ab138877

Sphingomyelin Assay Kit (Fluorometric)

Instructions for Use

For the detection of Sphingomyelin in blood, cell extracts and other solutions.

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

Version 3 Last Updated 20 November 2023

Table of Contents

1. 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.

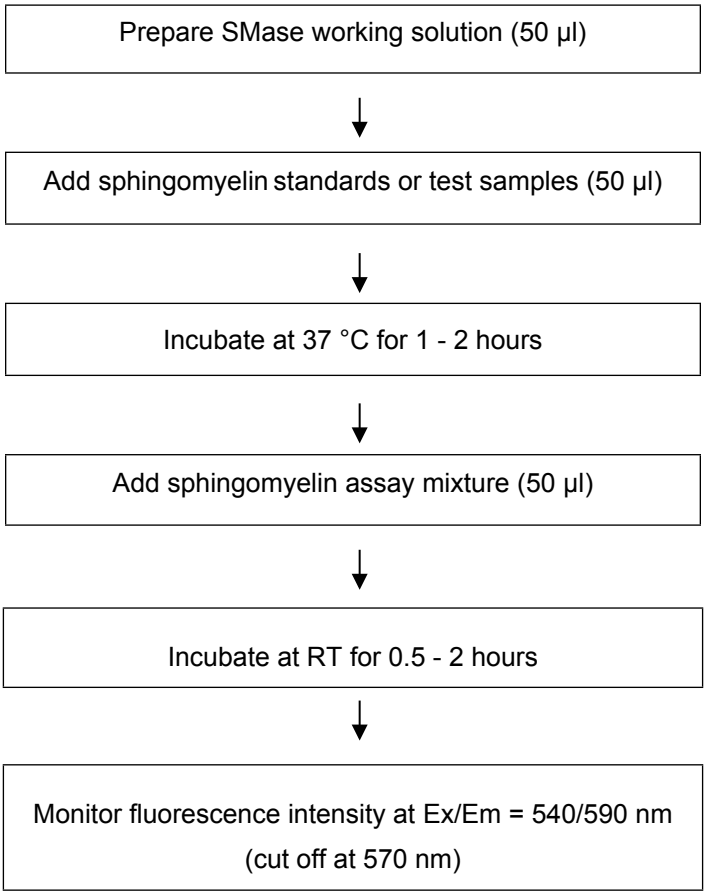
ab138877 Sphingomyelin Assay Kit (Fluorometric) provides the most sensitive method for detecting neutral SM activity or screening SM inhibitors. The kit uses AbRed Indicator as a fluorogenic probe to indirectly quantify the phosphocholine produced from the hydrolysis of sphingomyelin (SM) by sphingomyelinase (SMase). It can be used for measuring the amount of SM in blood, cell extracts or other solutions. The fluorescence intensity of AbRed Indicator is proportional to the formation of phosphocholine, therefore to the amount of SM. AbRed Indicator 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.

2. Protocol Summary

Summary for One 96-well Plate



Note: Thaw all the kit components to room temperature before starting the experiment.

3. Kit Contents

Components	Amount
Component A: Enzyme Mix	1 bottle (lyophilized)
Component B: Sphingomyelinase	1 vial (lyophilized)
Component C: AbRed Indicator	1 vial (lyophilized)
Component D: SMase Reaction Buffer	1 bottle (20 ml)
Component E: Assay Buffer	1 bottle (5ml)
Component F: 50 mM Sphingomyelin Standard	1 vial (20 µl)
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 solid black microplates
- Fluorescence microplate reader
- PBS
- 0.1% BSA

6. Sample preparation

Plasma

1. Collect blood using an anticoagulant.

2. Centrifuge the blood at 700-1,000 x g for 10 minutes at 4°C. Pipette off the top yellow plasma layer without disturbing the white buffy layer. Store plasma on ice. If not assaying the same day, freeze at -80°C.

Serum

1. Collect blood without using an anticoagulant.

2. Allow blood to clot for 30 minutes at 25°C.

3. Centrifuge the blood at 2,000 x g for 15 minutes at 4°C. Pipette off the top yellow serum layer without disturbing the white buffy layer. Store serum on ice. If not assaying the same day, freeze at -80°C.

The plasma and serum sample will be stable for one month while stored at -80°C.

Cell lysates

For preparing cell lysates we recommend using Mammalian Cell Lysis Buffer 5X (ab179835) to lyse the cells and use the supernatant for Sphingomyelin quantification.

7. Assay Protocol

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

A. Prepare Sphingomyelinase (SMase) working solution

1. Prepare 100X SMase stock solution: Add 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.

2. Prepare SMase working solution: Add 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.

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

1. Add 2 µl of 50 mM Sphingomyelin Standard (Component F) into 1000 µl of SMase Reaction Buffer (Component D) to get a 100 µM Sphingomyelin standard solution.

2. Take 200 µl of 100 µM Sphingomyelin standard solution to perform 1:3 serial dilutions to get 30, 10, 3, 1, 0.3, 0.1 and 0 µM serially diluted sphingomyelin standards.

3. Add the sphingomyelin standards and sphingomyelin-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.

BL	BL	TS	TS	….	….						
SM 1	SM 1	….	….	….	….						
SM 2	SM 2										
SM3	SM3										
SM4	SM4										
SM5	SM5										
SM6	SM6										
SM 7	SM 7										

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

Note: SM = Sphingomyelin Standards, BL = Blank Control, TS = Test Samples.

Sphingomyelin Standards	Blank Control	Test Sample
Serial Dilutions: 50 µl	Reaction Buffer: 50 µl	50 µl

Table 2 Reagent composition for each well

Note: Add the serially diluted sphingomyelin standards from 0.1 to 100 µM into wells from SM 1 to SM 7 in duplicate.

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

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

C. Prepare 200X AbRed Indicator stock solution:

Add 80 µl of DMSO (Component G) into the vial of AbRed Indicator (Component C) to make 200X AbRed Indicator stock solution.

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

Note 2: The AbRed 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. AbRed 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 sphingomyelin assay mixture:

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

2. Add 25 µl 200X AbRed Indicator 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.

E. Run sphingomyelin assay:

1. Add 50 µl of sphingomyelin assay mixture into each well of sphingomyelin 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 sphingomyelinase working solution and 25 µl of sphingomyelin assay mixture into each well.

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

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

8. 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.

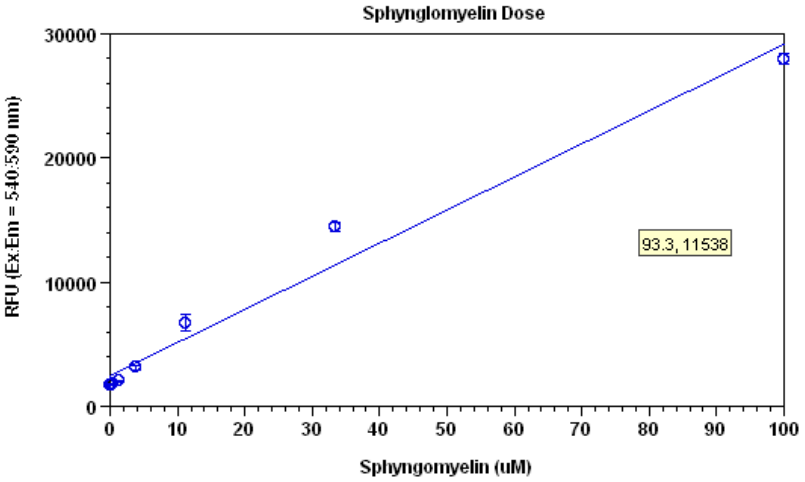


Figure 1. Sphingomyelin dose response was measured on a solid black 96-well plate with ab138877 using a fluorescence microplate reader. As low as 1 µM sphingomyelin can be detected with 60 minutes incubation (n=3).

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

9. 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).

For all technical and commercial enquires please go to:
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