Hytest | **Technotes**

Blood coagulation and Anemia • Bone Metabolism • Cardiac Markers • Fertility and Pregnancy • Hormone Markers • Immunology and Serology • Infectious Diseases • Inflammation • Kidney Diseases • Metabolic Syndrome • Neuroscience • Thyroid Diseases • Tumor Markers • Veterinary

Gangliosides

Gangliosides are a large group of sialylated glycosphingolipids that are widely expressed in mammalian tissues. Gangliosides are found in most tissues of the body, but they are particularly abundant in brain and nervous tissues. The differential distribution of gangliosides in various tissues is a strong indication that they play important roles in specific functions in different tissues.

Glycosphingolipids (neutral glycosphingolipids and gangliosides) are formed biosynthetically within the Golgi apparatus. Gangliosides are involved in a number of interaction processes with cell external ligands and cell membrane components. Gangliosides seem to be involved in cell-to-cell interaction and regulation of cell signaling. They can be receptors of proteins, viruses and bacteria (GM1 is a receptor of Cholera Toxin). Gangliosides are also playing a role in the cell proliferation. The differentiated cells in human melanoma are expressing GD3 and other b series gangliosides such as GQ1b, whereas GM3 can be involved in the differentiation of some lymphocytic cells.

Characterization of different cell type-specific disorders can be done using our ganglioside preparations. Incubation of cells in the presence of purified gangliosides leads to insertion of these glycolipids into the cell membranes, specifically altering the binding capacity of the membrane for hormones, bacterial toxins and growth factor.

Single-cell morphology as well as cell-cell interaction and differentiation can be studied by using ganglioside *in vivo* models. Immunization of animals with purified gangliosides produces anti-ganglioside antibodies. Animals injected with these antibodies are excellent models for studies of epilepsy and other neurological disorders. Removal of sialic acid from purified gangliosides leads to highly potent antigens – asialoglycolipids, which have been shown to be specific determinants of the immune system (B- and T-cell marker). In addition purified gangliosides can be used as biological substrates and inhibitors of glycosyltransferases and glycosidases in the study of the metabolic pathway of glycostructures.

High pure gangliosides can be used for characterization of different cell type-specific disorders.

Hytest offers a number of gangliosides belonging to ganglio-series. The main sources for gangliosides are bovine and human brains. Human gangliosides contain only N-acetylneuraminic acid residues, whereas bovine gangliosides may contain both N-acetyl- and N-glycosylneuraminic acid residues. The bovine brain gangliosides contain the residues of two main sphingosine bases, $C_{18:1}$ and $C_{20:1}$ in the ratio 3:2. The final HPLC-purified gangliosides have purity around 98 %. The purity is determined by TLC. We use two solvent systems for TLC of gangliosides: the neutral, chloroform – methanol – 15 mM aqueous $CaCl_2$, 60:40:9 (v/v/v) and the basic, chloroform – methanol – 2.5 N aqueous NH3, 60:40:9 (v/v/v). In both systems each ganglioside must be presented by one band (see figures 1 – 5).

The gangliosides are supplied in lyophilized form and almost all gangliosides are soluble in the mixture of chloroform-methanol, 2:1 (v/v). In case of polar gangliosides it cacacln be helpful to increase the amount of methanol or to use pure methanol. Polar gangliosides form micelles in water solution with the critical micelle concentration around $10^{-7} – 10^{-8}$ M. After sterilization by filtration aqueous ganglioside solutions do not require addition of preservative.
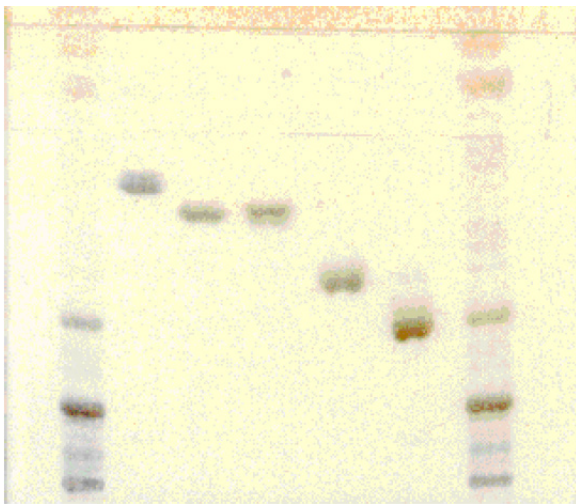
Figure 1.

HPTLC of monosialogangliosides in the chloroform – methanol – 15 mM aqueous $CaCl_2$, 60:40:9 (v/v/v), Kieselgel 60 (Merck).

Lines 1 and 7 – mixture of brain glycolipids
Line 2 – 8G16-5h (GM4 from human brain)
Line 3 – 8G16-4h (GM3 from human brain)
Line 4 – (GM3 from human liver)
Line 5 – 8G16-3h (GM2 from human brain)
Line 6 – 8G16-2h (GM1 from human brain)
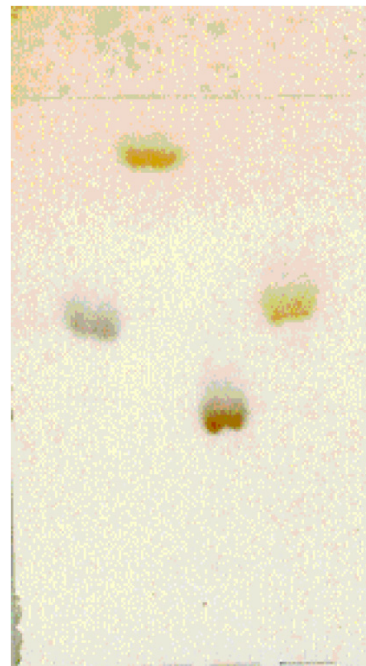
Figure 2.

HPTLC of asialo- and monosialogangliosides in the chloroform – methanol – 15 mM aqueous $CaCl_2$, 60:40:9 (v/v/v), Kieselgel 60 (Merck).

Line 1 – 8G16-3h (GM2 from human brain)
Line 3 – 8G16-2h (GM1 from human brain)
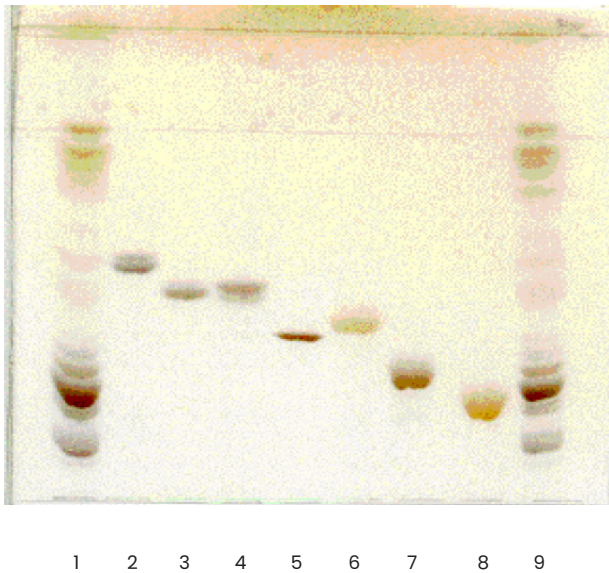Line 4 – 8G16-1h (Asialo-GM1 from human brain)

Figure 3.

HPTLC of monosialo- and asialogangliosides in the chloroform – methanol – 2.5 N aqueous NH3, 60:40:9 (v/v/v).

Lines 1 and 9 – mixture of brain glycolipids

Line 2 – 8G16-5h (GM4 from human brain)

Line 3 – 8G16-4h (GM3 from human brain)

Line 5 – 8G16-3h (GM2 from human brain)

Line 7 – 8G16-2h (GM1 from human brain)

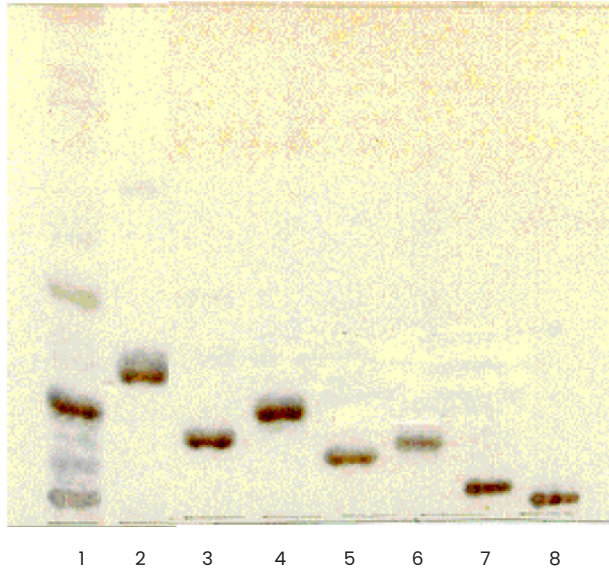Line 8 – 8G16-1h (Asialo-GM1 from human brain)

Figure 4.

HPTLC of di- and polysialogangliosides in the chloroform – methanol – 15 mM aqueous CaCl₂, 60:40:9 (v/v/v)

Line 1 – mixture of brain gangliosides

Line 2 – 8G16-9h (GD3 from human brain)

Line 3 – 8G16-8h (GD2 from human brain)

Line 4 – 8G16-6h (GD1a from human brain)

Line 5 – 8G16-7h (GD1b from human brain)

Line 6 – 8G16-11h (GT1a from human brain)

Line 7 – 8G16-10h (GT1b from human brain)
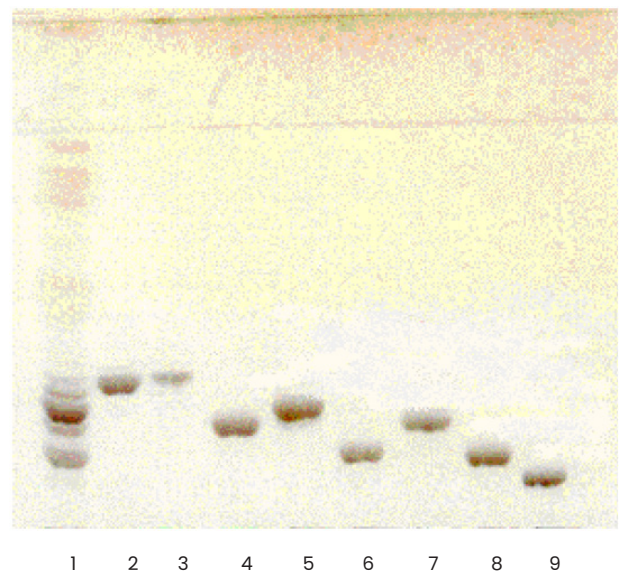
Line 8 – 8G16-12h (GQ1b from human brain)

Figure 5.

HPTLC of the same gangliosides as on Fig. 4, but in the chloroform – methanol – 2.5 N aqueous NH3, 60:40:9 (v/v/v).

REFERENCES

1. **Kolter T, et al. (2002)** Combinatorial Ganglioside Biosynthesis. J Biol Chem 277(29), 25859-25862.

2. **Jeyakumar M, et al. (2002)** Glycosphingolipid lysosomal storage diseases: therapy and pathogenesis. Neuropathol Appl Neurobiol 28(5), 343 357.

ORDERING INFORMATION

GANGLIOSIDES AND GLOBOSIDE

Product name	Cat. #	Purity	Source
Asialoganglioside GM1, bovine	8G16-1b	>98%	Bovine brain MW 1263
Asialoganglioside GM1, human	8G16-1h	>98%	Human brain MW 1263
Asialoganglioside GM2, bovine	8G16-15b	>98%	Bovine brain MW 1103
Disialoganglioside GD1a, bovine	8G16-6b	>98%	Bovine brain MW 1827
Disialoganglioside GD1a, human	8G16-6h	>98%	Human brain MW 1811
Disialoganglioside GD1a-NAcGal, bovine	8G16-17b	>98%	Bovine brain MW 2030
Disialoganglioside GD1b, bovine	8G16-7b	>98%	Bovine brain MW 1827
Disialoganglioside GD1b, human	8G16-7h	>98%	Human brain MW 1811
Disialoganglioside GD2, bovine	8G16-8b	>98%	Bovine brain MW 1665
Disialoganglioside GD2, human	8G16-8h	>98%	Human brain MW 1649
Disialoganglioside GD3, bovine	8G16-9b	>98%	Bovine brain MW 1461
Disialoganglioside GD3, human	8G16-9h	>98%	Human brain MW 1438
Monosialoganglioside GM1, bovine	8G16-2b	>98%	Bovine brain MW 1545
Monosialoganglioside GM1, human	8G16-2h	>98%	Human brain MW 1537
Monosialoganglioside GM2, bovine	8G16-3b	>98%	Bovine brain MW 1383
Monosialoganglioside GM2, human	8G16-3h	>98%	Human brain MW 1375
Monosialoganglioside GM3, bovine	8G16-4b	>98%	Bovine brain MW 1179
Monosialoganglioside GM3, human	8G16-4h	>98%	Human brain MW 1171
Monosialoganglioside GM4, bovine	8G16-5b	>98%	Bovine brain MW 1017
Monosialoganglioside GM4, human	8G16-5h	>98%	Human brain MW 1009
Tetrasialoganglioside GQ1b, bovine	8G16-12b	>98%	Bovine brain MW 2391
Tetrasialoganglioside GQ1b, human	8G16-12h	>98%	Human brain MW 2359
Trisialoganglioside GT1a, bovine	8G16-11b	>98%	Bovine brain MW 2109
Trisialoganglioside GT1b, bovine	8G16-10b	>98%	Bovine brain MW 2109
Trisialoganglioside GT1b, human	8G16-10h	>98%	Human brain MW 2085

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