Sphingomyelin Colorimetric Assay Kit

Item No. 10009928

www.caymanchem.com
Customer Service 800.364.9897
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1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity
700028	SM Buffer (5X)	1 vial
10010073	SM Color Detector	2 vials
10010074	SM Enzyme Mixture	2 vials
10010075	SM Alkaline Phosphatase	1 vial
10010076	Sphingomyelinase	1 vial
700027	Sphingomyelin Standard	1 vial
400014	96-Well Plate (Colorimetric Assay)	1 plate
400012	96-Well Cover Sheet	1 cover

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored at 4°C and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance between 585-600 nm.
2. Adjustable pipettes and a repeating pipettor.
3. A source of pure water; glass distilled water or HPLC-grade water is acceptable.

INTRODUCTION

Background

Sphingomyelin (ceramide phosphorylcholine) is an important lipid component of cell membranes and lipoproteins. It consists of a ceramide moiety linked *via* a phosphodiester bond to phosphorylcholine. Sphingomyelinases are a family of enzymes that can hydrolyze sphingomyelin into ceramide and phosphorylcholine (see Figure 1, on page 6).[1] Ceramides have been implicated as key mediators in signaling pathways, with outcomes as diverse as cell proliferation, differentiation, growth arrest, and apoptosis.[1,2] An inherited deficiency of acid sphingomyelinase activity results in the sphingomyelin storage disorder Niemann-Pick disease.[3] This disease results in the accumulation of sphinogmyelin in cells, tissues, and fluids. Since sphingomyelin has been implicated in the pathogenesis of several diseases, including atherosclerosis, sensitive and reliable techniques for its quantification are of considerable importance.[4-6]

About This Assay

Cayman's Sphingomyelin Colorimetric Assay provides a specific, sensitive, and convenient method for quantifying sphingomyelin in plasma or serum. In this assay, sphingomyelinase is first used to hydrolyze sphingomyelin to phosphorylcholine and ceramide. Alkaline phosphatase then generates choline from the phosphorylcholine and the newly formed choline is used to generate hydrogen peroxide in a reaction catalyzed by choline oxidase. Finally, with peroxidase as a catalyst, hydrogen peroxide reacts with DAOS and 4-aminoantipyrine to generate a blue color with an optimal absorption at 595 nm (see Figure 2, on page 7).[7]

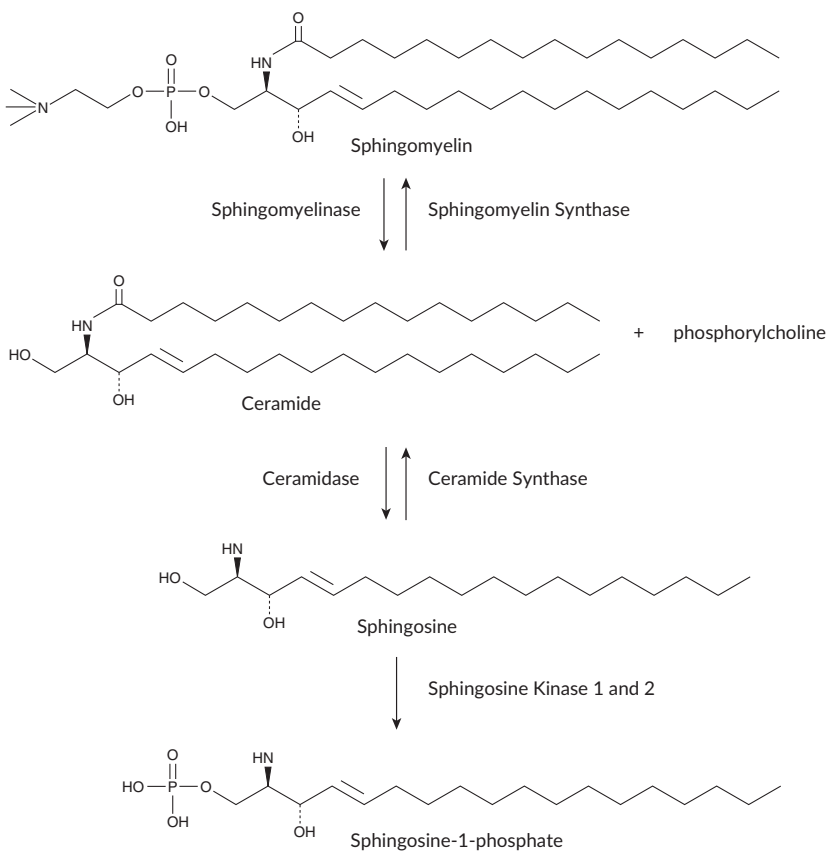


Figure 1. Ceramide/Sphingosine metabolism

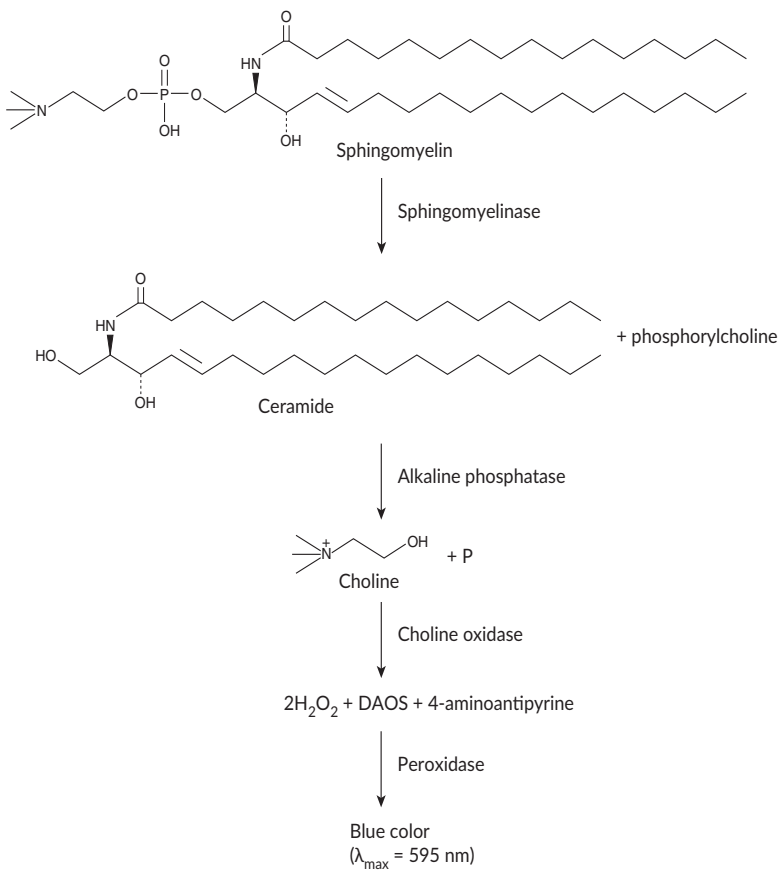


Figure 2. Assay scheme

PRE-ASSAY PREPARATION

Reagent Preparation

1. **SM Buffer (5X) - (Item No. 700028)**

   Dilute 6 ml of SM Buffer concentrate with 24 ml of HPLC-grade water. This final Buffer (50 mM Tris-HCl, pH 8.0, containing 0.66 mM $CaCl_2$ and 2% Nonidet™ P 40 Substitute) should be used for reconstituting the Color Reagent, Enzyme Mixture, Sphingomyelinase, and for diluting the Sphingomyelin Standard. When stored at 4°C, this diluted Buffer is stable for at least six months.

2. **SM Color Detector - (Item No. 10010073)**

   Each vial contains a lyophilized powder of DAOS (N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline) and 4-aminoantipyrine. Reconstitute the Color Detector with 3 ml of diluted SM Buffer. The reconstituted Color Detector is stable for eight hours at room temperature.

3. **SM Enzyme Mixture - (Item No. 10010074)**

   Each vial contains a lyophilized powder of choline oxidase and horseradish peroxidase. Prior to use in the assay, reconstitute the vial contents with 1 ml of diluted SM Buffer. Store the reconstituted Enzyme Mixture on ice until ready to use. The reconstituted Enzyme Mixture is stable for 24 hours at 4°C.

4. **SM Alkaline Phosphatase - (Item No. 10010075)**

   This vial contains a solution of Alkaline Phosphatase. It is ready to use as supplied.

5. **Sphingomyelinase - (Item No. 10010076)**

   This vial contains a lyophilized powder of sphingomyelinase from *Bacillus cereus*. Reconstitute the vial contents with 1 ml of diluted SM Buffer. Store the enzyme on ice until ready to use. Unused enzyme should be frozen at -20°C. It will be stable for one month.

6. **Sphingomyelin Standard - (Item No. 700027)**

   This vial contains a 2.5 mg of sphingomyelin in ethanol. Evaporate under nitrogen until dry, and bring up in 5 ml of 1X Assay Buffer to make a 50 mg/dl stock solution. Vortex well until all of the sphingomyelin is dissolved in solution. This stock solution will be used to make the dilutions for the standard curve. This solution is stable for 24 hours when stored at 4°C (see **Standard Preparation** on page 11).

Sample Preparation

Plasma

The typical concentration of sphingomyelin in human plasma is 25-60 mg/dl.[5,6]

1. Collect blood using an anticoagulant such as heparin, EDTA, or citrate.
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. The plasma sample will be stable for one month while stored at -80°C.
3. Plasma does not need to be diluted before assaying.

Serum

The typical concentration of sphingomyelin in human serum is 25-60 mg/dl.[4]

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 serum sample will be stable for one month while stored at -80°C.
4. Serum does not need to be diluted before assaying.

ASSAY PROTOCOL

Standard Preparation

Take seven clean glass test tubes and mark them A-G. Add the amount of SM Stock Standard (as prepared in previous section) and SM Buffer (dilute) to each tube as described in Table 1 (below). *NOTE: Bubbles may form in some of the standards, they will disperse in a few minutes and not affect the assay in any way. Diluted standards are stable for four hours.*

Tube	SM Stock Standard (µl) (50 mg/dl)	SM Buffer (µl)	SM Concentration (mg/dl)
A	0	500	0
B	50	450	5
C	100	400	10
D	200	300	20
E	300	200	30
F	400	100	40
G	500	0	50

Table 1. Sphingomyelin standards to be assayed along with plasma and serum samples.

Plate Set Up

There is no specific pattern for using the wells on the plate. A typical layout of sphingomyelin standards and samples to be measured in duplicate is given below in Figure 3. We suggest you record the contents of each well on the template sheet provided on page 18.

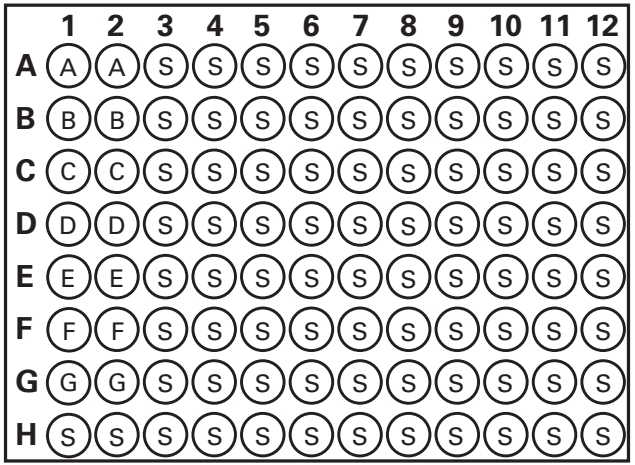


Figure 3. Sample plate format

Pipetting Hints

- It is recommended that a repeating pipettor be used to deliver reagents to the wells. This saves time and helps maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The final volume of the assay is 110 µl in all wells.
- The incubation temperature is 22°C.
- It is not necessary to use all the wells on the plate at one time.
- It is recommended that the standards and samples be assayed at least in duplicate.
- Twenty-seven samples can be assayed in triplicate or forty-one in duplicate.
- Monitor the absorbance at 585-600 nm using a plate reader.

Performing the Assay

1. **Preparation of Reaction Mixture** - To the SM Color Detector (Item No. 10010073) add the 1 ml of SM Enzyme Mixture (Item No. 10010074), 0.5 ml of Sphingomyelinase(Item No. 10010076), 10 µl of SM Alkaline Phosphatase (Item No. 10010075), and 490 µl of diluted Buffer for a final volume of 5 ml. This is enough Reaction Mixture to assay 50 wells. Prepare a second batch of Reaction Mixture using another set of reagent vials for additional samples. The Reaction Mixture is stable for 24 hours at 4°C.
2. **Sphingomyelin Standard Wells** - add 10 µl of Standard (tubes A-G) per well in the designated wells on the plate (see suggested plate configuration, Figure 3, page 12).
3. **Sample Wells** - add 10 µl of sample (either undiluted plasma or serum) to two or three wells. *NOTE: The amount of sample added to the well should always be 10 µl.*
4. Initiate the reactions by adding 100 µl of Reaction Mixture to each well.
5. Carefully shake the microwell plate for a few seconds to mix. Cover with plate cover.
6. Incubate the plate on a shaker for 60 minutes at room temperature. Read the absorbance at a wavelength between 585-600 nm using a plate reader.

ANALYSIS

Calculations

1. Determine the average absorbance of each standard and sample.
2. Subtract the absorbance of standard A from itself and all other standards and samples to yield the corrected absorbance value (CAV).
3. Graph the CAV of the standards as a function of the final sphingomyelin concentration (mg/dl) from Table 1 (on page 11). See Figure 4 (on page 16) for a typical standard curve.
4. Calculate the sphingomyelin concentration of the original samples using the equation obtained from the linear regression of the standard curve by substituting the CAV for each sample into the equation. *NOTE: The sphingomyelin concentration is calculated back to the original sample and not what is in the well.*

$$\text{Sphingomyelin (mg/dl)} = \left[\frac{\text{(CAV) - (y-intercept)}}{\text{Slope}} \right]$$

Performance Characteristics

Precision:

When a series of 16 human plasma and serum samples were assayed on the same day, the intra-assay coefficient of variation was 3.9% and 2.9%, respectively. When a series of 16 human plasma and serum samples were assayed on five different days under the same experimental conditions, the inter-assay coefficient of variation was 2.5% and 2.4%, respectively.

Assay Range:

Under the standardized conditions of the assay described in this booklet, the dynamic range of the kit is 5-50 mg/dl sphingomyelin.

Representative Sphingomyelin Standard Curve

The standard curve presented here is an example of the data typically provided with this kit; however, your results will not be identical to these. You must run a new standard curve - do not use these to determine the values of your samples.

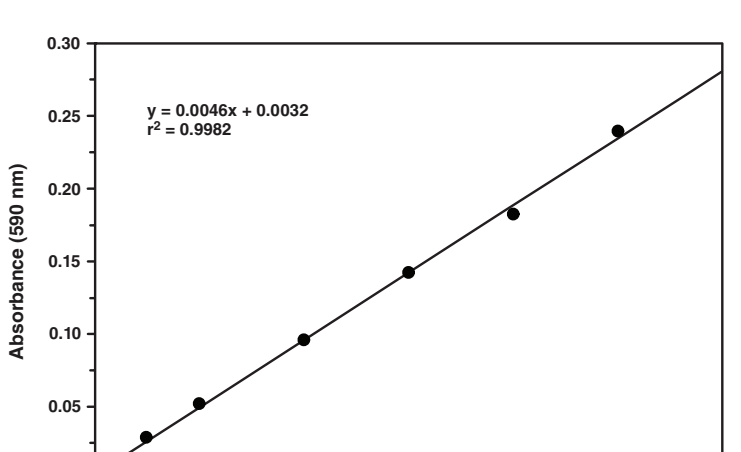


Figure 4. Sphingomyelin standard curve

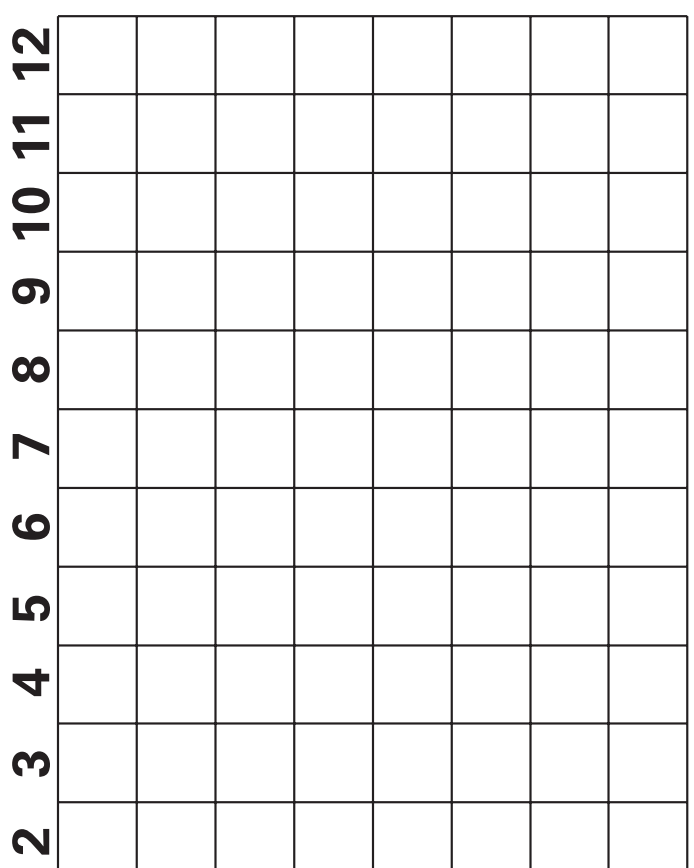
RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
No color development in any of the wells	The reaction mixture was not prepared correctly	Make sure to follow the directions when preparing the reaction mixture and re-assay
No color development in the standard wells	The standards were not diluted correctly	Make sure to follow the directions when preparing the standards and re-assay

References

1. Riboni, L., Viani, P., Bassi, R., *et al. Prog. Lipid Res.* **36**, 153-195 (1997).
2. Testi, R. *Trends Biochem. Sci.* **21**, 468-471 (1996).
3. He, X., Chen, F., McGovern, M.M., *et al. Anal. Biochem.* **306**, 115-123 (2002).
4. Schlitt, A., Hojjati, M.R., von Gizycki, H., *et al. J. Lipid Res.* **46(2)**, 196-200 (2005).
5. Schlitt, A., Blankenberg, S., Yan, D., *et al. Nutrition & Metabolism* **3(5)**, 1-8 (2006).
6. Jiang, X.-C., Paultre, F., Pearson, T.A., *et al. Arterioscler. Thromb. Vasc. Biol.* **20(12)**, 2614-2618 (2000).
7. Hojjati, M.R. and Jiang, X.-C. *J. Lipid Res.* **47(3)**, 673-676 (2006).



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.