ENZYMATIC ASSAY OF CATECHOL O-METHYL TRANSFERASE  
(EC 2.1.1.6)

Procedure

**Principle**

DHAP + SAM $\xrightarrow{\text{COMT}}$ S-adenosyl homocysteine + 3H4MAP + 4H3MAP

The increase in total O-methylated products is followed at an absorbance of 344nm.

**Conditions**

$T = 37^\circ C$, pH = 7.6, $A_{344nm}$, Light path = 1cm

**Method**

Stop rate determination.

**Reagents**

A. **0.5mM 3,4 Dihydroxyacetophenone**  
Prepare 10mL in deionized water using 3,4 Dihydroxyacetophenone (ex Fluorochem Product Number 002544 ). **Prepare fresh**.

B. **5mM S-Adenosyl-L-Methionine (SAM)**  
Prepare 1mL in deionized water using S-Adenosyl-L-Methionine Iodide Salt, Sigma Product Number A4377. **Prepare fresh and store on ice**.

C. **6mM Magnesium Chloride (MgCl$_2$)**  
Prepare 10mL in deionized water using Magnesium Chloride, Sigma Product Number M8266.

D. **20mM Dithiothreitol (DTT)**  
Prepare 10mL in deionized water using Dithiothreitol, Sigma Product No. D5545.

E. **0.2M N-Tris(hydroxymethyl)-methyl-2-aminoethane Sulphonic Acid (EnzymeDiluent Buffer)**  
Prepare 100mL in deionized water using N-Tris(hydroxymethyl)-methyl-2-aminoethane sulphonic acid, Sigma Product Number T6541. Adjust to pH 7.6 at 37°C with 1M NaOH.

F. **COMT Enzyme Solution**  
Immediately before use, prepare a solution containing an approximate 1000 units/ml of COMT in cold Reagent E. Further dilute in Reagent E at 1/10, 1/7 and 1/5 dilutions. Clarify by a short spin before use.

G. **0.4M Sodium Borate (Stop Solution)**  
Prepare 50mL in deionized water using Sodium Borate buffer, Sigma Product Number B0252. Adjust to pH 10.0 at 37°C with 1M NaOH.

**Test Method**
For each enzyme dilution test, pipette (in millilitres) the following reagents into suitable containers, in the order as described in the table below.

<table>
<thead>
<tr>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent A (DHAP)</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent B (SAM)</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent C (MgCl₂)</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent D (DTT)</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Mix by swirling and equilibrate to 37°C. Then add:

<table>
<thead>
<tr>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent F (Enzyme)</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Mix by swirling and incubated at 37°C for exactly 60 minutes. Then add:

<table>
<thead>
<tr>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent G (Stop solution)</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Mix immediately and record $A_{344\text{nm}}$ of Test and Blank with a suitable spectrophotometer.

**Calculation**

Units/ml = \( \frac{(A_{344\text{nm}} \text{ Test} - A_{344\text{nm}} \text{ Blank})(126.2)(\text{dilution factor})}{(0.1)} \)

0.1 = Enzyme volume added to reaction mixture in millilitres.
126.2 = Conversion factor in a 1ml reaction volume.

Units/mg protein = \( \frac{\text{units/ml}}{\text{mg/ml of protein}} \)

**Notes**

The assay method is based on the modification of the published paper in Anal Biochem, 58, 382-389, 1974.

The blank absorbances values should be between 0.3 and 0.5 at 344nm, otherwise repeat using different enzyme dilutions.

The enzyme reaction is sensitive to product inhibition. The product S-Adenosyl Homocysteine is inhibitory and hence appropriate concentration of S-Adenosyl Methionine should be used.

S-Adenosyl-L-Methionine is highly unstable and should be prepared fresh.
Where applicable, all concentrations of reagents indicated above are based on anhydrous molecular weight. Be sure to take into account % purity, salt, and water content.

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.