

Anti-CDw75, Mouse-Mono (FR9)

Catalog NO. FDV-0046

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Product Background

CDw75 (Neu5Ac α 2,6Gal β 1,4GlcNAc) 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, Western blotting and Flow cytometry, which makes glycan analysis much easier.

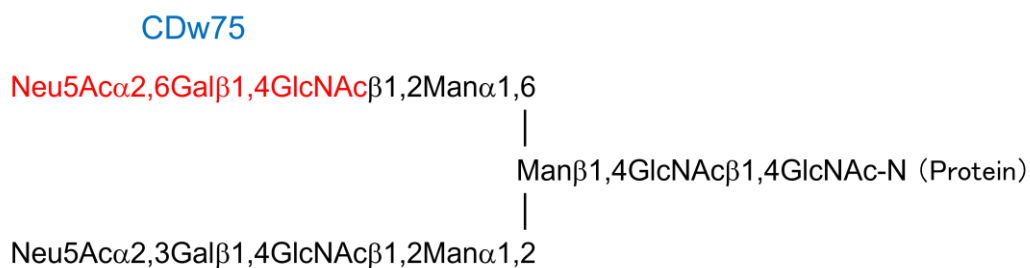


Figure 1. The Glycan structure of CDw75

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, -20°C. For long-term storage, -80°C storage is preferable.

Avoid repeated freeze-thaw cycles and avoid storage at 4°C.

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

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.

*Data was modified from Okuda *et al.* (2021)

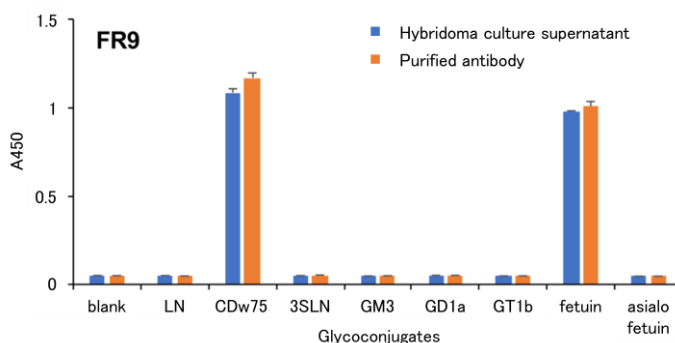
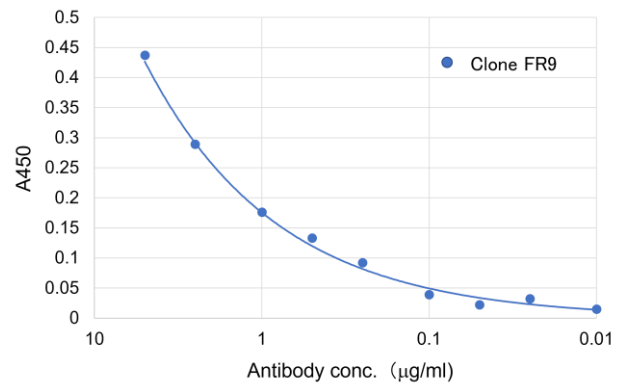


Table. Glycan structure of antigens used for ELISA

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

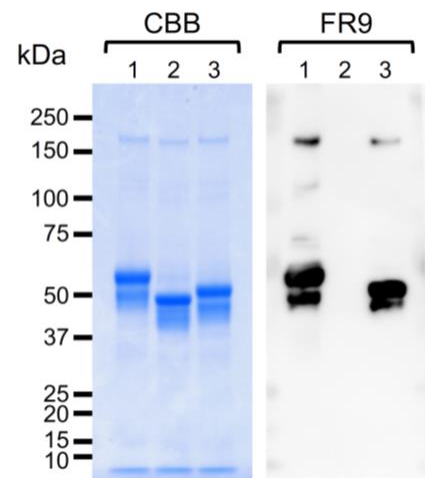
Serially diluted solutions of clone FR9 were applied to microplates coated with Fetuin, a glycoprotein containing CDw75. The reactivity of clone FR9 with Fetuin was examined using ELISA.



Immunoblot analysis of epitope specificity

The epitope specificity of clone FR9 was analyzed by immunoblotting. Fetuin (lane 1), asialofetuin (lane 2), and α 2,3-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.

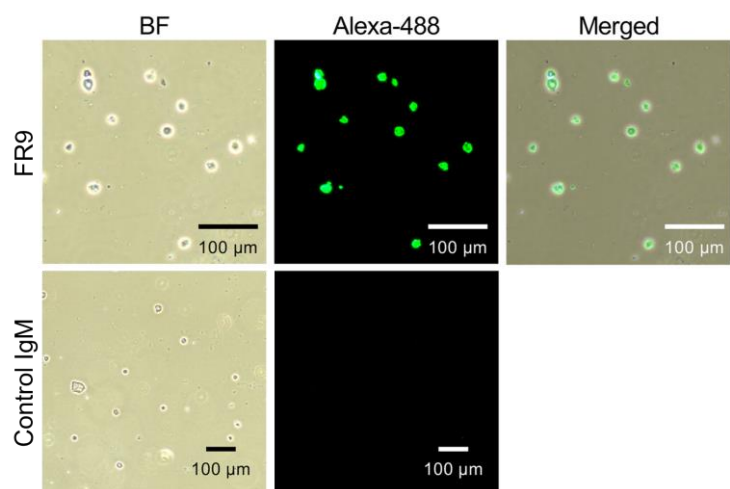
*Data was modified from Okuda *et al.* (2022)



Immunocytochemical analysis of FR9 epitopes expressed on cell surface

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.

*Data was modified from Okuda *et al.* (2022)



Reference

1. Okuda, T., *et al.*, *Sci Rep.*, **11**, 3233 (2021) Purification of anti-glycoconjugate monoclonal antibodies using newly developed porous zirconia particles.
2. Okuda, T., *et al.*, *Analytical Biochemistry*, **657**:114900 (2022) A zirconia-based column chromatography system optimized for the purification of IgM from hybridoma culture supernatants.

Related products

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

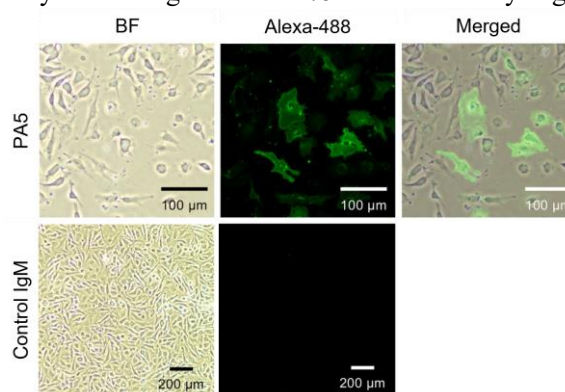
CDw75 (Neu5Ac α 2,6Gal β 1,4GlcNAc) is a sialylated glycan epitope found in mammalian N-linked glycoproteins and glycolipids. It is known as a marker of various cancers as well as an infection receptor for the human influenza virus. Thus, it is expected that CDw75 can be used as a diagnostic marker or 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-0047

Size 0.1 mg (0.5 mg/ml, 200 μ L)

Features

- Extremely high antigen specificity
- Can be used for immunocytochemistry, TLC immunoblotting, and Flow cytometry



Data was modified from Okuda *et al.* (2022)

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