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FRONTIERS IN LIFE SCIENCE

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Anti-Globotetraosylceramide (Gb4Cer), Mouse-Mono (PA5)

Catalog NO. FDV-0047

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Product Background

Globotetraosylceramide (Gb4Cer), the specific antigen of the Anti-Globotetraosylceramide antibody (clone PA5), is a glycolipid that belongs to a group called glycosphingolipid (GSL). GSLs are defined by a structure consisting of a mono- or oligosaccharide linked to a ceramide (Cer) backbone and are further classified into several subclasses. Gb4Cer belongs to a subclass called Globo-series. Biosynthesis of Gb4Cer occurs inside cells via the sequential addition of 4 monosaccharides to ceramide (Figure 1).

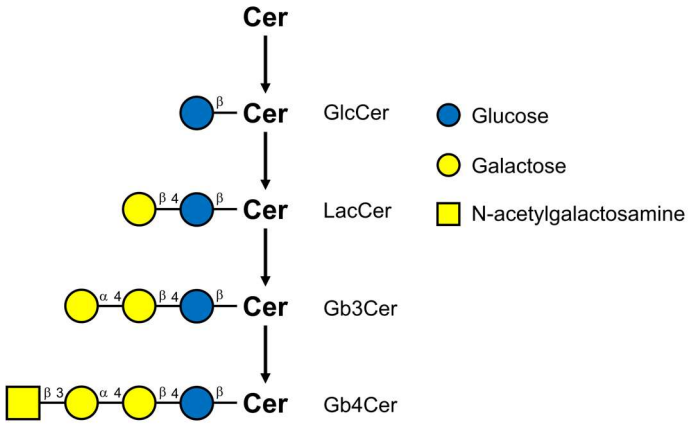


Figure 1. Biosynthesis pathway of Gb4Cer

Gb4Cer is a major glycolipid in human erythrocytes and is known as the P antigen of the human blood group. It is reported that stimulation with an inflammatory mediator, TNF-α, promotes the expression of Gb4Cer containing very-long-chain fatty acids (VLCFAs) in vascular endothelial cells. It is also indicated that Gb4Cer plays multiple roles related to the immune response, such as a cellular receptor of human parvovirus B19, an inhibitor of the TLR4-mediated endotoxin response, and a Shiga toxins receptor produced by a group of *E. coli*. In addition, recent studies reported that it promotes the proliferation of osteoblasts and differentiation of dental epithelial cells into ameloblasts, revealing novel aspects of its function.

Thus, Gb4Cer is an attractive target for research, but available methods were mainly limited to chemical analytical techniques such as mass spectrometry and thin-layer chromatography. Analysis by a simpler immunoassay requires a monoclonal antibody that specifically recognizes Gb4Cer. Although several antibodies have been reported so far, they cannot be used for immunostaining and are not commercially available. Dr. Okuda and co-workers have obtained the Anti-Globotetraosylceramide (Gb4Cer), Mouse-Mono (PA5) antibody via a novel and effective method for developing anti-glycan antibodies. This antibody shows extremely high specificity to Gb4Cer, and it does not recognize precursors and similar structures. It can be used for immunocytochemistry, TLC immunoblotting, and flow cytometry, which makes this antibody a convenient tool.

Description

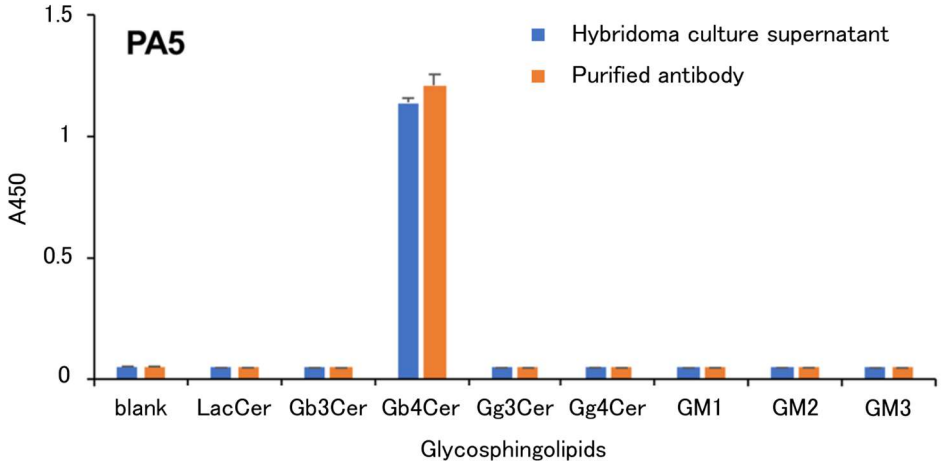
Catalog Number: FDV-0047
Size: 50 μg (0.5 mg/ml, 100 μL)
Formulation: Purified IgM in Phosphate Buffered Saline (PBS)
Host Species and Clonality: Mouse monoclonal
Clone name: PA5
Isotype and Subclass: IgM(κ)
Lot Number: See vial label
Specificity: This antibody specifically recognizes Globotetraosylceramide (Gb4Cer)
Storage: For short-term storage, -20ºC. For long-term storage, -80ºC storage is preferable.
Avoid repeated freeze-thaw cycles and avoid storage at 4ºC.

Application and Recommended usage

- Immunocytochemistry	The researcher should empirically determine optimal dilutions.
- TLC immunoblotting	The researcher should empirically determine optimal dilutions.
- Flow cytometry	The researcher should empirically determine optimal dilutions.

Reference data

Antigen specificity analysis of clone PA5 for glycosphingolipids using ELISA



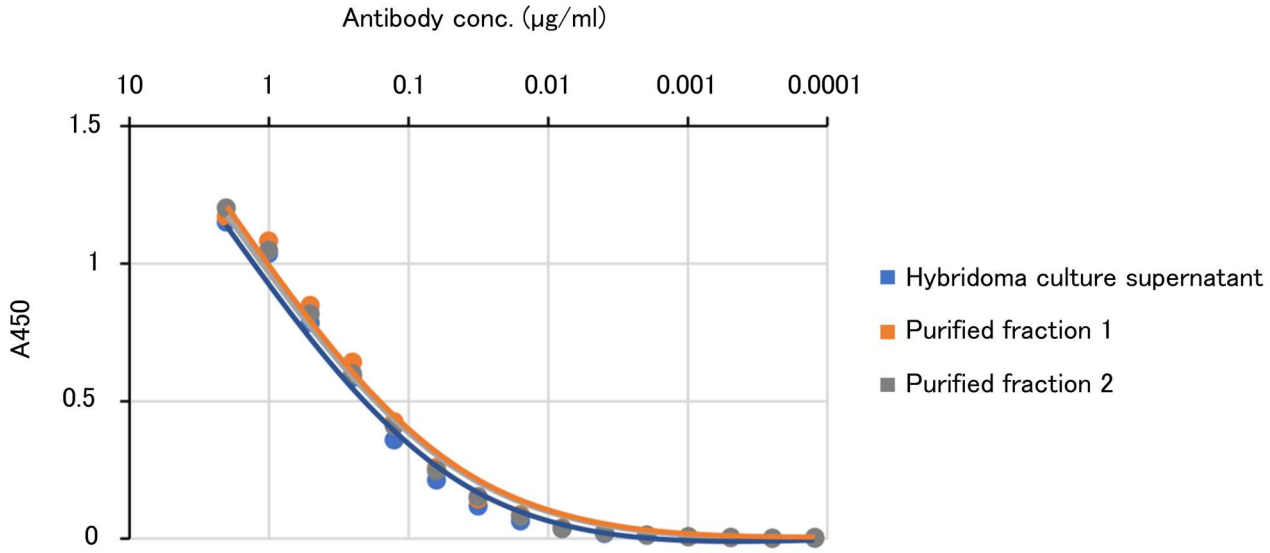
Modified from Okuda *et al.* (2021)

The antigen specificity of clone PA5 was examined by ELISA using microplates coated with various glycosphingolipids. As a result, a significant signal was only observed in wells coated with Gb4Cer. This result indicates that clone PA5 has exceptionally high specificity to Gb4Cer and does not recognize other glycosphingolipids, including its precursors such as LacCer and Gb3Cer.

Table. Glycosphingolipids used in the ELISA assay

Glycosphingolipids	Structure
LacCer	Galβ1,4GlcCer
Gb3Cer	Galα1,4Galβ1,4GlcCer
Gb4Cer	GalNAcβ1,3Galα1,4Galβ1,4GlcCer
Gg3Cer	GalNAcβ1,4Galβ1,4GlcCer
Gg4Cer	Galβ1,3GalNAcβ1,4Galβ1,4GlcCer
GM1	Galβ1,3GalNAcβ1,4(Siaα2,3)Galβ1,4GlcCer
GM2	GalNAcβ1,4(Siaα2,3)Galβ1,4GlcCer
GM3	Siaα2,3Galβ1,4GlcCer

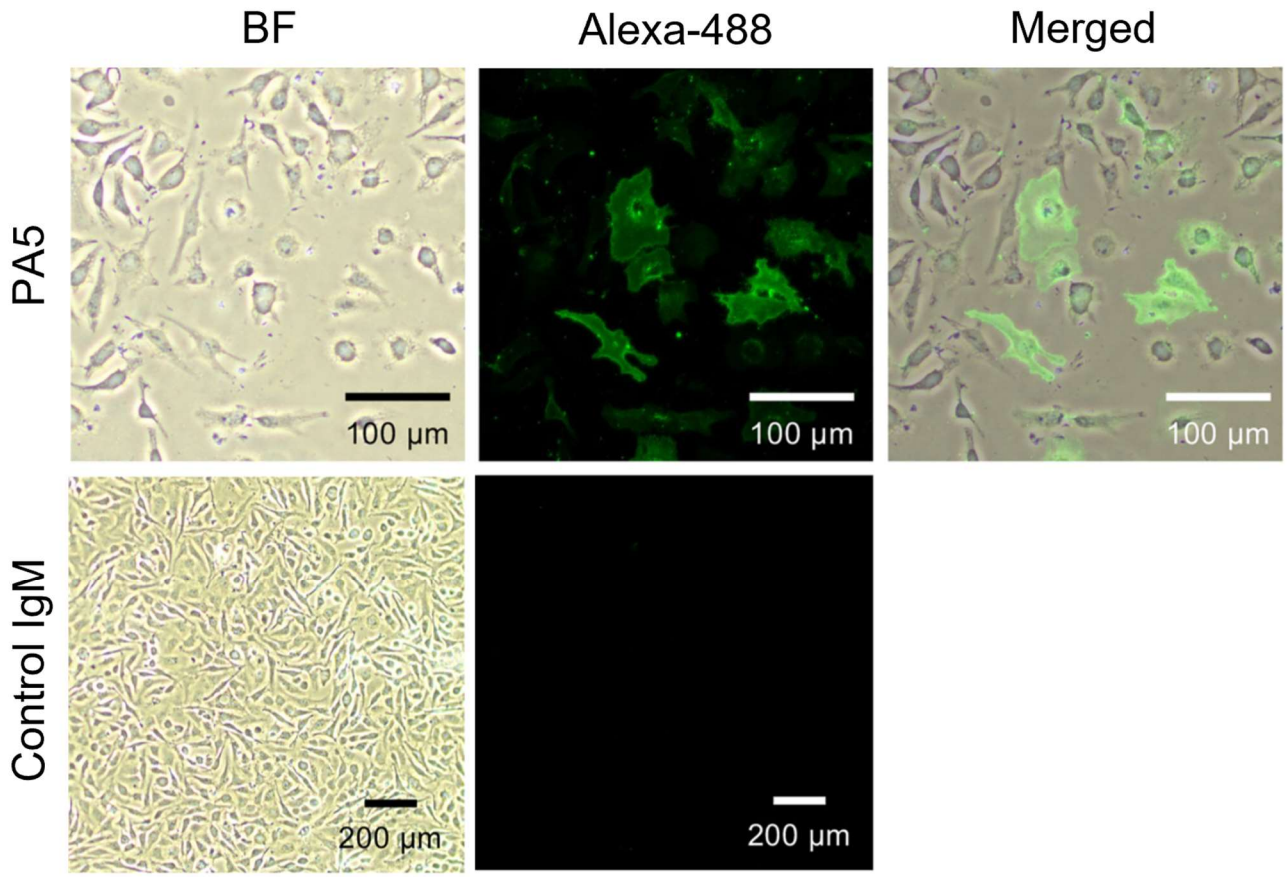
Reactivity of clone PA5 with Gb4Cer



Modified from Okuda *et al.* (2021)

Serially diluted solutions of clone PA5 were applied to microplates coated with Gb4Cer. The reactivity of the clone PA5 with Gb4Cer was examined using ELISA.

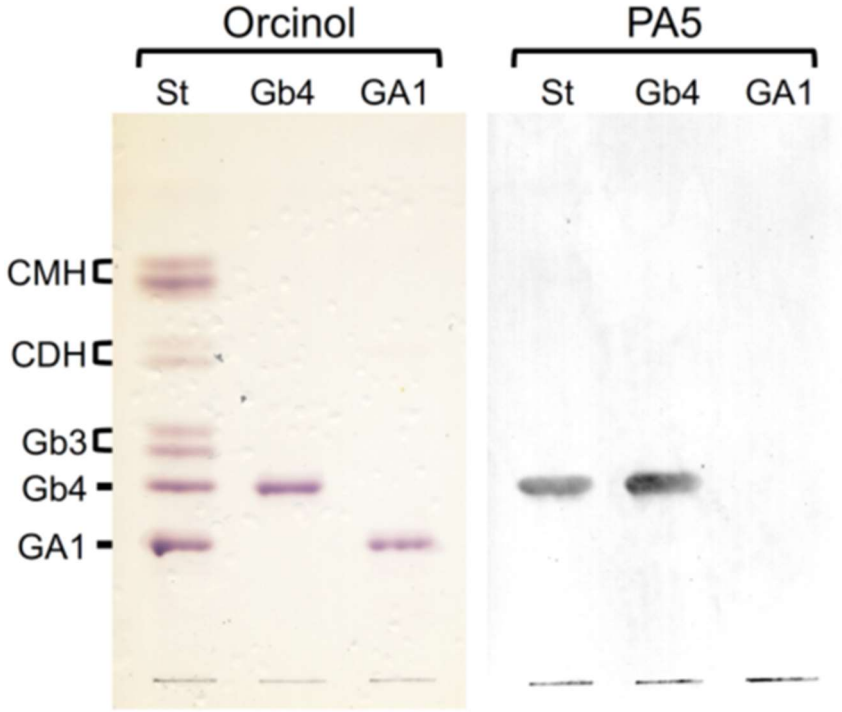
Immunocytochemical analysis of PA5 epitopes expressed on cell surface



Modified from Okuda *et al.* (2022)

Immunocytochemical analysis was performed using HeLa cells. Clone PA5 or isotype control IgM was used as the primary antibody, with the secondary antibody conjugated with Alexa488 applied for detection. Significant signal was detected on the surface of clone PA5 treated cells (top panels), but not in cells treated with control IgM (bottom panels). This result indicates that clone PA5 specifically recognizes cell surface antigens expressed in HeLa cells.

TLC immunoblot analysis of epitope specificity



Modified from Okuda *et al.* (2022)

Gb4Cer or control glycosphingolipids were separated on HPTLC plates with a solvent system consisting of chloroform/methanol/water (60:35:8, *v/v/v*) and were visualized by orcinol-H_2SO_4 (left panel) or PA5-immunostaining (right panel). Clone PA5 specifically recognized Gb4Cer, but not other glycosphingolipids.

St: Standard glycosphingolipids mixture
Gb4: Gb4Cer
GA1: Galβ1,3GalNAcβ1,4Galβ1,4GlcCer
CMH: ceramide monohexoside
CDH: ceramide dihexoside
Gb3: Gb3Cer

Reference

1. Okuda, T., *Biochem Biophys Res Commun.*, **487**, 76-82 (2017) PUGNAc treatment provokes globotetraosylceramide accumulation in human umbilical vein endothelial cells.

2. Okuda, T., *Data Brief*, **19**, 256-260 (2018) Data on immunoglobulin G antibodies induced by immunization of mice with globoside carrying very long-chain fatty acids.

3. Okuda, T., *Int. J. Mol. Sci.*, **21**, 3632 (2020) Isolation and characterization of antibodies induced by immunization with TNF-α inducible globotetraosylceramide.

4. Okuda, T., *et al.*, *Sci. Rep.*, **11**, 3233 (2021) Purification of anti-glycoconjugate monoclonal antibodies using newly developed porous zirconia particles.

5. Okuda, T., *et al.*, *Anal. Biochem.*, **657**, 114900 (2022) A zirconia-based column chromatography system optimized for the purification of IgM from hybridoma culture supernatants.

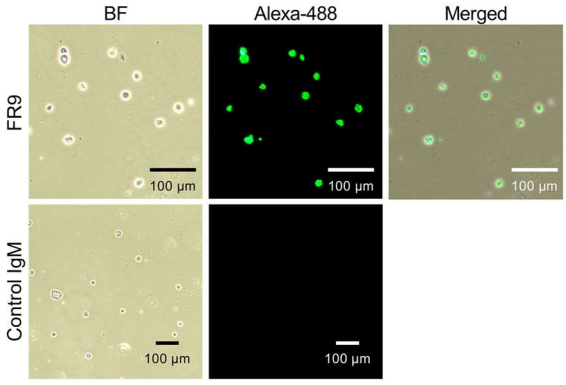
Related products

Anti-CDw75, Mouse-Mono (FR9)

CDw75 (Neu5Acα2,6Galβ1,4GlcNAc) is a sialylated glycan epitope found in mammalian N-linked glycoproteins and glycolipids. It is known as a marker of various cancers and an infection receptor for the human influenza virus. Thus, it is expected that CDw75 can be used as a diagnostic marker or therapeutic target for such diseases. Anti-CDw75, Mouse-Mono (FR9) is a novel monoclonal antibody that recognizes CDw75 with extremely high specificity.

Catalog No. FDV-0046
Size 0.1 mg (0.5 mg/ml, 200 µL)
Features

- Recognizes CDw75 in a manner independent of surrounding structures
- Extremely high antigen specificity
- Can be used for immunocytochemistry, Western blotting, and Flow cytometry



Modified from Okuda *et al.* (2022)

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