EnzyChrom™ Sphingomyelin Assay Kit (ESML-100)

Quantitative Colorimetric/Fluorimetric Determination of Sphingomyelin

DESCRIPTION

SPHINGOMYELINS (ceramide phosphocholine, SM) are key lipid components of cell membranes and lipoproteins. Sphingomyelinases (*SMases*) hydrolyze most sphingomyelins into ceramide and phosphocholine. This release of ceramide contributes to cell signaling pathways resulting in cell proliferation, differentiation, growth arrest, and apoptosis. Niemann-Pick disease is identified by the accumulation of sphingomyelin due to deficiencies in sphingomyelinase activity. Increased sphingomyelin levels have also been observed in several diseases, such as atherosclerosis, so simple, direct, and automation-ready procedures for measuring sphingomyelins are very desirable. BioAssay Systems' EnzyChrom™ Sphingomyelin Assay Kit provides a convenient colorimetric and fluorimetric method to quantify sphingomyelin in biological samples. In this assay, sphingomyelin is enzymatically converted to hydrogen peroxide, which forms a colored product with the dye reagent that is measurable at OD570 nm or fluorescence at λex/em = 530/585nm. The colored product is directly proportional to the sphingomyelin concentration in the sample.

KEY FEATURES

Safe. Non-radioactive assay.

Sensitive and accurate. Linear detection range in a 96-well plate: 1 - 50 mg/dL sphingomyelin for colorimetric assays and 0.07 - 7 mg/dL sphingomyelin for fluorimetric assays.

Convenient and high-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated to assay thousands of samples per day.

APPLICATIONS

For quantitative determination of sphingomyelin in biological samples (serum, plasma, etc.).

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer: 10 mL
Enzyme Mix: Dried
Dye Reagent: 120 µL
SMase: Dried
Hydrolase: 500 µL
Standard (20 mg/mL): 50 µL

Storage conditions. The kit is shipped on ice. Store all components at -20°C upon receipt. Shelf life: 6 months after receipt.

Precautions: Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to the Material Safety Data Sheet for detailed information.

COLORIMETRIC ASSAY

This assay is based on a kinetic reaction. To ensure identical incubation time, addition of the Working Reagent to samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended. Assays can be executed at room temperature.

Reagent Preparation: Prior to the assay, equilibrate all components to room temperature and briefly centrifuge tubes before opening. The Working Reagent should be prepared fresh for each assay run. Dissolve Enzyme Mix and SMase in 120 µL and 550 µL Assay Buffer, respectively. Both components are stable for 1 month at -20°C. *Note: a yellow precipitate may form after thawing reconstituted Enzyme Mix. If a precipitate forms, pellet it by centrifuging for 2 min at 14000 rpm and use the clear supernatant.*

Sample Preparation:

Serum and Plasma should be diluted at least 10x prior to the assay.

Standard Preparation:

Mix 5 µL of Standard and 195 µL of distilled water to prepare 50 mg/dL Standard Premix.

Prepare standards in 1.5-mL centrifuge tubes as follows:

No	Premix + dH2O	Vol (µL)	Sphingomyelin (mg/dL)
1	20 µL + 0 µL	20	50
2	12 µL + 8 µL	20	30
3	6 µL + 14 µL	20	15
4	0 µL + 20 µL	20	0

Procedure using 96-well plate:

1. Transfer 10 µL of each standard and 10 µL of each sample into separate wells of a clear flat-bottom 96-well plate.
2. Prepare enough Working Reagent by mixing, for each well, 1 µL Dye Reagent, 1 µL Enzyme Mix, 5 µL SMase, 5 µL Hydrolase, and 90 µL Assay Buffer. Add 90 µL Working Reagent to each sample well. Tap plate briefly to mix.
3. Incubate at room temperature for 30 minutes.
4. Read OD_{570nm}.

FLUORIMETRIC ASSAY

The fluorimetric assay procedure is similar to the colorimetric procedure except that 0, 2.1, 4.2 and 7 mg/dL sphingomyelin standards and a black 96-well plate are used. The Working Reagent is prepared the same and 10 µL standards or samples + 90 µL Working Reagent are still added to each well. Read fluorescence intensity at λex/em = 530/585 nm.

CALCULATION

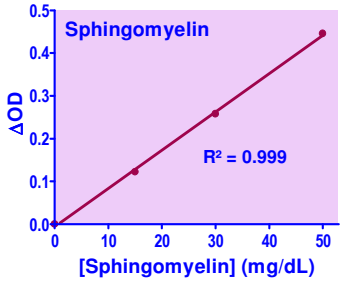
Subtract blank value (water, #4) from the standard values and plot the ΔOD or ΔF against standard concentrations. Determine the Slope and calculate sphingomyelin concentration in the samples:

$$[\text{Sphingomyelin}] = \frac{R_{Sample} - R_{Blank}}{\text{Slope}} \times \mathbf{n} \quad (\text{mg/dL})$$

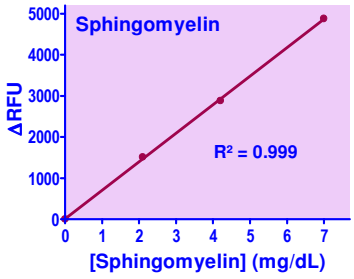
R_{Sample} and R_{Blank} are optical density or fluorescence intensity readings of the sample and blank (water, #4), respectively. **n** is the dilution factor. *Note: if the calculated sphingomyelin concentration of a sample is higher than 50 mg/dL in the Colorimetric Assay or 7 mg/dL in the Fluorimetric Assay, dilute samples in Assay Buffer and repeat the assay.*

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories (e.g. multi-channel pipettor), clear/black, flat-bottom 96-well plates (e.g. Corning Costar), centrifuge tubes and a plate reader.



Colorimetric Curve



Fluorimetric Curve

LITERATURE

1. Gault, C. R. et al (2010). An overview of sphingolipid metabolism: from synthesis to breakdown. *Adv in exp med biol*, 688, 1–23.

2. Pavoine, C. Pecker, F (2009). Sphingomyelinases: their regulation and roles in cardiovascular pathophysiology, *Cardiovascular Research*, 82(2), 175–183.

3. Slotte, J. P. (2013). Biological functions of sphingomyelins. *Progress in lipid research*, 52(4), 424–437.