Sphingomyelin Quantification Colorimetric Assay Kit (BN00827)

(Catalog #BN00827; 100 assays; Store at -20°C)

I. Introduction:

Sphingomyelin is an important structural lipid component of cell membranes and lipoproteins. Sphingomyelin has been implicated in the pathogenesis of several diseases; therefore a sensitive and reliable technique for its quantification is of considerable importance. Assay Genie's Sphingomyelin Quantification Assay Kit provides a simple and sensitive method for quantifying sphingomyelin using colorimetry. In the assay, sphingomyelin is hydrolyzed into ceramide and phosphorylcholine by sphingomyelinase. Alkaline phosphatase (ALP) dephosphorylates phosphorylcholine to choline, which then reacts with Sphingomyelin Enzyme Mix to produce an intermediate. The intermediate reacts with a highly specific probe to generate color (OD 570 nm). The kit can detect sphingomyelin in various biological samples as low as 1 µM.

II. Application:

- Sphingomyelin quantification in various biological samples
- Analysis of cell signaling pathway

III. Sample Type:

- Serum, plasma
- Tissue samples such as brain etc.
- Adhesion or suspension cells

IV. Kit Contents:

Components	BN00827	Cap Code	Part Number
SM Assay Buffer	100 ml	NM	BN00827-1
GenieRed Probe	0.2 ml	Red	BN00827-2A
Sphingomyelinase (Lyophilized)	1 vial	Purple	BN00827-3
ALP Enzyme (Lyophilized)	1 vial	Green	BN00827-4
SM Enzyme Mix (Lyophilized)	1 vial	Green, White Dot	BN00827-5
SM Standard (5 mM)	50 µl	Yellow	

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer capable of absorbance detection

VI. Storage and Handling:

Store kit at -20°C, protected from light. Warm SM Assay Buffer to room temperature before use. Read the entire protocol before performing the assay.

VII. Reagent Preparation and Storage Conditions:

- **GenieRed Probe:** Ready to use as supplied. Warm to room temperature before use. Store at -20°C.
- **Sphingomyelinase:** Reconstitute with 220 µl SM Assay Buffer. Store at 4°C. Stable for two months.
- **ALP Enzyme:** Reconstitute with 1.1 ml SM Assay Buffer. Store at 4°C. Stable for two months.
- **SM Enzyme Mix:** Reconstitute with 220 µl SM Assay Buffer. Store at 4°C. Stable for two months.
- **SM Standard:** Frozen storage may cause the SM Standard to separate from the aqueous phase. To re-dissolve, keep the cap tightly closed, place in a hot water bath (70-80°C) for 1 min., vortex for 30 sec., then cool to room temperature, spin for 5 sec. to bring condensate down and check for clarity. Repeat heating, if turbid. The SM Standard is now completely in solution, and ready to use. Use within two months.

VIII. Sphingomyelin Quantification Assay Protocol:

1. **Sample Preparation:** Homogenize 25 mg of sample (wet weight or cell pellet) in 0.5 ml of SM Assay Buffer. Centrifuge at 4°C for 5 min. at 10,000 x g and transfer the supernatant to a separate tube. Aliquot 20 µl of homogenate and add 20 µl of SM Assay Buffer. Heat the homogenate for 1-2 min. at 70°C or until it becomes cloudy. Allow the sample to cool at room temperature and repeat the heating step. Centrifuge at room temperature for 2 min. at 10,000 x g to remove the debris. Collect the supernatant. Add 1-5 µl of supernatant into desired well(s). Adjust the volume to 50 µl with SM Assay Buffer. Serum or plasma contains 0.5-1 mM of Sphingomyelin that can be tested directly using 2-10 µl sample.

   Note:

   a. For unknown samples, we suggest testing several doses of samples to ensure the readings are within the Standard Curve range.

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

2. **Standard Curve Preparation:** Dilute SM Standard to 100 µM by adding 5 µl of 5 mM Standard to 245 µl of SM Assay Buffer. Add 0, 10, 20, 30, 40 and 50 µl of the diluted SM Standard into a series of wells in 96-well plate to generate 0, 1, 2, 3, 4 and 5 nmol/well of SM Standard. Adjust the volume to 50 µl with SM Assay Buffer.
3. **Reaction Mix:** Mix enough reagents for the number of assays (samples and Standards) to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix	* Background Control Mix
SM Assay Buffer	34 µl	36 µl
Sphingomyelinase	2 µl	----
ALP Enzyme	10 µl	10 µl
SM Enzyme Mix	2 µl	2 µl
GenieRed Probe	2 µl	2 µl

Mix well. Add 50 µl of the Reaction Mix to each well containing the SM Standard and samples. Mix well.

* For samples having high background, add 50 µl of Background Control Mix to sample background control well(s). Mix well.

4. **Measurement**: Incubate the plate at 37°C for 1 hr. Measure absorbance (570 nm) in a microplate reader.
5. **Calculations:** Subtract 0 Standard reading from all readings. If sample background control reading is significant then subtract sample background control reading from sample reading. Plot the SM Standard Curve. Apply corrected sample reading to the Standard Curve to get B nmol of sphingomylein in the sample well.

Sample Sphinomylein concentration (C) = B/V X D = nmol/µl or µmol/ml or mM

Where: **B** is the Sphingomyelin amount from Standard Curve (nmol)
V is the sample volume added in sample well (µl)
D is the dilution factor

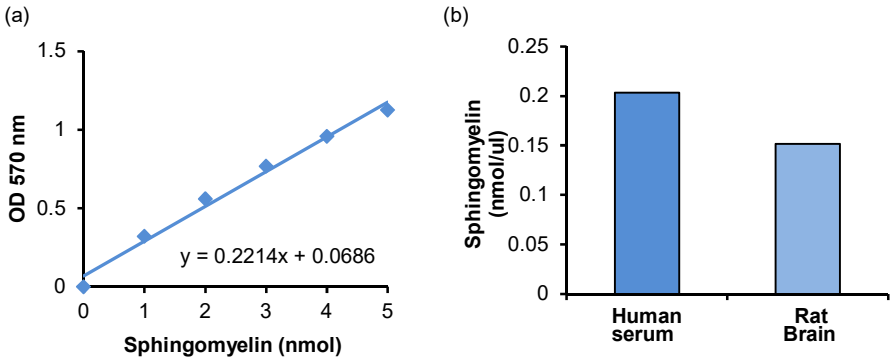

Figure: Sphingomyelin Standard Curve (a). Quantification of Sphingomyelin levels in human serum (1 µl) and rat brain (1 µg). Assays were performed following the kit protocol.

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