3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Sphingomyelin Quantification Assay Kit

Catalog Number **MAK262**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description
Sphingomyelin, a sphingolipid consisting of a phospholipid (typically phosphocholine) attached to a ceramide moiety, is a major component of cellular membranes. Sphingomylein is enriched in the exoplasmic leaflet of the cell membrane. Within the cell membrane, sphingomyelin interacts with cholesterol to form lipid rafts. The metabolism of sphingomyelin contributes to cellular signaling via the release of ceramide, a second messenger that participates in multiple cellular signaling pathways. Sphingomyelin accumulates in Niemann-Pick disease due to deficiencies in sphingomyelinase activity.

The Sphingomyelin Quantification Assay Kit is a simple and sensitive method for quantifying sphingomyelin. The assay is based on the hydrolysis of sphingomyelin into ceramide and phosphocholine by sphingomyelinase. Alkaline phosphatase (ALP) dephosphorylates phosphocholine to choline producing a reaction that generates a colorimetric signal (A_{570}). The assay is sensitive to 1 μM of sphingomyelin in a variety of samples.

The assay kit is suitable for use with serum, plasma, and other biological fluids, and tissue and cell culture samples.

Components
The kit is sufficient for 100 assays in 96 well plates.

SM Assay Buffer Catalog Number MAK262A	100 mL
Red Probe Catalog Number MAK262B	0.2 mL
Sphingomyelinase Catalog Number MAK262C	1 vl
ALP Enzyme Catalog Number MAK262D	1 vl
SM Enzyme Mix Catalog Number MAK262E	1vl
SM Standard (5 mM) Catalog Number MAK262F	50 μL

Reagents and Equipment Required but Not Provided.
- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

SM Assay Buffer – Allow buffer to come to room temperature before use.

Red Probe - Warm to room temperature prior to use. Store at –20 °C. Upon thawing, the reagent is ready-to-use as supplied.

Sphingomyelinase – Reconstitute with 220 μL of SM Assay Buffer. Mix well by pipetting (do not vortex). Store at 2–8 °C. Use within 2 months of reconstitution.

ALP Enzyme – Reconstitute with 1.1 mL of SM Assay Buffer. Mix well by pipetting (do not vortex), and store at 2–8 °C. Use within 2 months of reconstitution.

SM Enzyme Mix – Reconstitute with 220 μL of SM Assay Buffer. Mix well by pipetting (do not vortex), and store at 2–8 °C. Use within 2 months of reconstitution.

SM Standard –Storage at –20 °C may cause the SM Standard to separate from the aqueous phase. Redissolve by placing in a hot water bath (70–80 °C) for 1 minute. Vortex for 30 seconds and then cool to room temperature. Centrifuge for 5 seconds and check for clarity. Repeat heating if solution is turbid. Stable for 2 months.

Storage/Stability
The kit is shipped on wet ice. Storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

SM Standards for Colorimetric Detection
Dilute 5 μL of the 5 mM SM Standard with 245 μL of SM Assay Buffer to prepare a 100 μM standard solution. Add 0, 10, 20, 30, 40, and 50 μL of the diluted SM standard solution into a 96 well plate, generating 0 (blank), 1, 2, 3, 4, and 5 nmole/well standards. Add SM Assay Buffer to each well to bring the volume to 50 μL.

Sample Preparation
The colorimetric assay requires 50 μL of sample for each reaction (well).

Serum or plasma samples containing 0.5–1 mM of sphingomyelin can be assayed directly using 2–10 μL of sample.

Tissue or cells (~25 mg) can be homogenized in 0.5 mL of SM Assay Buffer. Centrifuge the samples at 10,000 × *g* for 5 minutes at 4 °C and transfer the supernatant to a separate tube. Aliquot 20 μL of the homogenate and add 20 μL of SM Assay Buffer. Heat the homogenate for 1–2 minutes at 70 °C, or until it becomes cloudy. Allow the sample to cool to room temperature and then repeat the heating step. Centrifuge at 10,000 × *g* for 2 minutes at room temperature to remove insoluble materials. Collect the supernatant.

Add 1–5 μL samples into wells of a 96 well plate. Bring samples to a final volume of 50 μL with SM Assay Buffer.

Notes: For unknown samples, it is suggested to test several sample volumes to make sure the readings are within the range of the standard curve.

For samples having high background, prepare parallel sample well(s) as sample background control(s).

Assay Reaction
1. Set up the Master Reaction Mix according to the scheme in Table 1. 50 μL of the Master Reaction Mix is required for each reaction (well).

Table 1.
Master Reaction Mix

Reagent	**Samples and Standards**	**Sample Control**
SM Assay Buffer	34 μL	36 μL
Red Probe	2 μL	2 μL
Sphingomyelinase	2 μL	–
ALP Enzyme	10 μL	10 μL
SM Enzyme Mix	2 μL	2 μL

2. Add 50 μL of the Master Reaction Mix to each sample and standard control well. If using a sample background control, add 50 μL of Sample Control Mix to sample control wells. Mix well using a horizontal shaker or by pipetting.

3. Incubate the plate for 60 minutes at 37 °C.

4. Measure the absorbance at 570 nm (A_{570}).

Results

Calculations

The reagent background for the assay is the value obtained for the 0 (assay blank) SM Standard. Correct for the background by subtracting the 0 (assay blank) value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate SM standards to plot a standard curve.

Notes: A new standard curve must be set up each time the assay is run.

Subtract the Sample Background Control value from the sample readings to obtain the corrected colorimetric measurement. Using the corrected measurement, determine the amount of Sphingomyelin present in the sample from the standard curve.

Concentration of Sphingomyelin

$$S_a/S_v = C$$

S_a = Amount of Sphingomyelin in the unknown sample (nmole) from standard curve
S_v = Sample volume (μL) added into the wells
C = Concentration of Sphingomyelin in sample

Sample Calculation

Amount of Sphingomyelin (S_a) = 3.84 nmole (from standard curve)
Sample volume (S_v) = 50.0 μL

Concentration of Sphingomyelin in sample

$$3.84 \text{ nmole}/50.0 \text{ μL} = 0.0768 \text{ nmole/μL}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay Not Working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

KVG,SJ,JAC,MAM 02/18-1