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

PRINCIPLE:

\[ ^{14}C(CH_3)\text{-SAM} + \text{Protocatechuic Acid} \xrightarrow{\text{CMT}} \text{SAH} + ^{14}C\text{-Methyl Protocatechuic Acid} \]

Abbreviations used:

- \(^{14}C(CH_3)\text{-SAM} = \text{S-Adenosyl-L(Methyl-}^{14}\text{C)-Methionine} \)
- \(\text{CMT} = \text{Catechol-O-Methyl Transferase} \)
- \(\text{SAH} = \text{S-Adenosyl-Homocysteine} \)

CONDITIONS: \(T = 37^\circ C, \text{pH} 7.9 \)

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. 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.)

C. 100 mM DL-Dithiothreitol Solution (DTT)
(Prepare 10 ml in deionized water using DL-Dithiothreitol.)

D. 10 mM Ethylenediaminetetraacetic Acid Solution (EDTA)
(Prepare 10 ml in deionized water using Ethylenediaminetetraacetic Acid, Tetrasodium Salt, Hydrate.)

E. 10 mM Protocatechuic Acid Solution (PCA)
(Prepare 20 ml in deionized water using Protocatechuic Acid.)
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REAGENTS: (continued)

F. S-Adenosyl-L-(Methyl-\(^{14}\)C)-Methionine Solution (Hot SAM)
   (Use 60 mCi/mmol, 25 µCi/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, 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, and DL-Dithiothreitol.)

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.)

K. Ethyl Acetate Solution (ETOAC)
   (Use Ethyl Acetate.)

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 µl of the reaction cocktail to 37°C. Keep in a N\(_2\) 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):

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
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</thead>
<tbody>
<tr>
<td>Reaction Cocktail</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>------</td>
<td>0.01</td>
</tr>
<tr>
<td>Reagent I (Enzyme Solution)</td>
<td>0.01</td>
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</tbody>
</table>

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

<table>
<thead>
<tr>
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<tr>
<td>1 M HCl</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Reagent K (ETOAC)</td>
<td>5.00</td>
<td>5.00</td>
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</tbody>
</table>

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  |

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)  
\(\text{df}\) = 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
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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(methyl \textsuperscript{14}C)-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 \textit{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:


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.

This procedure is for informational purposes.