# Amplite™ Fluorimetric Sphingomyelin Assay Kit *Red Fluorescence*

Catalog number: 13625
Unit size: 100 Tests

| Component | Storage | Amount |
|---|---|---|
| Component A: Enzyme Mix | Freeze (<-15 °C), Dessicated, Avoid Light | 1 bottle (lyophilized powder) |
| Component B: Sphingomyelinase | Freeze (<-15 °C), Dessicated, Avoid Light | 1 vial (lyophilized powder) |
| Component C: Amplite™ Red | Freeze (<-15 °C), Dessicated, Avoid Light | 1 vial (lyophilized powder) |
| Component D: SMase Reaction Buffer | Freeze (<-15 °C), Avoid Light | 1 bottle (20 mL) |
| Component E: Assay Buffer | Freeze (<-15 °C), Avoid Light | 1 bottle (5 mL) |
| Component F: 50 mM Sphingomyelin | Freeze (<-15 °C), Avoid Light | 1 vial (20 µL ) |
| Component G: DMSO | Freeze (<-15 °C), Avoid Light | 1 vial (200 µL) |

## OVERVIEW

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. It 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 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). It can be used for measuring the 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 either in fluorescence intensity or absorption mode. 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.

## AT A GLANCE

**Protocol summary**

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

**Important** Thaw kit components at room temperature before starting your experiment.

## KEY PARAMETERS

| | |
|---|---|
| Instrument: | Fluorescence microplate reader |
| Excitation: | 540 nm |
| Emission: | 590 nm |
| Cutoff: | 570 nm |
| Recommended plate: | Solid black |

## PREPARATION OF STOCK SOLUTIONS

*Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.*

*1. SMase stock solution (100X):*
Add 50 µL of PBS with 0.1% BSA into the vial of Sphingomyelinase (Component B) to make SMase stock solution (100X).

*2. Amplite™ Red stock solution (200X):*
Add 80 µL of DMSO (Component G) into the vial of Amplite™ Red (Component C) to make 200X Amplite™ Red stock solution. Keep from light.

***Note*** 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. pH 7.4 is recommended for the assay buffer.

## PREPARATION OF STANDARD SOLUTION

**Sphingomyelin standard**

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/13625

Add 2 µL of 50 mM Sphingomyelin (Component F) into 1000 µL of SMase Reaction Buffer (Component D) to get a 100 µM Sphingomyelin standard solution (SM7). Take 100 µM Sphingomyelin standard solution to perform 1:3 serial dilutions to get serially diluted sphingomyelin standards (SM6 - SM1).

## PREPARATION OF WORKING SOLUTION

*1. Sphingomyelinase (SMase) working solution:*
Prepare SMase working solution by adding the whole content (50 µL) of 100X SMase stock solution into 5 mL of SMase Reaction Buffer (Component D) and mix well.

***Note*** The SMase working solution should be used promptly.

*2. Sphingomyelin working solution:*
Add the whole content (5 mL) of Assay Buffer (Component E) into the bottle of Enzyme Mix (Component A) and mix well. Add 25 µL 200X Amplite™ Red stock solution into the bottle of Enzyme Mix solution to make the sphingomyelin assay mixture before starting the assay.

***Note*** The sphingomyelin assay mixture should be used promptly and kept from light; longer storage is likely to cause high assay background.

## PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

## SAMPLE EXPERIMENTAL PROTOCOL

**Table 1.** Layout of sphingomyelin standards and test samples in a solid black 96-well microplate. SM = Sphingomyelin Standards (SM1 - SM7, 0.1 to 100 µM), BL = Blank Control, TS = Test Samples.

| BL | BL | TS | TS |
|---|---|---|---|
| SM1 | SM1 | ... | ... |
| SM2 | SM2 | ... | ... |
| SM3 | SM3 | | |
| SM4 | SM4 | | |
| SM5 | SM5 | | |
| SM6 | SM6 | | |
| SM7 | SM7 | | |

**Table 2.** Reagent composition for each well.

| Well | Volume | Reagent |
|---|---|---|
| SM1 - SM7 | 50 µL | Serial Dilutions (0.1 to 100 µM) |
| BL | 50 µL | SMase Reaction Buffer |
| TS | 50 µL | test sample |

1. Prepare sphingomyelin standards (SM), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

   ***Note*** Treat your cells or tissue samples as desired.

2. Add 50 µL of SMase working solution to each well of sphingomyelin standard, blank control, and test samples to make the total sphingomyelin assay volume of 100 µL/well. For a 384-well plate, add 25 µL of SMase working solution into each well instead, for a total volume of 50 µL/well.

3. Incubate the reaction mixture at 37°C for 1 - 2 hours.

4. Add 50 µL of sphingomyelin assay mixture to each well of sphingomyelin standard, blank control, and test samples to make the total sphingomyelin assay volume of 150 µL/well. For a 384-well plate, add 25 µL of sphingomyelin assay mixture into each well instead, for a total volume of 75 µL/well.

5. Incubate the reaction mixture for 1 - 2 hours at room temperature (protected from light).

6. Monitor the fluorescence increase with a fluorescence microplate reader at Ex/Em = 540/590 nm (cut off at 570 nm).

## EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Sphingomyelin samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

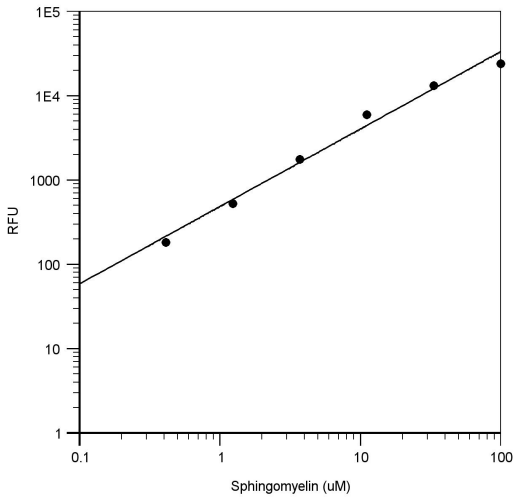


**Figure 1.** Sphingomyelin dose response was measured on a solid black 96-well plate with Amplite™ Fluorimetric Sphingomyelin Assay Kit using a Gemini fluorescence microplate reader (Molecular Devices).

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