Purification of anti-glycoconjugate monoclonal antibodies using newly developed porous zirconia particles

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Supplementary Information

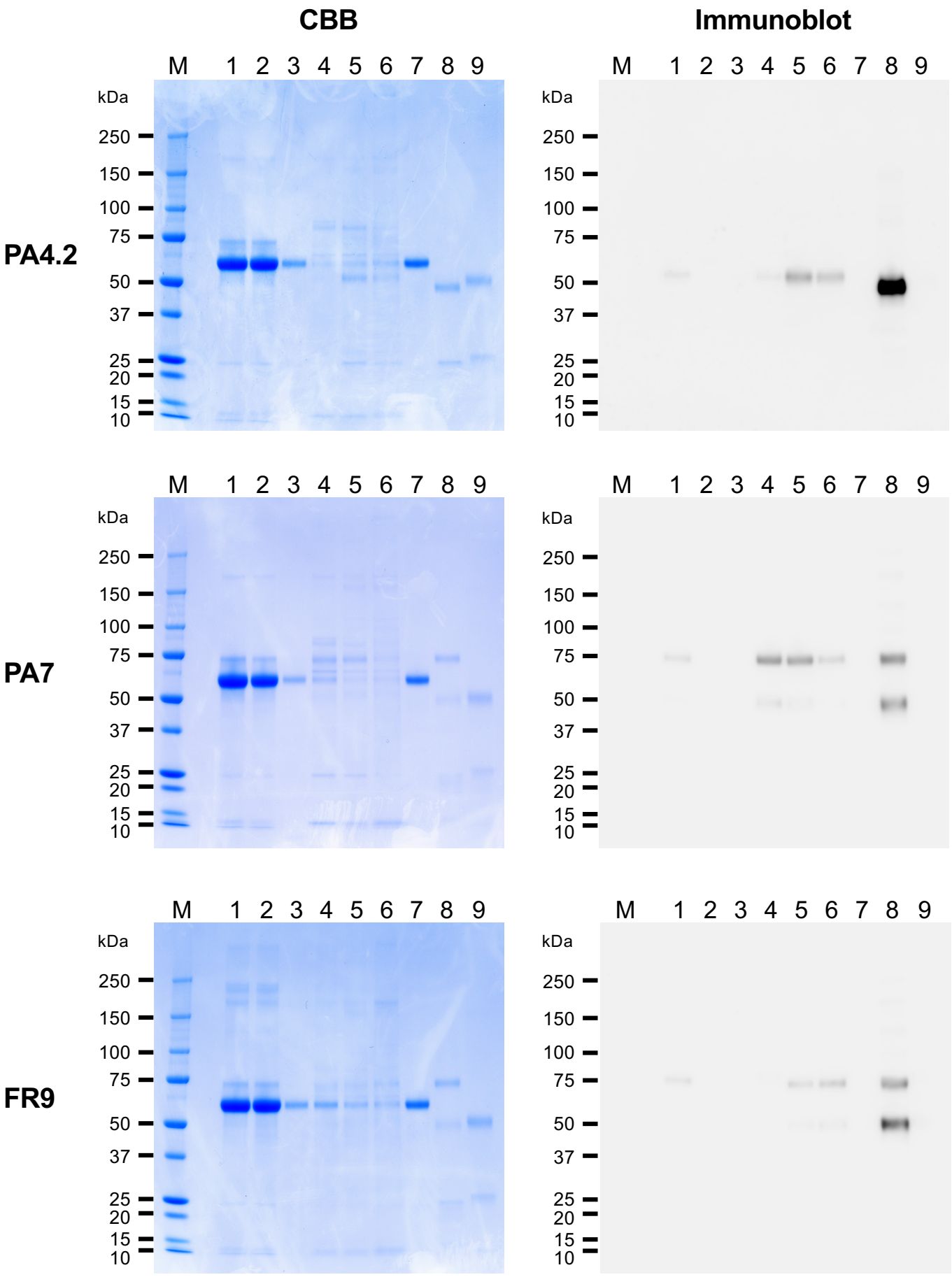

Figure S1. SDS-PAGE analysis of PZP-purified monoclonal antibodies.
Upper panels, PA4.2 IgG3; middle panels, PA7 IgM; bottom panels, FR9 IgM. Left panels, CBB staining; right panels, immunoblotting. Lane 1, hybridoma supernatant; 2, non-adsorbed fraction; 3, wash fraction; 4, 100 mM PB-eluted fraction; 5, 200 mM PB-eluted fraction; 6, 500 mM PB-eluted fraction; 7, standard BSA; 8, standard mouse IgG3 (for PA4.2) or IgM (for PA7 or FR9); 9, standard bovine IgG.

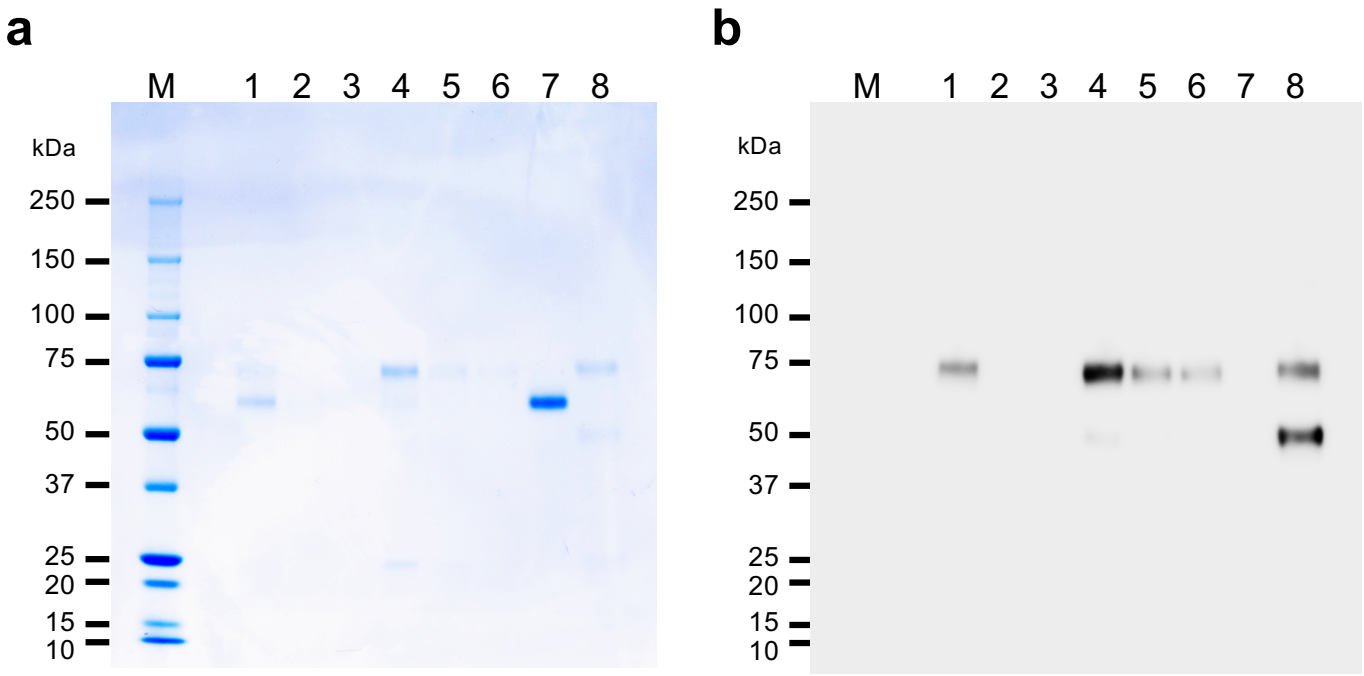

Figure S2. SDS-PAGE analysis of IgM composed by λ-light chains after PZP purification. Ascites fluid containing monoclonal IgM composed of λ-light chains (MOPC104E) was diluted with 10 mM PB (pH 7.0), and 1 mL of sample containing approximately 60 μg of these antibodies was purified using PZPs. The following PZP purification fractions were analyzed by SDS-PAGE with CBB staining **(a)** or anti-mouse IgM immunoblotting **(b)**, as described in the *Methods*. Lane 1, PB-diluted ascites; 2, non-adsorbed fraction; 3, wash fraction; 4, 100 mM PB-eluted fraction; 5, 200 mM PB-eluted fraction; 6, 500 mM PB-eluted fraction; 7, standard BSA; 8, standard mouse IgM; M, molecular weight marker. The protein band detected near the 50-kDa marker in lanes 4 and 8 is a degradation product of the immunoglobulin μ-chain.

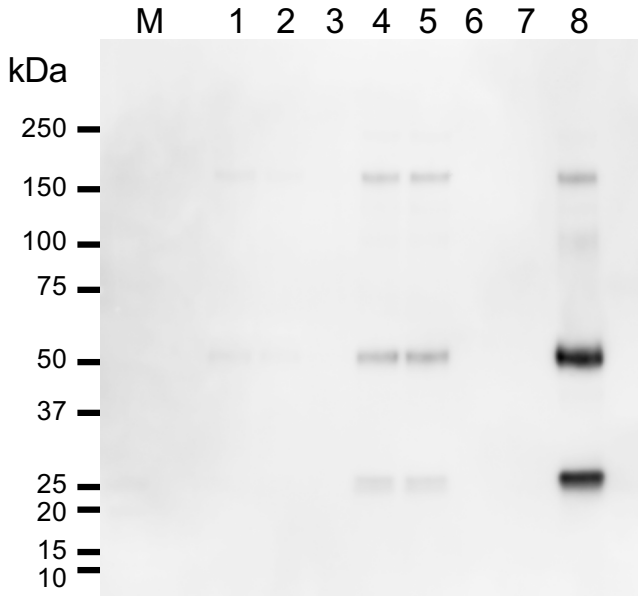

Figure S3. Anti-bovine IgG immunoblot analysis of PA5 purified from hybridoma supernatant using PZPs.
Bovine IgG in the SDS-PAGE sample (Fig. 2a) was detected by immunoblotting using HRP-labeled anti-bovine IgG as described in the *Methods*. Lane 1, hybridoma culture supernatant; 2, non-adsorbed fraction; 3, wash fraction; 4, 100 mM PB-eluted fraction; 5, 200 mM PB-eluted fraction; 6, standard BSA; 7, standard mouse IgM; 8, standard bovine IgG .

Glycoconjugate	Structure
LacCer	Galβ1,4GlcCer
Gb3Cer	Galα1,4Galβ1,4GlcCer
Gb4Cer	GalNAcβ1,3Galα1,4Galβ1,4GlcCer
Gg3Cer	GalNAcβ1,4Galβ1,4GlcCer
Gg4Cer	Galβ1,3GalNAcβ1,4Galβ1,4GlcCer
GM3	Siaα2,3Galβ1,4GlcCer
GM2	GalNAcβ1,4(Siaα2,3)Galβ1,4GlcCer
GM1	Galβ1,3GalNAcβ1,4(Siaα2,3)Galβ1,4GlcCer
GD1a	Siaα2,3Galβ1,3GalNAcβ1,4(Siaα2,3)Galβ1,4GlcCer
GT1b	Siaα2,3Galβ1,3GalNAcβ1,4(Siaα2,8Siaα2,3)Galβ1,4GlcCer
LacNAcCerA	Galβ1,4GlcNAcCerA
α2,6-sialyl LacNAcCerA	Siaα2,6Galβ1,4GlcNAcCerA
α2,3-sialyl LacNAcCerA	Siaα2,3Galβ1,4GlcNAcCerA
fetuin*	Siaα2,6Galβ1,4GlcNAc
asialo-fetuin*	Galβ1,4GlcNAc

Table S1. Structures of oligosaccharides in glycoconjugates used in this study. *Main structures of the non-reducing terminal oligosaccharides in these glycoproteins are presented. Abbreviations: Cer, ceramide; Sia, sialic acid (Neu5Ac); CerA, ceramide analogue.