-adenosyl-L-(methyl-¹⁴C)-methionine and 50 units catechol-O-methyl transferase.

NOTES:

1. The TCR determines the number of counts in the reaction mix before enzyme reaction.
2. The recovery of ¹⁴C material using the Ethyl Acetate extraction and correcting for the removal of only 4 ml of the layer is only 56% efficient, so a correction must be made for the counts.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.