Tribioscience

Phenylethanolamine N-Methyl Transferase, Enzyme Activity

Catalog	Unit
TBP0083-1MG	1 mg
TBP0083-5MG	5 mg

Product Details

Form: Freeze-dried

Solubility: Soluble in distilled water or dilute buffer

Stability: -20° C; -4° F

Activity: 50-100 U/mg protein

Unit Definition

The amount of enzyme which will convert one nanomole of normetanephrine to metanephrine per hour at pH 8.5 at 37°C.

Assay Method

Enzyme activity is determined by measuring the amount of 14C incorporated into the metanephrine formed during the reaction. S- adenosyl-L-(methyl 14C)-methionine serves as a methyl donor.

Applications

Phenylethanolamine N-methyltransferase (PNMT) (EC 2.1.1.28) is the enzyme which catalyzes the N-methylation of norepinephrine thereby resulting in the formation of epinephrine as shown below:

$$\text{Norepinephrine} + \text{S-Adenosyl methionine (SAM)} \xrightarrow{\text{PNMT}} \text{Epinephrine}$$

The mechanism involves transfer of an active methyl group from S-adenosylmethionine (SAM) to the primary amino group of norepinephrine. Although it is primarily localized in the adrenal medulla, PNMT activity has also been demonstrated in the brain and heart tissues of several mammalian species including humans. PNMT purified from ox, rat and rabbit adrenal medulla have molecular weights in the range of 37,000-38,000. Analysis of PNMT activity could provide valuable information in the evaluation of catecholamine metabolism.

Reagents

1. 1.0 mM S-adenosylmethionine (SAM), (0.435 mg/ml). 20 µl. (This product must be of highest purity and must not contain traces of S-adenosylhomocysteine).
2. 1.0 M Tris-HCl, pH 8.5 in distilled water. 200 µl.
3. 30 mM D,L Normetanephrine, (5.1 mg/ml) in distilled water. 100 µl.
4. 14C S-adenosylmethionine, 10 µCi (55 mCi/mM). Distilled water is added to this solution to make 0.5 ml. This solution must be kept on ice until use. Note: The assay solution used for PNMT assay is prepared by mixing the above four reagents in the amounts indicated.
5. 1% Bovine serum albumin (BSA) solution.
6. 0.5 M Sodium borate, (100.65 g/L), pH 10.0.
7. Toluene:isoamyl alcohol (3:2, v/v).
8. PNMT (enzyme) solution. Prepare a suitable dilution of the enzyme using cold 1% BSA. Prepare fresh prior to assay.

Calculation

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

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