ab138876

Sphingomyelinase Assay Kit (Colorimetric)

Instructions for Use

For measuring Sphingomyelinase activity in blood, cell extracts or other solutions.

This product is for research use only and is not intended for diagnostic use.

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1. 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.

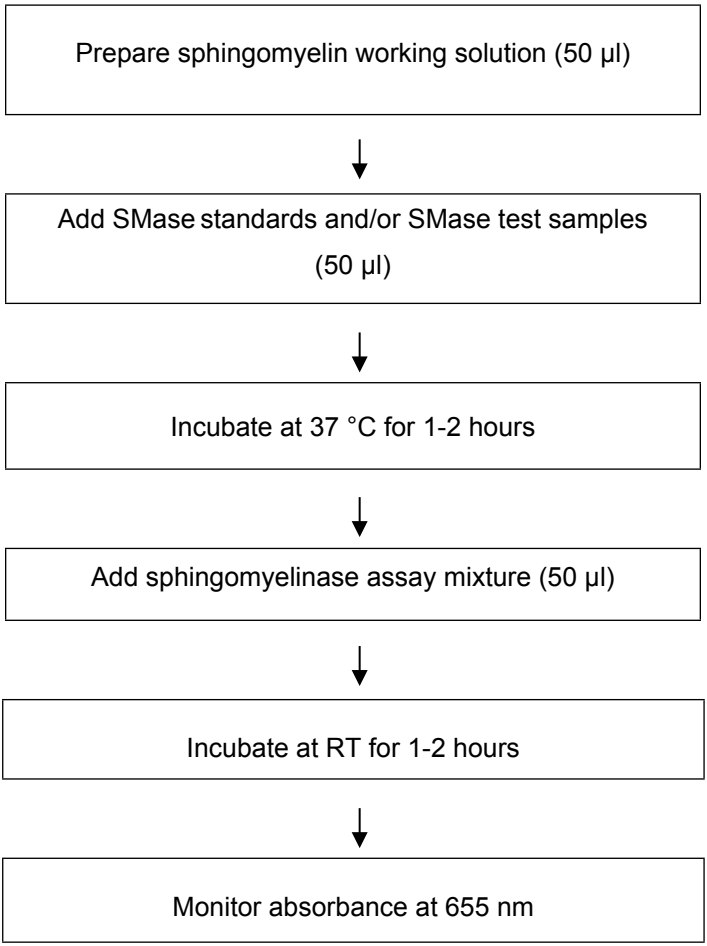
ab138876 Sphingomyelinase Assay Kit (Colorimetric) provides a sensitive method for detecting neutral SMase activity or screening its inhibitors. The kit uses our proprietary AbBlue Indicator as a colorimetric probe to indirectly quantify the phosphocholine produced from the hydrolysis of sphingomyelin (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 0.08 mU/ml sphingomyelinase in solution.
- **Continuous:** Easily adapted to automation without a separation step.
- **Convenient:** Formulated to have minimal hands-on time.

2. Protocol Summary

Summary for One 96-well Plate



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

3. Kit Contents

Components	Amount
Component A: Enzyme Mix	2 bottles (lyophilized powder)
Component B: Sphingomyelin	1 vial (100 µl)
Component C: AbBlue Indicator	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)

4. Storage and Handling

Keep at -20°C. Avoid exposure to light.

5. Additional Materials Required

- 96 or 384-well white wall/clear bottom or clear microplates
- Microplate reader
- PBS
- 0.1% BSA

6. Assay Protocol

Note: This protocol is for one 96 - well plate.

A. Prepare sphingomyelin working solution

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

Note: The sphingomyelin working solution should be used promptly.

B. Prepare sphingomyelinase standards and/or sphingomyelinase-containing samples

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. Add 1 µl of 10 units/ml sphingomyelinase standard stock solution 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.

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.

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.

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										

Table 1. Layout of sphingomyelinase standards and test samples in a white wall/clear bottom 96-well microplate.

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

Sphingomyelinase Standard	Blank Control	Test Sample
Serial Dilutions: 50 µl	Assay Buffer: 50 µl	50 µl

Table 2. Reagent composition for each well.

Note: Add the serially diluted sphingomyelinase standards from 0.078 to 5 mU/ml into wells from SMase 1 to SMase 7 in duplicate.

5. Add 50 µl of sphingomyelin working solution into each well of sphingomyelinase standards, blank control and test samples.

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

C. Prepare 200X AbBlue Indicator stock solution:

Add 100 µL of DMSO (Component G) into the vial of AbBlue Indicator (Component C) to make 200X AbBlue Indicator stock solution.

Note 1: The unused AbBlue Indicator stock solution should be aliquoted and stored at -20 °C (kept from light).

Note 2: The AbBlue Indicator 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. AbBlue Indicator 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.

D. Prepare sphingomyelinase assay mixture:

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

2. Add 50 μl of 200X AbBlue Indicator stock solution into the bottle of Enzyme Mix solution 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.

E. Run sphingomyelinase assay

1. Add 50 µl of sphingomyelinase assay mixture into each well of sphingomyelinase standards, blank control, and test samples 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.

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

3. Monitor the absorbance increase with an absorbance microplate reader at 655 nm.

7. 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.

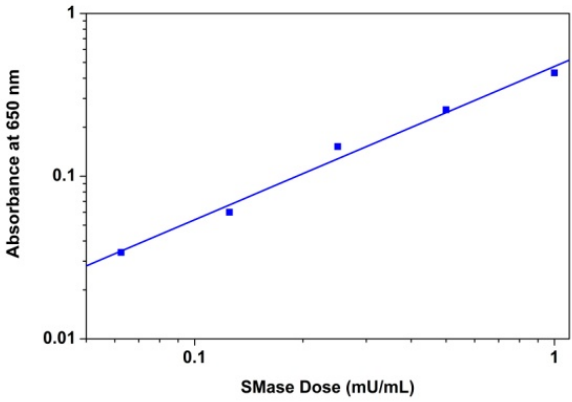


Figure 1. Sphingomyelinase dose response was measured on a 96-well plate with ab138876 using a microplate reader. As low as 0.08 mU/ml sphingomyelinase can be detected with 60 minutes incubation (n=3).

Note: The absorbance background increases with time. It is important to subtract the absorbance value of the blank wells for each data point.

8. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)*** or ***Deproteinizing sample preparation kit (ab93299)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet

	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

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