Amplite™ Colorimetric Sphingomyelinase Assay Kit

Blue Color

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 13620 (200 assays)	Keep in freezer and avoid exposure to light	Absorbance microplate readers

Introduction

Sphingomyelinase (SMase) is an enzyme that is responsible for cleaving sphingomyelin (SM) to phosphocholine and ceramide. Activation of SMases in cells plays an important role in the cellular responses. 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, the lysosomal acidic SMase and the magnesium-dependent neutral SMase are considered major candidates for the production of ceramide in the cellular response to stress.

Our Amplite™ Colorimetric Sphingomyelinase Assay Kit provides a sensitive method for detecting neutral SMase activity or screening its inhibitors. The kit uses our proprietary Amplite™ UltraBlue as a colorimetric probe to indirectly quantify the phosphocholine produced from the hydrolysis of sphingomyelin (SM) by sphingomyelinase (SMase). It can be used for measuring the SMase activity in blood, cell extracts or other solutions. The absorbance of light at 655 nm is proportional to the formation of phosphocholine, therefore to the SMase activity. The kit is an optimized "mix and read" assay that is compatible with HTS liquid handling instruments.

Kit Key Features

Broad Application:	Used for quantifying neutral sphingomyelinase in blood, cell extracts and solutions.
Sensitive:	Detect as low as of 0.1 mU/mL 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™ UltraBlue	1 vial (lyophilized powder)
Component D: SMase Reaction Buffer	1 bottle (10 mL)
Component E: Assay Buffer	1 bottle (20 mL)
Component F: Sphingomyelinase Standard	0.2 unit (lyophilized powder)
Component G: DMSO	1 vial (200 µL)

Assay Protocol for One 96-well Plate

Brief Summary

Prepare sphingomyelin working solution (50 µL) → Add SMase standards and/or SMase test samples (50 µL) → Incubate at 37 °C for 1-2 hours→ Add sphingomyelinase assay mixture (50 µL) → Incubate at RT for 1-2 hours → Monitor absorbance at 655 nm

Note: Thaw one vial (or bottle) of each kit component at room temperature before starting your experiment.

1. Prepare sphingomyelin working solution:

Add 50 µL of Sphingomyelin (Component B) into 5 mL SMase Reaction Buffer (Component D), and mix well.

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Note: The sphingomyelin working solution should be used promptly.

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

2.1 Add 20 µL of PBS with 0.1% BSA into the vial of Sphingomyelinase Standard (Component F) to make a 10 units/mL sphingomyelinase standard stock solution.
Note: The unused sphingomyelinase standard stock solution should be aliquoted and stored at -20 °C.

2.2 Add 1 µL of 10 units/mL sphingomyelinase standard stock solution (from Step 2.1) into 1000 µL assay buffer (Component E) to generate a 10 mU/mL sphingomyelinase standard.
Note: Diluted sphingomyelinase standard stock solution is unstable, should be used within 4 hours.

2.3 Take 500 µL of 10 mU/mL sphingomyelinase standard to perform 1 to 2 serial dilutions to get 5, 2.5, 1.25, 0.625, 0.313, 0.156, 0.078, and 0 mU/mL serially diluted sphingomyelinase standards.

2.4 Add the serially diluted sphingomyelinase standards and/or sphingomyelinase-containing test samples into white wall/clear bottom or a clear 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 white wall/clear bottom 96-well microplate

BL	BL	TS	TS	….	….						
SMase 1	SMase 1	….	….	….	….						
SMase 2	SMase 2										
SMase 3	SMase 3										
SMase 4	SMase 4										
SMase 5	SMase 5										
SMase 6	SMase 6										
SMase 7	SMase 7										

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

Table 2 Reagent composition for each well

Sphingomyelinase Standards	**Blank Control**	**Test Sample**
Serial Dilutions: 50 µL	Assay buffer: 50 µL	50 µL

Note: Add the serially diluted sphingomyelinase standards from 0.078 to5 mU/mL into wells from SMase 1 to SMase 7 in duplicate.

2.5 Add 50 µL of sphingomyelin working solution (from Step 1) into each well of sphingomyelinase standards, blank control and test samples (from Step 2.4).

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

3. Prepare 200X Amplite™ UltraBlue stock solution:

Add 100 µL of DMSO (Component G) into the vial of Amplite™ UltraBlue (Component C) to make 200X Amplite™ UltraBlue stock solution.
Note 1: The unused Amplite™ UltraBlue stock solution should be aliquoted and stored at -20 °C (kept from light).

Note 2: The Amplite™ UltraBlue 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™ UltraBlue 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.

4. Prepare sphingomyelinase assay mixture:

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

4.2 Add 50 µL of 200X Amplite™ UltraBlue 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 1: The sphingomyelinase assay mixture should be used promptly and kept from light; longer storage is likely to cause high assay background.

Note 2: The cloudiness of the mixture is normal; it will not interfere with the assay performance.

5. Run sphingomyelinase assay:

5.1 Add 50 µL of sphingomyelinase assay mixture (from Step 4.2) into each well of 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 of sphingomyelinase 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 absorbance increase with an absorbance microplate reader at 655 nm.

Data Analysis

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

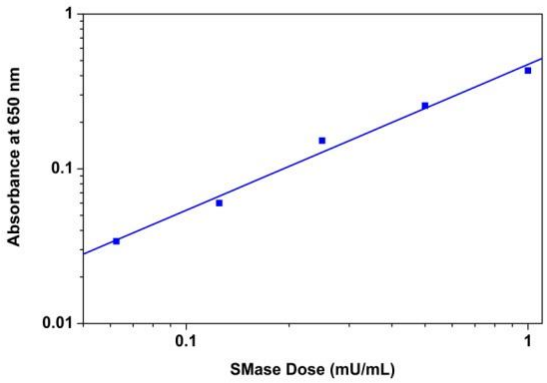

Figure 1 Sphingomyelinase dose response was measured on a 96-well white wall/clear bottom plate with Amplite™ Colorimetric Sphingomyelinase Assay Kit using a SpectraMax microplate reader (Molecular Devices). As low as 0.1 mU/mL sphingomyelinase can be detected with 60 minutes incubation (n=3). *Note: The absorbance background increases with time. It is important to subtract the absorbance value of the blank wells for each data point.*

References

1. 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.
2. Kentaro Hanada, et al. (2000). "Neutral sphingomyelinase activity dependent on Mg2+ and anionic phospholipids in the intraerythrocytic malaria parasite Plasmodium falciparum". Biochem. J. (2000) 346, 671-677.
3. 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