

**Enzymatic Assay of PHENYLETHANOLAMINE N-METHYLTRANSFERASE
(EC 2.1.1.28)**

PRINCIPLE:



Abbreviations:

${}^{14}\text{C} (\text{CH}_3)\text{-SAM}$ = S-Adenosyl-L-[Methyl- ${}^{14}\text{C}$]Methionine

PNMT = Phenylethanolamine N-Methyltransferase

SAM = S-Adenosyl-L-Methionine

CONDITIONS: T = 37°C, pH = 8.5

METHOD: Radiolabelled Stop Reaction

REAGENTS:

- A. 400 mM Tris HCl Buffer, pH 8.5 at 37°C
(Prepare 100 ml in deionized water using Trizma Base, Prod. No. T-1503. Adjust to pH 8.5 at 37°C with 1 M HCl.)
- B. 300 mM DL-Normetanephrine Solution
(Prepare 10 ml in deionized water using DL-Normetanephrine, Hydrochloride, Prod. No. N-7127.)
- C. S-Adenosyl-L-[Methyl- ${}^{14}\text{C}$]Methionine (${}^{14}\text{C} (\text{CH}_3)\text{-SAM}$)
(Use 60 mCi/mmol, 25 μ Ci/ml.)
- D. 0.2% (w/v) S-Adenosyl-L-Methionine (SAM)
(Prepare 5 ml in deionized water using S-Adenosyl-L-Methionine, p-Toluenesulfonate Salt, Prod. No. A-2408. **PREPARE FRESH.**)
- E. 500 mM Borate Buffer, pH 10.0 at 37°C
(Prepare 100 ml in deionized water using Boric Acid, Prod. No. B-0252. Adjust to pH 10.0 at 37°C with 5 M NaOH.)
- F. Toluene:Isoamyl Alcohol (3:2)
(Prepare 100 ml using Toluene and Isoamyl Alcohol, Prod. No. I-3643.)

**Enzymatic Assay of PHENYLETHANOLAMINE N-METHYLTRANSFERASE
(EC 2.1.1.28)**

REAGENTS: (continued)

- G. Phenylethanolamine N-Methyltransferase Enzyme Solution (PNMT)
(Immediately before use, prepare a solution containing approximately 100 units/ml in cold deionized water.)
- H. Scintillation Cocktail
(Use Sigma-Fluor Universal LSC Cocktail for Aqueous Samples, Prod. No. S-4273.)

PROCEDURE:

Prepare a reaction cocktail by pipetting (in milliliters) the following reagents into a suitable container:

Reagent A (Tris HCl Buffer)	2.50
Reagent B (DL-Normetanephine)	0.50
Reagent D (SAM)	0.14
	8
Deionized Water	1.84
	2
Reagent C (¹⁴ C (CH ₃)-SAM)	0.010

Pipette (in milliliters) the following reagents into suitable plastic tubes:

	<u>Test</u>	<u>Blank</u>
Reaction Cocktail	0.25	0.25

Equilibrate for 5 minutes at 37°C. Then add:

Reagent G (PNMT)	0.015	-----
Deionized Water	-----	0.015

Immediately mix by swirling and incubate for 15 - 30 minutes at 37°C. Remove a 0.050 ml aliquot from the test mixture and add it to 0.50 ml of Reagent E to stop the reaction.

Extract the ¹⁴C-Methylated product by the addition of 2.5 ml of Reagent F and mix (by vortexing) for 15 seconds. Centrifuge at 2500 RPM for 5 minutes.

Remove 2 ml of the organic upper layer, and place in 7.0 ml glass scintillation vials with 5 ml of Reagent H. Count for 2 minutes with a suitable scintillation counter.

**Enzymatic Assay of PHENYLETHANOLAMINE N-METHYLTRANSFERASE
(EC 2.1.1.28)**

PROCEDURES: (continued)

To determine the total potential decays per minute (dpm), pipette 0.02 ml of the reaction cocktail into 7.0 ml glass scintillation vials. Add 2.00 ml of Reagent F and 5.00 ml of Reagent H. Mix thoroughly and count for 2 minutes with a suitable scintillation counter.

CALCULATION:

$$\text{nmoles of cold SAM} = \frac{(\text{mg/ml in 5.0 ml RM}) (1000) (1000)}{\text{FW}}$$

1000 = Conversion factor from nmoles to μ moles
1000 = Conversion factor from μ moles to nmoles
FW = Formula Weight of SAM used
RM = Reaction Mixture

$$\text{nmoles of } ^{14}\text{C-SAM} = \frac{(\mu\text{Ci/ml}) (1000) (0.010)}{(\text{mCi/mmole}) (5.0)}$$

1000 = Conversion factor from μ moles to nmoles
0.010 = Aliquot volume for $^{14}\text{C-SAM}$
5 = Volume of scintillation fluid
Total nmoles of SAM = nmole cold SAM plus nmole $^{14}\text{C-SAM}$

$$\text{Potential dpm/nmole} = \frac{\text{dpm of 0.02 ml RM}}{\text{Total nmoles of SAM}}$$

RM = Reaction Mixture

$$\text{units/mg enzyme} = \frac{(\text{dpm Test} - \text{dpm Blank})(60)(0.265)(2.5)}{(\text{potential dpm/nmole})(T)(\text{mg enzyme/RM})(0.05)(2.0)}$$

T = Time of reaction
60 = Unit definition of 60 minutes
0.265 = Total volume of Reaction Mixture
2.5 = Total volume of organic layer
0.05 = Volume of Reaction Mixture removed and stopped
2.0 = Volume of organic layer counted

**Enzymatic Assay of PHENYLETHANOLAMINE N-METHYLTRANSFERASE
(EC 2.1.1.28)**

UNIT DEFINITION:

One unit will convert 1.0 nanomole of normetanephrine to metanephrine per hour at pH 8.5 at 37°C.

FINAL ASSAY CONCENTRATIONS:

In a 0.265 ml reaction mix, the final concentrations are 189 mM Tris, 28 mM DL-normetanephrine, 0.006% (w/v) SAM, 0.047 μCi ^{14}C (CH₃)-SAM and 1.5 units phenylethanolamine N-methyltransferase.

REFERENCES:

Axelrod, J. (1962) *Journal of Biological Chemistry* **237**, 1657

NOTES:

1. All products and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.