

Amplite™ Fluorimetric Sphingomyelin Assay Kit

Red Fluorescence

Ordering Information	Storage Conditions	Instrument Platform
Product Number: 13625 (100 assays)	Keep in freezer and avoid exposure to light	Fluorescence microplate readers

Introduction

Sphingomyelin (SM) is largely found in the exoplasmic leaflet of the cell membrane, primarily in nervous tissue. It plays an important role in signal transduction. Sphingomyelin accumulates abnormally in Niemann-Pick disease and Abetalipoproteinemia.

Our Amplite™ Fluorimetric Sphingomyelin Assay Kit provides the most sensitive method for detecting neutral SM activity or screening SM inhibitors. The kit uses Amplite™ Red as a fluorogenic probe to indirectly quantify the phosphocholine produced from the hydrolysis of sphingomyelin (SM) by sphingomyelinase (SMase). It can be used for measuring the amount of SM in blood, cell extracts or other solutions. The fluorescence intensity of Amplite™ Red is proportional to the formation of phosphocholine, therefore to the amount of SM. Amplite™ Red enables the assay readable by either a fluorescence reader or an absorbance reader. The kit is an optimized “mix and read” assay that can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step.

Kit Key Features

Broad Application:	Used for quantifying sphingomyelin in blood, cell extracts and solutions.
Sensitive:	Detect as low as 1 μM sphingomyelin in solution.
Continuous:	Easily adapted to automation without a separation step.
Convenient:	Formulated to have minimal hands-on time.

Kit Components

Components	Amount
Component A: Enzyme Mix	1 bottle (lyophilized powder)
Component B: Sphingomyelinase	1 vial (lyophilized powder)
Component C: Amplite™ Red	1 vial (lyophilized powder)
Component D: SMase Reaction Buffer	1 bottle (20 mL)
Component E: Assay Buffer	1 bottle (5 mL)
Component F: 50 mM Sphingomyelin	1 vial (20 μL)
Component G: DMSO	1 vial (200 μL)

Assay Protocol for One 96-well Plate

Brief Summary

Prepare SMase working solution (50 μL) → Add sphingomyelin standards or test samples (50 μL) → Incubate at 37 °C for 1 - 2 hours → Add sphingomyelin assay mixture (50 μL) → Incubate at RT for 0.5 - 2 hours → Monitor fluorescence intensity at Ex/Em = 540/590 nm (cut off at 570 nm)

Note: Thaw kit components at room temperature before starting your experiment.

1. Prepare Sphingomyelinase (SMase) working solution:

- 1.1 Prepare 100X SMase stock solution by adding 50 μL of PBS with 0.1% BSA into the vial of Sphingomyelinase (Component B).

Note: The unused 100X SMase stock solution should be aliquoted and stored at -20°C

5. Run sphingomyelin assay:

- 5.1 Add 50 μL of sphingomyelin assay mixture (from Step 4.2) into each well of sphingomyelin standards, blank control, and test samples (from Step 2.5) to make the total sphingomyelinase assay volume of 150 μL /well. *Note: For a 384-well plate, add 25 μL of sample, 25 μL of sphingomyelinase working solution and 25 μL of sphingomyelin assay mixture into each well.*
- 5.2 Incubate the reaction mixture for 1-2 hours at room temperature (protected from light).
- 5.3 Monitor the fluorescence increase with a fluorescence microplate reader at Ex/Em = 540/590 nm (cut off at 570 nm).

Data Analysis

The fluorescence in blank wells (with the assay buffer only) is used as a control, and is subtracted from the values for those wells with the sphingomyelin reactions. A sphingomyelin standard curve is shown in Figure 1.

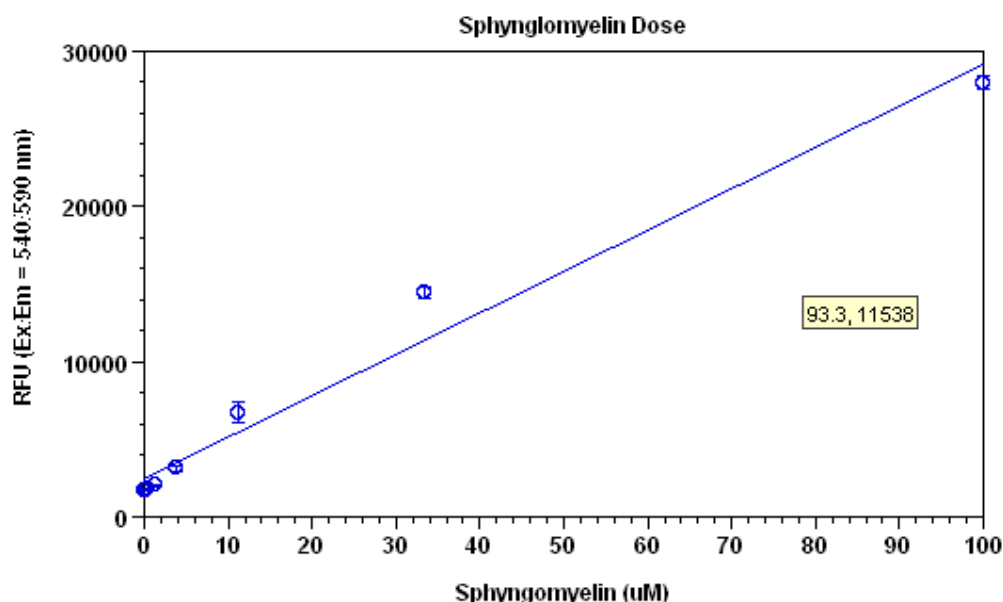


Figure 1 Sphingomyelin dose response was measured on a solid black 96-well plate with Amplite™ Fluorimetric Sphingomyelin Assay Kit using a Gemini fluorescence microplate reader (Molecular Devices). As low as 1 μM sphingomyelin can be detected with 60 minutes incubation (n=3). *Note: The fluorescence background increases with time. It is important to subtract the fluorescence intensity value of the blank wells for each data point.*

References

1. Kentaro Hanada, et al. (2000). "Neutral sphingomyelinase activity dependent on Mg^{2+} and anionic phospholipids in the intraerythrocytic malaria parasite *Plasmodium falciparum*". *Biochem. J.* (2000) 346, 671-677.
2. Bin Liu, et al. (1998). "Purification and Characterization of a Membrane Bound Neutral pH Optimum Magnesium-dependent and Phosphatidylserine-stimulated Sphingomyelinase from Rat Brain". *The Journal of Biological Chemistry*, (1998) 273(51), 34472-34479

Warning: This kit is only sold to end users. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest. Chemical analysis of kit components is strictly prohibited. Please call us at 408-733-1055 or e-mail us at info@aatbio.com if you have any questions.