

Catechol O-Methyl Transferase, Enzyme Activity

Catalog	Unit
TBP0061-1MG	1 mg
TBP0061-5MG	5 mg

Product Details

Form: Freeze-dried powder

Solubility: Distilled water or dilute buffer

Stability: Store at -20° C (-4° F)

Activity: 4000 U/mg protein

Protein: 90%

Contaminants: PNMT <0.01%

Unit Definition

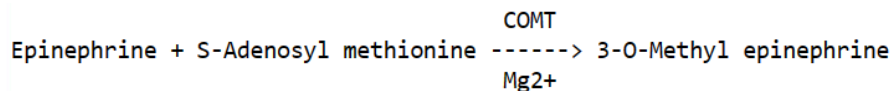
The amount of enzyme which will catalyze the methylation of one nanomole of dihydroxybenzoic acid per hour at 37°C, pH 8.0, using S-adenosyl-L-(methyl 14C)-methionine as the methyl donor.

Assay Method

Enzyme activity is determined by measuring the amount of 14C incorporated into the methylated substrate (3,4-Dihydroxybenzoic acid).

Applications

The enzyme, catechol O-methyltransferase (COMT) (EC 2.1.1.6) can O-methylate a wide variety of substituted catechols such as dopamine, epinephrine and norepinephrine. This is an important step in the metabolic detoxification of catecholamines. A representative reaction is shown below:



Reagents

- 1 mM S-Adenosylmethionine (SAM), (0.435 mg/ml) 100µl. (This product must be of highest purity and must not contain traces of S-adenosylhomocysteine).
- 10 mM Magnesium chloride, in distilled water (0.952 mg/ml). 100µl.
- 5 mM Dithiothreitol (0.61 mg/ml) in distilled water. 100 µl.
- 0.5 M Tris-HCl buffer, pH 8.0 in distilled water. 200 µl.
- 10-50 mM 3,4 Dihydroxybenzoic acid, (1.56-7.81 mg/ml) in water. 200 µl.
- 14C S-Adenosylmethionine, (55mCi/mMole). Distilled water is added to this solution to make 1 ml. It must be kept on ice until needed. Use 10µCi in the assay mixture.
Note: The assay solution used for COMT assay is prepared by mixing the above reagents in the amounts indicated.
- 1% Bovine serum albumin (BSA) solution. Dissolve 1.0 g BSA in 100 ml distilled water. Albumin should be of highest purity.
- COMT (enzyme) solution: Prepare a suitable dilution of the enzyme using cold 1% BSA. Prepare fresh prior to assay.
- Hydrochloric acid (conc.).
- Toluene: 3-methyl butanol, 7:3 (v/v).

Calculation

$$\text{Activity (U/mg)} = \frac{(\text{CPM/sample})(6)(\text{Enz. Diln.})}{(\text{CPM/nmole SAM})(\text{mg Enz./ml})}$$

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