proteomics

Development of a Novel Antibody-Based Resin for the Depletion of Human Albumin and IgG

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Application Notes

- Small, single-chain antibody resin with 2-4 times the serum capacity compared to conventional antibody-based resins
- Displays higher specificity than Cibacron Blue resins and conventional antibody-based resins
- Included buffers are directly compatible with 2DE, thereby eliminating the need for protein precipitation procedures prior to electrophoresis
- Facilitates fast, efficient and specific removal of albumin and IgG from serum

Introduction

The study of the human serum proteome is an area of great interest, especially the pharmaceutical potential for identifying disease biomarkers. The study of this proteome is challenging because most of the proteins of pharmaceutical interest appear at low concentrations. Albumin and IgGs make up greater than 70% of the proteins in serum. Depletion of these high abundance proteins allows for 1) visualization of proteins co-migrating with albumin and IgG on a 2DE gel and 2) each individual protein can be loaded at a 4- to 5-fold higher level for improved visualization of lower copy number proteins.

Antibody-based high abundance protein depletion resins display higher specificity than dye-based resins, making them preferred over materials such as Cibacron Blue. Antibody-based resins have much higher specificity for albumin and IgG, but typically have lower protein-binding capacity. A novel high-binding capacity antibody-based resin for the depletion of human albumin and IgG has been developed. The recombinant antibody ligands are small (12 kDa) single-chain proteins, which are expressed in yeast. The small, single-chain antibody ligands confer the high specificity of conventional 150 kDa IgG antibodies but are more stable and produce a higher density of binding sites, thus increasing the antigen-binding capacity of the resin.

The ProteoPrep Immunoaffinity Equilibration Buffer is a low salt Tris buffer. Figure 1 is a schematic diagram of the PROT-IA procedure. The optimized protocol keeps sample dilution low, moreover the low salt content of the equilibration buffer makes the depleted serum directly compatible with 2DE, thereby eliminating the need for protein precipitation procedures prior to electrophoresis. Without the need for post-depletion sample concentration or precipitation, the depletion process is fast, and can be carried out in less than 30 min.

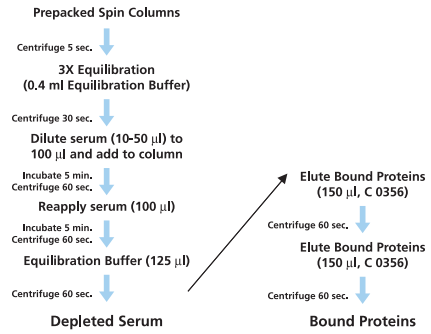

Figure 1. Flow chart illustrating the workflow required for the use of PROT-IA.

Benefits of albumin and IgG depletion

The 2DE gels clearly demonstrate the benefits for albumin and IgG depletion of serum (Figure 2). Depletion clears the upper portion of the gel, thereby bringing into focus the higher molecular weight proteins. Removal of albumin and IgG also reduces contamination of spots, which can interfere with MALDI-TOF mass spectrometric identification. Finally, depletion allows for increased (4-5 fold) serum loads onto a gel so that lower copy number proteins can be visualized and identified.

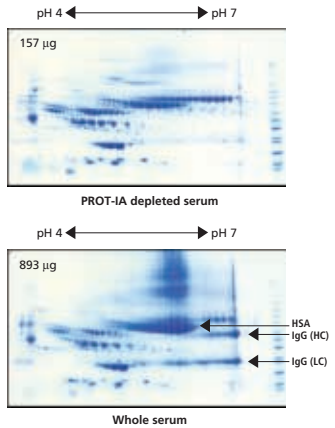

Figure 2. Benefits of albumin and IgG depletion of serum. *A 50 µl sample of human serum was depleted of albumin and IgG using the ProteoPrep Immunoaffinity Albumin and IgG Depletion Kit (Product Code PROT-IA). Two-dimensional electrophoresis was carried out on a 15 µl serum sample and volume normalized depleted serum using 11 cm, pH 4-7 IPG strips. For both albumin and IgG the percent depletion was determined by ELISA to be 99%. The protein load is detailed in the upper left-hand corner of each gel. Protein concentration was determined by Bradford Protein Assay (Product Code B 6916).*

The human albumin and IgG depletion capacity of PROT-IA and a conventional antibody-based resin were compared (Figure 3, Panels A and B). Various volumes of human serum were applied to the resins and the percent (%) depletion of albumin and IgG determined by ELISA. The PROT-IA Kit demonstrates higher binding capacity (2-4 times) for human albumin and IgG than does the conventional antibody resin. PROT-IA depletes greater than 98% of albumin from 50 µl of serum whereas to have a similar depletion efficiency, the conventional resin must use 15 µl of serum or less (Figure 3, Panel A). Similarly, PROT-IA will deplete greater than 98% of IgG from 50 µl of serum whereas to have similar depletion efficiency, the conventional resin must use 20 µl of serum or less (Figure 3, Panel B).

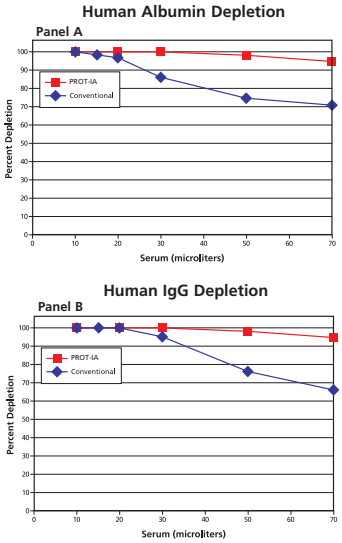

Figure 3. Comparison of human albumin and IgG depletion capacity. *The PROT-IA resin (350 µl packed resin) and a conventional antibody based resin (375 µl packed resin) were used to deplete albumin and IgG from human serum (10, 15, 20, 30, 50, and 70 µl). Depleted serum from each resin was analyzed for residual albumin and IgG by ELISA. The percent depletion was determined by comparison to albumin and IgG levels in undepleted serum.*

The binding specificity of PROT-IA (Figure 4, Panel A) was compared to a Cibacron Blue-based resin (Figure 4, Panel B) and a conventional antibody-based resin (Figure 4, Panel C). Higher specificity was seen for the PROT-IA Kit than for either the Cibacron Blue resin or the conventional antibody-based resin. The 2DE analysis software (Nonlinear Dynamics) detected 390 spots on the PROT-IA gel whereas 324 spots were detected on the Cibacron Blue resin gel and 279 spots were detected on the conventional antibody resin gel. One hundred twenty five (125) protein spots were detected on the PROT-IA gel, which were not matched on the Cibacron Blue gel whereas eleven (11) spots detected on the Cibacron Blue gel were not matched to the PROT-IA gel. Ninety three (93) protein spots were detected on the PROT-IA gel, which were not matched on the conventional gel whereas only seven (7) spots detected on the conventional resin gel were not matched to the PROT-IA gel.

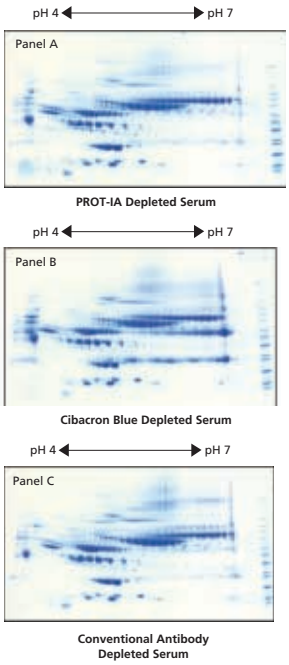

Figure 4. Specificity comparison of PROT-IA to a Cibacron Blue and conventional antibody-based resin. *A 50 µl sample of human serum was depleted of albumin and IgG using the ProteoPrep Immunoaffinity Albumin and IgG Depletion Kit (Product Code PROT-IA) following the protocol outlined in Figure 1 (Panel A). A 50 µl sample of human serum was depleted of albumin only using a commercially available Cibacron Blue-based albumin depletion kit (Panel B). Albumin and IgG were also depleted from 15 µl of human serum using a commercially available conventional antibody-based albumin depletion kit following the protocol included with the kit (Panel C). The depleted serum (515 µl final volume) obtained from the conventional antibody resin was acetone precipitated to remove salts and concentrate the proteins. Two-dimensional electrophoresis was carried out on volume normalized depleted serum (15 µl) using 11 cm, pH 4-7 IPG strips. The amount of protein loaded on each gel was 157 µg for PROT-IA treated sample (Panel A), 181 µg for the Cibacron Blue-treated sample (Panel B) and 164 µg for the Conventional antibody treated sample (Panel C). Protein concentration was determined by Bradford Protein Assay (Product Code B 6916).*

Ordering Information

Product	Description	Unit
PROT-IA	ProteoPrep Immunoaffinity Albumin and IgG Depletion Kit	1 kit