

**Enzymatic Assay of SPHINGOMYELINASE
(EC 3.1.4.12)**

PRINCIPLE:

[N-Methyl-¹⁴C]Sphingomyelin $\xrightarrow{\text{SM}}$ ¹⁴C-Choline Phosphate + N-Acylsphingosine

Abbreviation used:

SM = Sphingomyelinase

CONDITIONS: T = 37°C, pH = 5.0

METHOD: Radiolabelled Stop Reaction

REAGENTS:

- A. 100 mM Potassium Acetate Buffer with 0.5% (w/v) Triton X-100 and 0.5% (w/v) Human Serum Albumin, pH 5.0 at 37°C (THS)
(Prepare 100 ml in deionized water using Potassium Acetate, Sigma Prod. No. P-1147, Triton X-100, Sigma Stock No. X-100, and Albumin, Human, Sigma Prod. No. A-1653. Adjust to pH 5.0 at 37°C with 1 M HCl.)
- B. 6.1 N Trichloroacetic Acid Solution (TCA)
(Use Trichloroacetic Acid, 6.1 N Solution, approximately 100% w/v, Sigma Stock No. 490-10.)
- C. 1.0% (w/v) Human Serum Albumin Solution (HSA)
(Prepare 100 ml in deionized water using Albumin, Human, Sigma Prod. No. A-1653.)
- D. 0.08 mM Sphingomyelin Solution (Sphingomyelin)
(Prepare by adding 1 mg of Sphingomyelin, Sigma Prod. No. S-7004, and 30 µl of ¹⁴C-Sphingomyelin, Amersham Prod. No. CFASGG (50-62 Ci/mmol, 25mCi/ml) to 15.6 ml of Reagent A. Sonicate to ensure complete dissolution.)
- E. 0.25% (w/v) Triton X-100 with 0.1% (w/v) Human Serum Albumin (Enz Dil)
(Prepare 25 ml in deionized water using Triton X-100, Sigma Stock No. X-100, and Reagent C.)

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REAGENTS: (continued)

- F. Sphingomyelinase Enzyme Solution
(Immediately before use, prepare a solution containing 5 - 10 units/ml of Sphingomyelinase in Reagent E.)
- G. Scintillation Cocktail
(Use Sigma-Fluor Universal LSC Cocktail for Aqueous Samples, Sigma Prod. No. S-4273.)
- H. 1:1 Methylethyl Cellosolve
(Prepare by adding equal volumes of Ethylene Glycol Monoethyl Ether, Sigma Prod. No. E-2632, and Ethylene Glycol Monomethyl Ether, Sigma Prod. No. E-5378.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable containers:

	<u>Test</u>	<u>Blank</u>
Reagent D (Sphingomyelin)	0.150	0.150

Equilibrate to 37°C. Then add:

Reagent F (Enzyme Solution)	0.05	-----
Reagent E (Enz Dil)	-----	0.05

Mix by swirling and incubate at 37°C for exactly 20 minutes. Then add:

Reagent C (HSA)	1.00	1.00
Reagent B (TCA)	0.10	0.10

Mix by swirling and centrifuge for 5 minutes. Remove 0.10 ml of the supernatant from both the Test and Blank and place each into a scintillation vial containing 2 ml of Reagent H (Methylethyl Cellosolve) followed by the addition of 5 ml of Reagent G (Scintillation Cocktail). Count the radioactivity in a suitable scintillation counter.

Prepare potential counts by pipetting 0.10 ml of Reagent D (Sphingomyelin) into 2.0 ml of Reagent H (Methylethyl Cellosolve) followed by the addition of 5 ml of Reagent G (Scintillation Cocktail). Count the radioactivity in a suitable scintillation counter. Calculate the DPM/nmole sphingomyelin.

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CALCULATIONS:

$$\text{DPM/nmole} = \frac{\text{DPM of Potential}}{\text{nmoles of Sphingomyelin in potential}}$$

$$\text{Units/ml enzyme} = \frac{(\text{DPM Test} - \text{DPM Blank})(3)(1.3)(\text{df})}{(\text{DPM/nmole of Sphingomyelin})(0.1)(0.05)}$$

DPM = Disintegrations per minute

3 = Conversion factor from 20 minutes to 1 hour as per the Unit Definition

1.3 = Total volume (in milliliters) of stopped reaction

df = Dilution factor

0.1 = Volume (in milliliter) of supernatant which is added to the scintillation fluid

0.05 = Volume (in milliliter) of enzyme used

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

UNIT DEFINITION:

One unit will hydrolyze 1.0 nanomole of sphingomyelin to N-acylsphingosine and choline phosphate per hour at pH 5.0 at 37°C.

FINAL ASSAY CONCENTRATION:

In a 0.20 ml reaction mix, the final concentrations are 75 mM potassium acetate, 0.4% (w/v) Triton X-100, 0.4% (w/v) human serum albumin, 0.06 mM sphingomyelin, and 0.25 - 0.50 unit sphingomyelinase.

REFERENCE:

Pentchev, P.G., Brady, R.O., Gal, A.E., and Hibbert, S.R. (1977) *Biochimica et Biophysica Acta* **488**, 312-321

NOTES:

1. Not more than 20% of the available counts should be converted during the reaction to ensure linearity.
2. This assay is based on the cited reference.

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NOTES: (continued)

3. 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.