ab287874 – Sphingomyelinase Assay Kit

For the quantitative measurement of neutral-SMase enzymatic activity using fluorescence.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/287874

Storage and Stability

Store kit at -20°C, protected from light.

Materials Supplied

Item	Quantity	Storage Condition
SMase Assay Buffer	28 ml	-20°C
SMase Probe (DMSO)	0.2 ml	-20°C
SMase Substrate (Lyophilized)	1 vial	-20°C
SMase Enzyme Mix I (Lyophilized)	1 vial	-20°C
SMase Enzyme Mix II (Lyophilized)	1 vial	-20°C
Choline Standard (Lyophilized)	1 vial	-20°C
SMase Positive Control (Lyophilized)	1 vial	-20°C
SMase Extraction Detergent	1 ml	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer.

Reagent Preparation

- Briefly centrifuge small vials prior to opening.
- Read the entire protocol before performing the assay.

SMase Assay Buffer: Warm SMase Assay Buffer to room temperature (RT) before use. Store at 4°C or -20°C.

SMase Probe: Store at -20°C. Avoid light exposure. Warm to RT before use. Use within two months.

SMase Substrate: Reconstitute with 110 µl SMase Assay Buffer. Store at -20°C. Keep on ice while in use. Use within two months.

SMase Enzyme Mix I: Reconstitute with 220 µl SMase Assay Buffer. Store at -20°C. Keep on ice while in use. Use within two months.

SMase Enzyme Mix II: Reconstitute with 1.1 ml of SMase Assay Buffer. Store at -20°C. Use within two months.

Choline Standard: Reconstitute with 100 µl SMase Assay Buffer to generate 50 mM Choline Standard stock. Store at -20°C. Use within two months.

SMase Positive Control: Reconstitute with 100 µl of SMase Assay Buffer. Aliquot and store at -20°C. Use within two months.

SMase Extraction Detergent: Store at RT. Vortex quickly before use.

Standard Preparation

- Dilute Choline Standard to 0.5 mM by adding 10 µl of 50 mM Choline Standard into 990 µl of SMase Assay Buffer and mix well.
- Dilute further to 50 µM by adding 10 µl of 0.5 mM Choline Standard into 90 µl of SMase Assay Buffer and mix well.
- Add 0, 2, 4, 6, and 8 µl of the diluted 50 µM Choline Standard into a series of wells in 96-well plate.
- Adjust the volume to 50 µl/well with the SMase Assay Buffer to generate 0, 100, 200, 300 and 400 pmol/well of Choline Standard.

Sample Preparation

- Add 92 µl of SMase Assay Buffer and 8 µl of SMase Extraction Detergent to 10 mg of sample (wet weight or cell pellet).
- Homogenize on ice using a Dounce homogenizer (BV Cat.# 1998). Centrifuge at 10,000 X g, 4°C for 5 min. Collect the supernatant.
- Add 5-50 µl of supernatant into desired well(s) in 96-well plate and adjust the volume to 50 µl with SMase Assay Buffer.
- Add 5-10 µl of SMase Positive Control into desired positive control well(s) and adjust the final volume to 50 µl with SMase Assay Buffer.

Δ Note: *We recommend adding a Protease Inhibitor Cocktail in 1:1000 ratio while preparing the samples.*

Δ Note: *Cell & tissue lysate can be stored at -80°C for future experiments.*

Δ Note: *For unknown samples, we suggest doing a pilot experiment & test several doses to ensure the readings are within the Standard Curve range.*

Δ Note: *For samples with a high background signal, prepare parallel well(s) containing the same amount of sample as in the test well (see below). Adjust the volume to 50 µl with SMase Assay Buffer.*

Assay Protocol

Mix enough reagents for the number of assays (samples, Standards & Positive Control) to be performed. Dilute SMase Probe 1:10 (1 µl Probe in 9 µl SMase Assay Buffer) before use. For each well, prepare 50 µl Reaction Mix containing:

Component	Reaction Mix	Background Control Mix
SMase Assay Buffer	35 µl	36 µl
SMase Enzyme Mix I	2 µl	2 µl
SMase Enzyme Mix II	10 µl	10 µl
SMase Substrate	1 µl	--
SMase Probe	2 µl	2 µl

Mix well. Add 50 µl of Reaction Mix to each well containing Choline Standards, Positive Control and samples. Mix well.

For samples with a high background signal, add 50 µl of Background Control Mix to Sample Background Control well(s). Mix well.

Measurement

Incubate for 30 min. at 37°C and measure fluorescence (Ex/Em = 535/587 nm).

Δ Note: *Incubation time depends on the SMase Activity in the samples. We recommend measuring fluorescence in kinetic mode, and choosing two time points (T1 and T2) in the linear range to calculate the SMase activity of the samples. We recommend running the assay for at least 1 hr. in kinetic mode. The Standard Curve can be read in end point mode (i.e. at the end of incubation time).*

Calculation:

Subtract 0 Choline Standard reading from all readings. Plot the Choline Standard Curve. If the Sample Background Control reading is significant, subtract Background Control reading from sample readings. Calculate SMase activity of test sample:

ΔRFU = RFU2 – RFU1. Apply ΔRFU to Standard Curve to get B pmol of Choline generated by SMase during the reaction time (ΔT = T2 – T1).

Sample Sphingomyelinase Activity = B/(ΔT X V) x D = pmol/min/ml = µU/ml

Where:

- **B** = SMase amount from the Standard Curve (pmol)
- **ΔT** = reaction time (min.)
- **V** = sample volume added into the reaction well (ml)
- **D** = sample dilution factor

Sphingomyelinase specific activity can be expressed as mU/mg or as µU/mg of protein

Technical Support

For all technical or commercial enquiries please go to:
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www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)