funakoshi
FRONTIERS IN LIFE SCIENCE

9-7 Hongo 2-Chome, Bunkyo-Ku
Tokyo 113-0033, Japan

Anti-CDw75, Mouse-Mono (FR9)

Catalog NO. FDV-0046

This product has been commercialized under a license from The National Institute of Advanced Industrial Science and Technology (AIST).

Product Background

CDw75 (Neu5Acα2,6Galβ1,4GlcNAc) is a sialylated glycan epitope found in mammalian N-linked glycoproteins and glycolipids. It is known as a surface marker of B-cell lymphoma and as a novel tumor marker that correlates with stomach and colorectal cancer grade. CDw75 is also known as 6SLN, 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. So far, several monoclonal antibodies targeting CDw75 have been obtained. However, these antibodies show different antigen specificity, suggesting that these antibodies recognize the glycan structure of CDw75 and nearby surrounding regions. Hence, a novel antibody showing higher specificity only for CDw75 has been desired. Although it was very difficult to obtain anti-glycan antibodies in general because of glycan's low immunogenicity to mammals, an effective method has been developed by Dr. Okuda and co-workers. Anti-CDw75, Mouse-Mono (FR9) was obtained by this method. It recognizes CDw75 in a manner independent of surrounding structures, and it does not recognize precursors and similar structures. Thus, it has extremely high antigen specificity. It can be used for immunocytochemistry and Western blotting, which makes glycan analysis much easier.

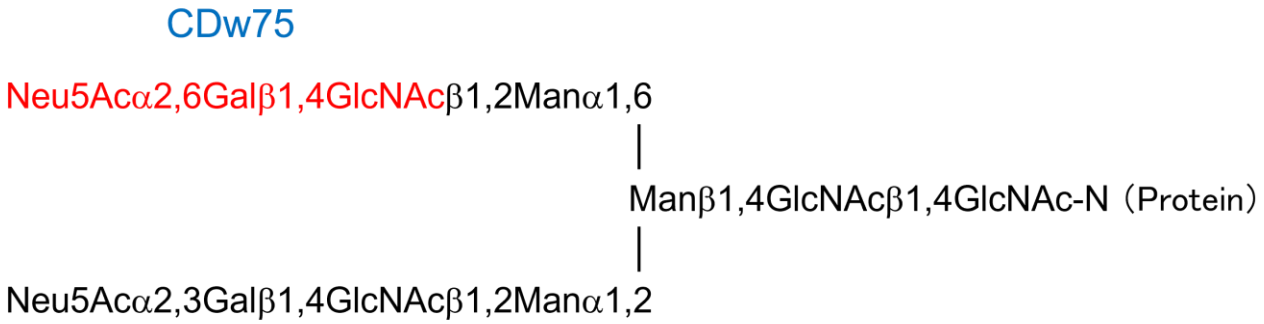


Figure 1. The Glycan structure of CDw75

Description

Catalog Number: FDV-0046
Size: 0.1 mg (0.5 mg/ml, 200 μL)
Formulation: Purified IgM in Phosphate Buffered Saline (PBS)
Host Species and Clonality: Mouse monoclonal
Clone name: FR9
Isotype and Subclass: IgM(κ)
Lot Number: See vial label
Specificity: This antibody specifically recognizes CDw75 (Neu5Acα2,6Galβ1,4GlcNAc) in glycoproteins and glycolipids expressed in mammalian cells.
Storage: For short-term storage, -20ºC. For long-term storage, -80ºC storage is preferable.
Avoid repeated freeze-thaw cycles and avoid storage at 4ºC.

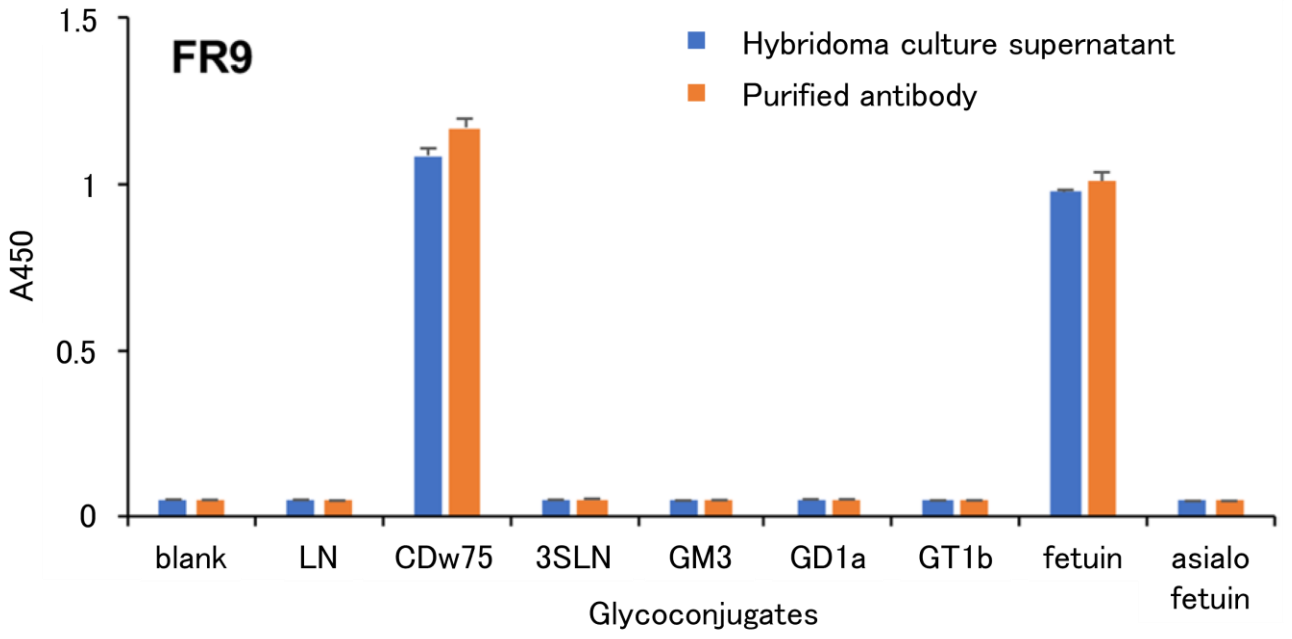
Application and Recommended usage

- Western blotting	1/1,000 – 1/250
- Immunocytochemistry	Optimal dilutions should be determined by the researcher.
- Flow cytometry	Optimal dilutions should be determined by the researcher.

*Skim milk is not suitable for blocking

Reference data

Specificity analysis of clone FR9 for its antigens using ELISA



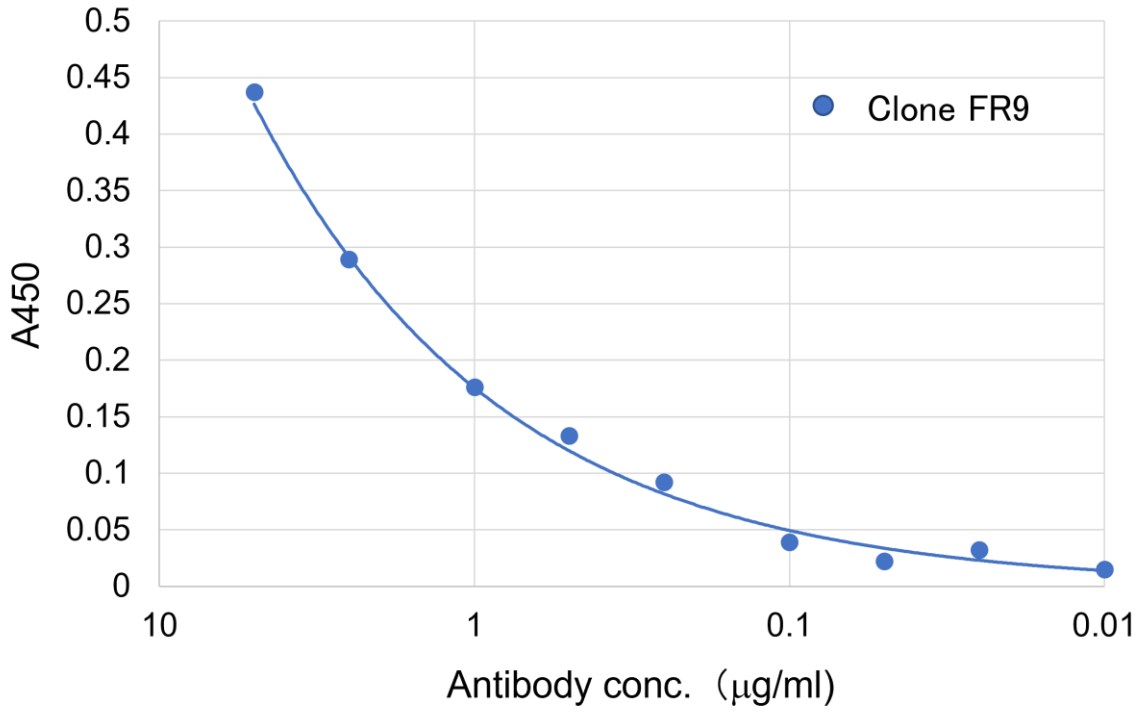
Modified from Okuda et al. (2021)

The specificity of clone FR9 was examined by ELISA using microplates coated with various glycoconjugates. As a result, a significant signal was observed only in glycoconjugates containing CDw75. This result indicates that clone FR9 has exceptionally high specificity to CDw75 and does not recognize similar glycan structures.

Table. Glycan structure of antigens used for ELISA

Glycoconjugate	Structure
LN	Galβ1,4GlcNAcCerA
CDw75	Neu5Acα2,6Galβ1,4GlcNAcCerA
3SLN	Neu5Acα2,3Galβ1,4GlcNAcCerA
GM3	Neu5Acα2,3Galβ1,4GlcCer
GD1a	Neu5Acα2,3Galβ1,3GalNAcβ1,4(Neu5Acα2,3)Galβ1,4GlcCer
GT1b	Neu5Acα2,3Galβ1,3GalNAcβ1,4(Neu5Acα2,8Neu5Ac α2,3)Galβ1,4GlcCer
fetuin	Neu5Acα2,6Galβ1,4GlcNAc-R, Neu5Acα2,3Galβ1,4GlcNAc-R, etc.
asialofetuin	Galβ1,4GlcNAc-R, etc.

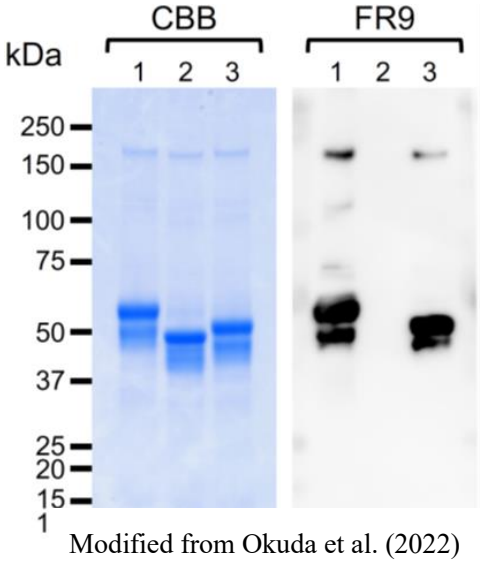
Reactivity of clone FR9 with its antigen



Serially diluted solutions of clone FR9 were applied to microplates coated with Fetuin, a glycoprotein containing CDw75. The reactivity of clone FR9 with Fetuin was examined using ELISA.

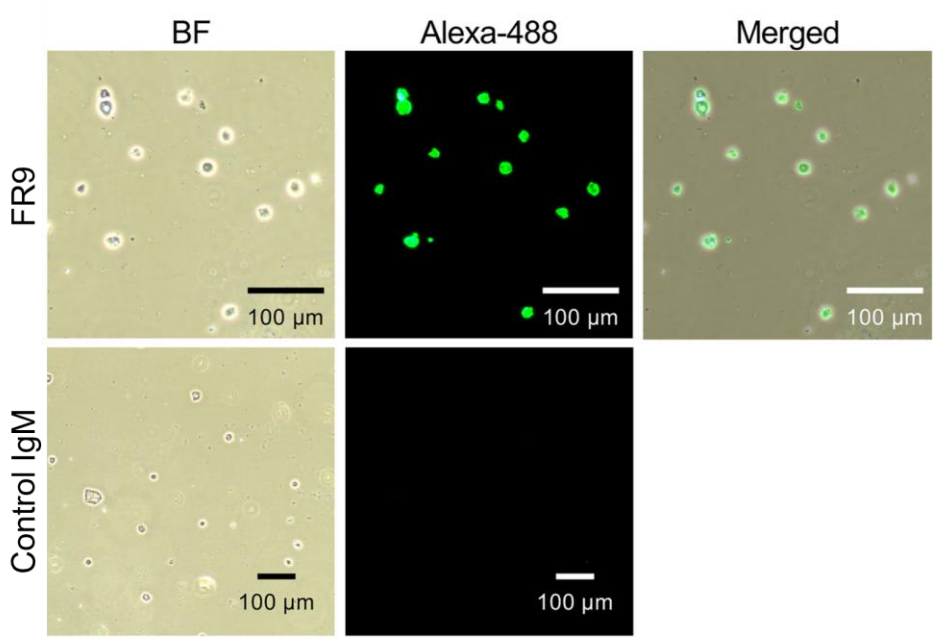
Immunoblot analysis of epitope specificity

The epitope specificity of clone FR9 was analyzed by immunoblotting. Fetuin (lane 1), asialofetuin (lane 2), and α2,3-linked sialic acid-removed fetuin (lane 3) were separated by SDS-PAGE and then analyzed by Coomassie Brilliant Blue staining (CBB, left panel) or immunoblotting (right panel). Clone FR9 specifically recognized the antigens containing CDw75, but not the antigen lacking CDw75.



Modified from Okuda et al. (2022)

Immunocytochemical analysis of FR9 epitopes expressed on cell surface



Modified from Okuda et al. (2022)

Immunocytochemical analysis was performed using Burkitt's lymphoma cells (Raji cells). Clone FR9 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 FR9 treated cells (top panels), but not in cells treated with control IgM (bottom panels). This result indicates that clone FR9 specifically recognizes cell surface antigens expressed in Raji cells.

Reference

1. Okuda, T., et al., “Purification of anti-glycoconjugate monoclonal antibodies using newly developed porous zirconia particles.”, Sci Rep 11, 3233. doi: 10.1038/s41598-021-82457-0., (2021). [PMID: 33564002]

2. Okuda, T., et al., "A zirconia-based column chromatography system optimized for the purification of IgM from hybridoma culture supernatants.", Analytical Biochemistry, 657:114900. doi: 10.1016/j.ab.2022.114900., (2022). [PMID: 36122604]
