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°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.

	Test	Blank
Water	---	0.10
Reagent A (DHAP)	0.10	0.10
Reagent B (SAM)	0.10	---
Reagent C (MgCl2)	0.10	0.10
Reagent D (DTT)	0.10	0.10

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

	Test	Blank
Reagent F (Enzyme)	0.10	0.10

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

	Test	Blank
Reagent G (Stop solution)	0.50	0.50

Mix immediately and record A_{344nm} of Test and Blank [8.2] with a suitable spectrophotometer.

Calculation

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

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

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

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.