Amplite™ Fluorimetric Acidic Sphingomyelinase Assay Kit

Red Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 13622 (200 assays)	Keep in freezer and avoid exposure to light	Fluorescence microplate readers

Introduction

Sphingomyelinase (SMase) is an enzyme that is responsible for cleaving sphingomyelin (SM) to phosphocholine and ceramide. Activation of SMase plays an important role in the cellular responses such as regulation of cell growth, cell differentiation, cell cycle arrest and programmed cell death. Five types of sphingomyelinase (SMase) have been identified based on their cation dependence and pH optima of action. They are lysosomal acid SMase, secreted zinc-dependent acid SMase, magnesium-dependent neutral SMase, magnesium-independent neutral SMase and alkaline SMase. Among the five types of sphingomyelinase, lysosomal acidic SMase and magnesium-dependent neutral SMase are considered to be the major factors for the production of ceramide in cellular stress responses.

Our Amplite™ Fluorimetric Acidic Sphingomyelinase Assay Kit provides one of the most sensitive methods for detecting acidic SMase activity or screening its 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). The fluorescence intensity of Amplite™ Red is proportional to the formation of phosphocholine, therefore to the SMase activity. It can be used for measuring the SMase activity in blood, cell extracts or other solutions. The kit is an optimized "mix and read" assay which is compatible with HTS liquid handling instruments.

Kit Key Features

Broad Application:	Used for quantifying acidic sphingomyelinase in blood, cell extracts and solutions.
Sensitive:	Detect as low as 1 unit/mL acidic sphingomyelinase 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	2 bottles (lyophilized powder)
Component B: Sphingomyelin	1 vial (100 µL)
Component C: Amplite™ Red	1 vial (lyophilized powder)
Component D: SMase Reaction Buffer	1 bottle (10 mL)
Component E: Assay Buffer	1 bottle (10 mL)
Component F: DMSO	1 vial (200 µL)

Assay Protocol for One 96-well Plate

Brief Summary

Prepare sphingomyelin working solution (50 µL) → Add SMase standards or SMase test samples (50 µL) → Incubate at 37 °C for 2-3 hours→ Add sphingomyelinase assay mixture (50 µL) → Incubate at RT for 1-2 hours → Monitor fluorescence increase at Ex/Em = 540/590 nm (cut off at 570 nm)

Note: Thaw1 vial (or bottle) of each kit component to room temperature before starting your experiment.

1. Prepare sphingomyelin working solution:

Add 50 µL of Sphingomyelin (Component B) to 5 mL of SMase Reaction Buffer (Component D) and mix well.
Note: The sphingomyelin working solution should be used promptly.

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2. Prepare sphingomyelinase standards and/or sphingomyelinase-containing samples:

2.1 Dilute sphingomyelinase sotck solution in 20 mM sodium acetate buffer (pH = 5.0, not provided in the kit). We recommend the concentration range from 10 U/mL to 0.5 U/mL.
Note 1: Acidic sphingomyelinase standard (from human placenta) was from Sigma-Aldrich (S-5383).
Note 2: Diluted sphingomyelinase standard solution is unstable, and should be used within 4 hours.

2.2 Add the sphingomyelinase standards and sphingomyelinase-containing test samples into a solid black 96-well microplate as shown in Tables 1 and 2.
Note: Treat your cells or tissue samples as desired.

Table 1 Layout of sphingomyelinase standards and test samples in a solid black 96-well microplate

BL	BL	TS	TS	….	….						
SMase1	SMase 1	….	….	….	….						
SMase 2	SMase 2										
SMase 3	SMase 3										
SMase 4	SMase 4										
….	….										
….	….										
….	….										

Note: SMase = Sphingomyelinase Standards, BL = Blank Control, TS = Test Samples

Table 2 Reagent composition for each well

Sphingomyelinase Standard	**Blank Control**	**Test Sample**
Serial dilutions: 50 µL	20 mM sodium acetate buffer (pH = 5): 50 µL	50 µL

Note: Add the diluted sphingomyelinase standards in duplicate.

2.3 Add 50 µL of sphingomyelin working solution (from Step 1) into each well of the sphingomyelinase standards, blank control and test samples (from Step 2.2).

2.4 Incubate the reaction mixture at 37 °C for 2-3 hours.

3. Prepare 200X Amplite™ Red stock solution:

Add 80 µL of DMSO (Component F) into the vial of Amplite™ Red (Component C) to make 200X Amplite™ stock solution.
Note 1: The unused Amplite™ Red stock solution should be aliquoted and stored at -20°C (protected from light).
Note 2: The Amplite™ Red is unstable in the presence of thiols (such as DTT) and 2-mercaptoethanol). The final concentration of DTT or 2-mercaptoethanol in the reaction should be lower than 10 µM. Amplite™ Red is also unstable at high pH (>8.5). The reactions should be performed at pH 7–8. The assay buffer at pH 7.4 is recommended.

4. Prepare sphingomyelinase assay mixture:

4.1 Add 5 mL of Assay Buffer (Component E) to the bottle of Enzyme Mix (Component A) and mix well.

4.2 Add 25 µL of 200X Amplite™ Red stock solution (from Step 3) into the bottle of Enzyme Mix solution (from Step 4.1) to make the sphingomyelinase assay mixture before starting the assay.
Note: The sphingomyelinase assay mixture should be used promptly and kept from light; longer storage is likely to cause high assay background.

5. Run sphingomyelinase assay:

5.1 Add 50 µL of sphingomyelinase assay mixture (from Step 4.2) into each well of the sphingomyelinase standards, blank control, and test samples (from Step 2.4) 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 sphingomyelin working solution, and 25 µL sphingomyelinase assay mixture into each well.

5.2 Incubate the enzyme 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

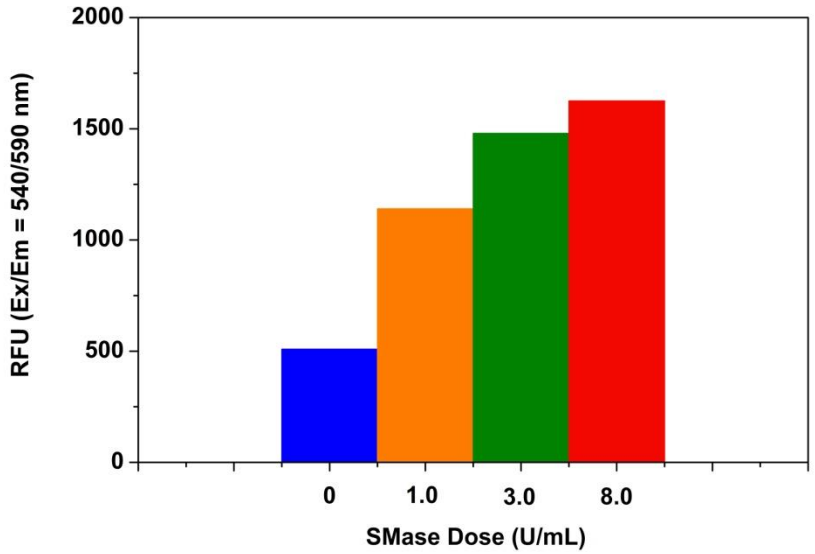

Figure 1 Sphingomyelinase (from human placenta) dose response was measured on a 96-well half-area black plate with Amplite™ Fluorimetric Acidic Sphingomyelinase Assay Kit (13622) using a Gemini fluorescence microplate reader (Molecular Devices). 20 µL of SMase standard or control was incubated with 20 µL of sphingomyelin working solution at 37 °C for 3 hours, and then 20 µL of sphingomyelinase assay mixture was added into each well. The signals shown in the figure are the readings at Ex/Em = 540/590 nm (cut off at 570 nm) after 2 hours incubation at room temperature.

References

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3. Xu M, Liu K, Southall N, Marugan JJ, Remaley AT, Zheng W. A high-throughput sphingomyelinase assay using natural substrate. *Anal Bioanal Chem.* 2012 404(2):407-14. doi: 10.1007/s00216-012-6174-5