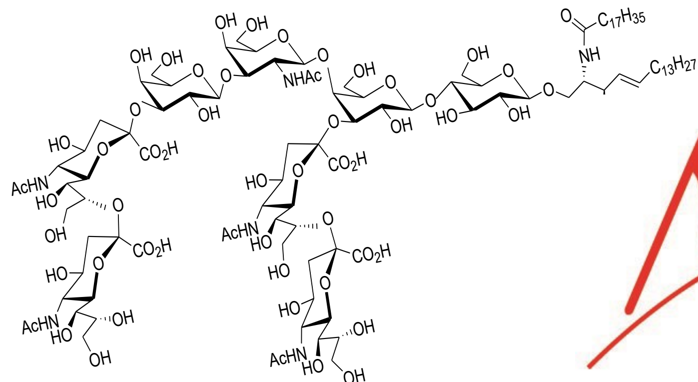
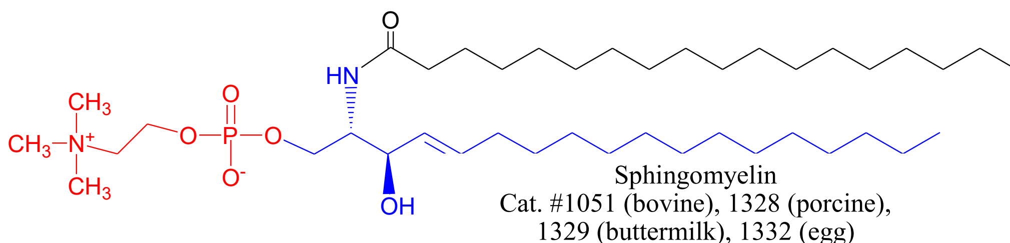
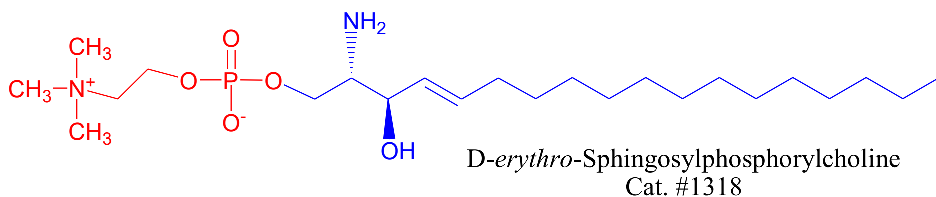


NEWSLETTER FOR GLYCO/SPHINGOLIPID RESEARCH
AUGUST 2019

Sphingomyelin and *lyso*-Sphingomyelin: Vital Membrane Components



Sphingomyelin
Cat. #1051 (bovine), 1328 (porcine), 1329 (buttermilk), 1332 (egg)



D-*erythro*-Sphingosylphosphorylcholine
Cat. #1318

Sphingomyelin, sphingosylphosphorylcholine, and dihydrosphingomyelin are major and important phosphosphingolipids found in mammalian cell membranes, especially in the membranes of the myelin sheath. Sphingomyelin is the most abundant sphingolipid in mammals and is found mostly in the exoplasmic leaflet of the membrane. Sphingomyelin has numerous critical cellular functions including signal transduction, apoptosis, free sphingosine and ceramide metabolism, and myelin sheath formation [1]. Sphingomyelin also plays a significant role in Niemann-pick disease, types A and B, multiple sclerosis, neonatal respiratory distress syndrome, and abetalipoproteinemia.

The ratio of sphingomyelin to ceramide in different cell types plays a critical role in cellular function [2]. Sphingomyelin is an important amphiphilic component when plasma lipoprotein pools expand in response to large lipid loads or metabolic abnormalities [3]. In contrast to ceramides, N-hexanoyl-sphingomyelin does not initiate vesicle formation in cells [4] but has been used to enhance the uptake of anti-tumor drugs by cancer cells, thereby increasing their cytotoxicity [5]. Sphingosylphosphorylcholine, the deacylated form of sphingomyelin, has been shown to induce intracellular calcium release while its short chain analog, N-acetyl sphingosylphosphoylcholine, requires a significantly higher concentration to initiate the same level of response [6].

Sphingomyelin also has important implications in several severe diseases. Niemann-Pick disease is a rare lysosomal storage disorder with debilitating effects and is characterized by a deficiency of the enzyme acid sphingomyelinase. This results in the accumulation of sphingomyelin leading to hepatosplenomegaly, liver dysfunction, interstitial lung disease, thrombocytopenia, anemia, an atherogenic lipid profile, bone disease, and neurodegeneration [7,8]. Low levels of sphingomyelin is considered a blood biomarker for multiple sclerosis, although whether sphingomyelin is an active species in the disease remains unclear [9]. In neonatal respiratory distress syndrome the ratio of lecithin/sphingomyelin in amniotic fluid has been used to predict risk of

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the disease [10]. An excess of sphingomyelin in red blood cells leads to abetalipoproteinemia, causing decreased membrane fluidity [11].

Sphingosylphosphorylcholine (SPC) has been identified in normal blood plasma, ascites, and various other tissues. SPC is similar in structure to sphingosine-1-phosphate and lysophosphatidylcholine and has at least low-binding affinity to some of the same receptors, such as the sphingosine-1-phosphate receptor. It is a bioactive lipid that acts as an intracellular and extracellular signaling molecule in numerous biological processes such as vasoconstriction, vasodilation, angiogenesis, stress fiber formation, cytoskeletal rearrangements, proliferation, differentiation, migration, wound healing, and stimulation of DNA synthesis. SPC can also inhibit the growth of various cell types, including tumor cells, causing much interest in its possible role as an anti-tumor therapy. It is a high-affinity ligand for the orphan receptor ovarian cancer G-protein-coupled receptor 1 (OGR1). The specific binding of SPC to OGR1 also activates p42/44 mitogen-activated protein kinases (MAP kinases) and inhibits cell proliferation [12]. SPC may be able to help treat inflammatory kidney diseases and has been found to trigger proteins known to reduce inflammation. SPC has also been shown to cause an increase in urine production in the kidneys with an abnormal accumulation of salt in the urine [13]. SPC acts as an inhibitor for calmodulin, a highly prevalent intracellular calcium sensor in eukaryotic cells [14].

The extracellular effects of SPC appear to be stereospecific while intracellular effects may not be. D-*erythro*-SPC, but not L-*threo*-SPC, stereoselectively stimulates the proliferation of human adipose tissue-derived mesenchymal stem cells and stimulates an increase in calcium concentration and cellular proliferation [15]. Both the L-*threo*-SPC isomer and the D-*erythro*-SPC isomer can act as second messengers by releasing calcium from internal stores.

Dihydrosphingomyelin, containing a saturated sphingosine chain, has been identified as a minor lipid component in many mammalian tissues but has recently been reported to be present in significant amounts in bovine brain and bovine milk [16]. It is also found in much greater amounts in human lens membranes (half of all the phospholipids) where it has a critical role in ocular function and perhaps in age-related nuclear cataracts [17]. However, dihydrosphingomyelin has been reported to occur only in small amounts in the lens membranes of other mammals. Dihydrosphingomyelin demonstrates good mixing properties with both sterols and sphingomyelin indicating that it could function as a membrane organizer and this may be the reason it is present in large amounts in human lens membranes where cholesterol is also enriched [18]. The enzyme *sphingomyelinase* is active towards dihydrosphingomyelin and readily converts it to dihydroceramide. Recent evidence has been presented that indicates that dihydrosphingomyelin impairs HIV-1 infection by rigidifying liquid-ordered membrane domains, a finding that could have great potential in providing a therapeutic treatment for this debilitating disease [19].

Cat. #	Amount	Purity	Product Name
1051	25 mg	98^+%	Sphingomyelin (bovine)
1328	25 mg	98^+%	Sphingomyelin (porcine)
1329	25 mg	98^+%	Sphingomyelin (buttermilk)
1332	25 mg	98^+%	Sphingomyelin (egg)
1907	5 mg	98^+%	N-C2:0-Sphingosylphosphorylcholine
1890	5 mg	98^+%	N-C17:0-Sphingosylphosphorylcholine
1911	5 mg	98^+%	N-C18:0-Sphingosylphosphorylcholine
1917	500 µg	98^+%	N-C20:0-Sphingosylphosphorylcholine
1918	500 µg	98^+%	N-C22:0-Sphingosylphosphorylcholine
2200	1 mg	98^+%	N-1-^{13}C-C16:0-Sphingosylphosphorylcholine
1912	100 µg	98^+%	NBD-C6:0-Sphingosylphosphorylcholine
1619	100 µg	98^+%	NBD-C12:0-Sphingosylphosphorylcholine
1318	5 mg	98^+%	D-*erythro*-Sphingosylphosphorylcholine
1319	5 mg	98^+%	L-*threo*-Sphingosylphosphorylcholine
1321	10 mg	98^+%	D-*erythro*/L-*threo*-Sphingosylphosphorylcholine
1913	1 mg	98^+%	D-*erythro*/L-*threo*-Dihydrosphingosylphosphorylcholine
1327	5 mg	98^+%	N-Acyl-Sphingosylphosphorylethanolamine

GI

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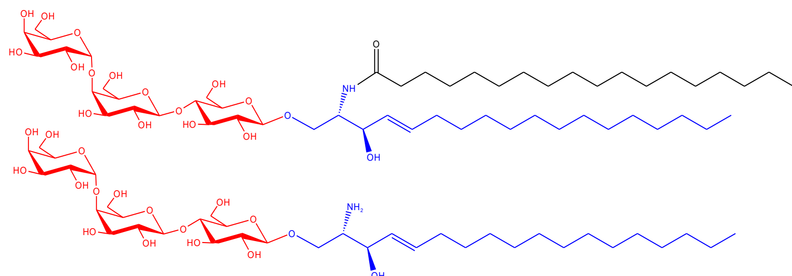
Biomarker Standards for Fabry Disease Diagnosis and Monitoring



Globotriaosylceramide (Gb_3, CTH) catalog #1067

Globotriaosylsphingosine (*lyso*-Gb_3, *lyso*-CTH) catalog #1520

Lysosomal storage disorders are a set of more than 70 inherited conditions that result in the accumulation of various lipids in cells due to an inability to enzymatically degrade them. Fabry disease (FD) is one such disorder that is characterized by a deficiency of the enzyme α-galactosidase A, resulting in an accumulation of globotriaosylceramide (Gb_3), globotriaosylsphingosine (*lyso*-Gb_3), galabiaosylceramide (Ga_2), and blood group B glycolipids. This debilitating disease is an X-linked chromosomal disorder and early diagnosis is critical as progression will lead to multiorgan dysfunction and early death. To aid in timely detection and treatment monitoring several disease biomarkers have been identified, including Gb3 and *lyso*-Gb3.

The use of *lyso*-Gb_3 for the determination of Fabry disease has now been well established, resulting in the development of reproducible and highly sensitive methods that require extremely small plasma, dried blood spot, or urine samples.[1,2] *lyso*-Gb_3 has been demonstrated to be an effective biomarker for FD in symptomatic patients, showing higher diagnostic sensitivity than Gb_3.[1] However, this lipid has not always been found to be a good candidate biomarker for asymptomatic females.[3] Heterozygous females can also manifest symptoms of FD making early detection of critical importance in these cases as well. Urinary Gb_3 (but not plasma Gb_3) has been reported to be elevated in both symptomatic and asymptomatic males and females even though it's sensitivity is lower than *lyso*-Gb_3. Although levels of Gb_3 do not necessarily correlate with disease severity, and though false positives do occur using this biomarker, nevertheless Gb_3 has been recognized as a useful diagnostic marker and may also indicate the formation of antibodies during enzyme replacement therapy.[3] Other possible biomarkers for FD include galabiaosylceramide (Ga_2)[4] and blood group B glycolipids.

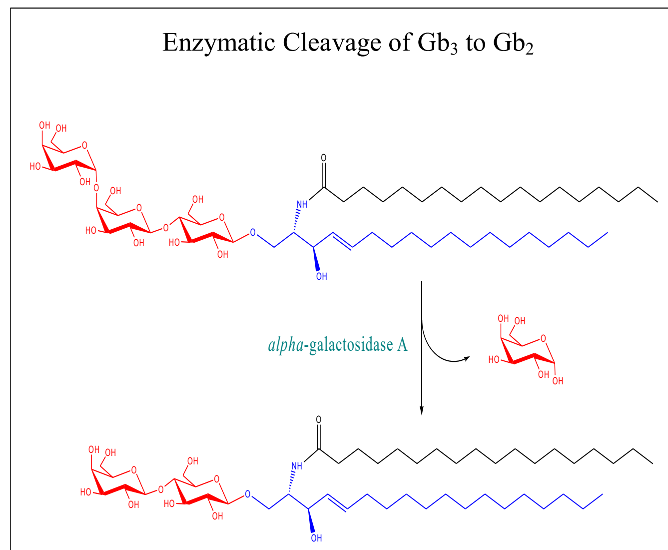
Currently FD is most often treated by enzyme replacement therapy (ERT) and the levels of Gb_3 and *lyso*-Gb_3 have also been found to be useful in monitoring the levels of replacement enzymes needed as well as disease progression. ERT has shown a reduction not only in plasma Gb_3 but also in plasma *lyso*-Gb_3. Because FD is

a result of deficient enzyme activity, ERT is able to successfully halt the disease progress in many patients. This is especially evident in patients that begin treatment early in the disease progression as untreated patients can quickly develop irreversible organ damage.[5]

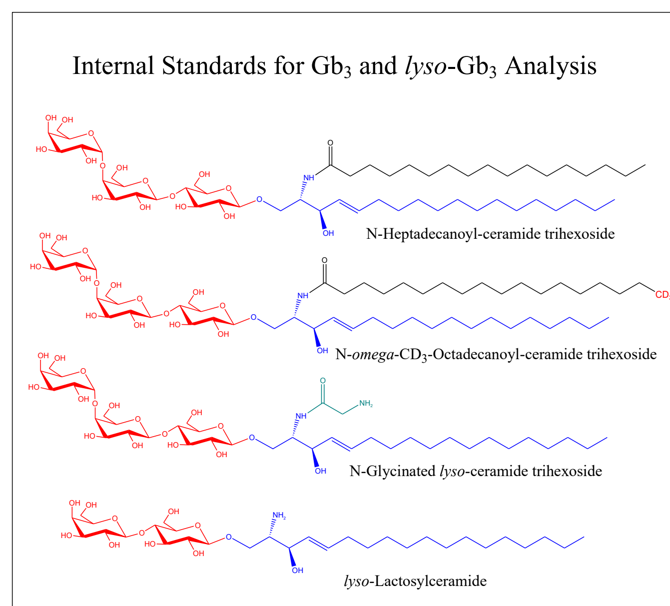
Internal standards for Gb_3 and *lyso*-Gb_3 are critical for the effective quantitation of these FD biomarkers. Glycinated *lyso*-Gb_3 (cat. #1530) was explored as an internal standard and the physical and chemical properties were found to be almost identical to that of natural *lyso*-Gb_3 in terms of extraction, stability, and sensitivity, making it an excellent internal standard for clinical work.[6] This internal standard contains a glycine molecule attached to the amine of *lyso*-Gb_3, preserving the key primary amine functionality. Another commonly used internal standard for *lyso*-Gb_3 analysis is *lyso*-lactosylsphingosine (*lyso*-Gb_2, cat. #1517) which lacks the terminal galactose of *lyso*-Gb3 but does contain a primary amine.[6,2] For Gb_3 analysis the most useful internal standard is a stable isotope labeled standard (such as deuterated octadecanoyl-Gb_3, cat. #1537) or a well-defined Gb_3 containing an unusual fatty acid (such as heptadecanoyl-Gb_3, cat. #1523). Armed with these internal standards, diagnosis and monitoring of FD can go forward steadily.

An example of the ongoing development of sensitive and efficient analyses of *lyso*-Gb_3 in FD is seen in the work by J. Lukas and coworkers[2]: three 3.2 mm dried blood spot samples were extracted with DMSO:water 1:1 in the presence of *lyso*-Gb_2 (as an internal standard) in ethanol with agitation and sonication. After particle filtration by centrifugation the sample was ready for analysis. Similarly, 25 µL of plasma was extracted in ethanol in the presence of *lyso*-Gb_2 (again as an internal standard). After protein precipitation the supernatant was filtrated by centrifugation and analyzed. Anal-

Enzymatic Cleavage of Gb_3 to Gb_2



Internal Standards for Gb_3 and *lyso*-Gb_3 Analysis



Product Name	Catalog #	Amount	Purity
Ceramide trihexoside (Globotriaosylceramide, Gb_3)	1067	1 mg	98^+%
lyso-Ceramide trihexoside (Globotriaosylsphingosine, *lyso*-Gb_3)	1520	1 mg	98^+%
N-Glycinated *lyso*-ceramide trihexoside	1530	1 mg	98^+%
N-Hexadecanoyl-ceramide trihexoside	1528	1 mg	98^+%
N-Heptadecanoyl-ceramide trihexoside	1523	500 µg	98^+%
N-Octadecanoyl-ceramide trihexoside	1529	500 µg	98^+%
N-Tricosanoyl-ceramide trihexoside	1524	500 µg	98^+%
N-*omega*-CD_3-Octadecanoyl-ceramide trihexoside	1537	500 µg	98^+%
lyso-Lactosylceramide, synthetic	2088	1 mg	98^+%
lyso-Lactosylceramide, bovine buttermilk	1517	1 mg	98^+%

Please visit www.matreya.com for a full list of ceramide trihexosides and other lipid standards

ysis was performed by UPLC/triple quadrupole mass spectrometer in MRM mode, monitoring the mass transitions of both *lyso*-Gb_3 and *lyso*-Gb_2.

Another method has been evaluated for the analysis of *lyso*-Gb_3 from dried blood spots by B. Johnson and colleagues.[7] This HPLC-MS/MS method yielded reproducible results in patients with Fabry disease, although the method was found to be unsuitable for newborn screening and late onset females.

A method that takes advantage of detectable *lyso*-Gb_3 in urine is reported by H. Gold and coworkers and includes the use of a stable isotope labeled *lyso*-Gb_3 (not yet commercially available).[1] Urine was extracted in methanol/chloroform along with the internal standard. The sample was centrifuged and the supernatant diluted with chloroform/water to make an upper and lower layer. The upper methanol/water phase was collected, evaporated, and partitioned with butanol/water. The upper butanol phase was collected, evaporated, and re-dissolved in methanol for analysis by UPLC-ESI-MS/MS.

It will be interesting to see what the future holds for enhanced early diagnosis, treatment, and monitoring of FD. As methods become more refined we hope to see such techniques as solid phase microextraction being developed for even more efficient analysis. Equipped with an expanding array of standards, researchers can continue to delve deeper into the role of accumulated lipids in this disease's devastating pathogenesis. The recent and ongoing push to make newborn screening for α-galactosidase A more prevalent will undoubtedly have tremendous benefits for the generations ahead.

References:

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