

proteomics

Superior Performance of the ProteoPrep™ Blue Albumin Depletion Kit for 2D Electrophoresis

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Application Notes

- ProteoPrep Blue typically removes > 95% albumin and > 80% IgG from 75 μ l of human serum
- Tris-buffered urea equilibration solution for superior 2DE without the need for precipitation
- A unique blue ligand-based resin which displays higher specificity for human albumin from serum than other commercially available kits
- A protocol, which is fast, reproducible and minimizes dilution of the depleted serum

Introduction

The study of the human serum proteome is an area of great interest, especially the pharmaceutical potential for biomarkers for diseases. Albumin and IgGs make up greater than 70% of the proteins in human serum. Depletion of these proteins allows for: 1) visualization of proteins co-migrating with albumin and IgG on a one-dimensional SDS-PAGE gel or 2DE gel and 2) higher sample loads (4-5 fold) for improved visualization of lower abundance proteins.

The most common method for depletion of albumin is the use of Cibacron Blue resin equilibrated with a buffered salt solution. Cibacron Blue typically has high capacity but displays lower specificity. Low specificity may result in the loss of proteins of interest. Antibody-based albumin depletion resins display higher specificity but have lower capacity and are more expensive.

Most protocols for albumin depletion of serum utilize sodium chloride or potassium chloride to reduce non-specific binding to the resin. The presence of salts tends to decrease the resolution of proteins on a 2DE gel. Samples containing salts typically require precipitation of the protein sample, which adds extra steps to the process and may lead to a loss of other proteins.

Sigma-Aldrich has developed an albumin/IgG depletion kit (ProteoPrep™ Blue, Product Code [P 1120](#)) with specificity for albumin and IgG depletion superior to other commercially available blue ligand-based resins. It utilizes a urea-based equilibration buffer, which is superior to salt-based buffers for subsequent 2DE analysis (Figure 1).

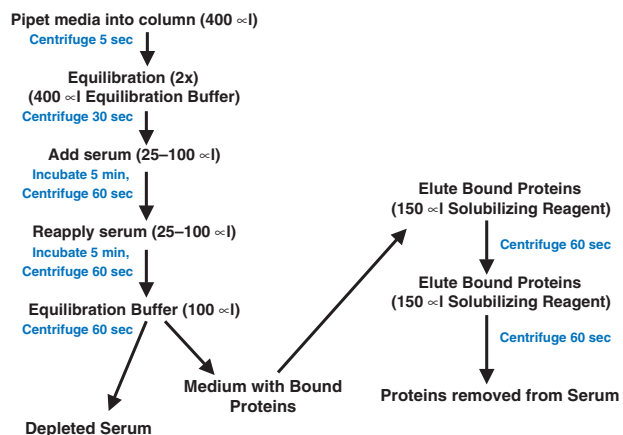


Figure 1. Albumin depletion with ProteoPrep Blue.

Complete kit for human serum samples

The importance of depleting human serum of the albumin and IgG's can be seen in Figure 2. Lower abundance proteins, which co-migrate with albumin and IgG, are unmasked after depletion. Removing albumin also reduces smearing in the upper portion of the gel, which can obscure the higher molecular weight proteins. The removal of albumin and IgG will lead to reduced contamination of spots, which can interfere with MALDI identification. In addition, depletion of the high abundance proteins allows for increased (4-5 fold) amounts of serum that can be loaded onto a gel (data not shown). ProteoPrep Blue typically removes >95% human albumin and >80% human IgG from 75 μ l serum which allows for subsequent detection of lower abundance proteins.

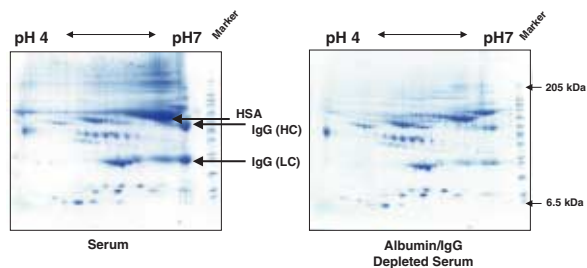


Figure 2. Benefits of albumin and IgG depletion of human serum for 2DE analysis. A 75- μ l sample of human serum was depleted of albumin and IgG using the ProteoPrep Blue Albumin Depletion Kit. Two-dimensional electrophoresis was carried out on a 5 μ l-serum sample and the depleted serum using pH 4-7 IPG strips. The percent depletion of albumin and IgG was determined by ELISA to be 96% and 81%, respectively.

Urea-based kit superior to salt-based kit

The presence of salt in 2DE samples can negatively impact 2DE, as shown in Figure 3. Increased salt concentration causes an area depleted of focused proteins on the high pH end. The proteins that do focus tend to shift toward the low pH end and are less resolved. Also, an increased fraction of the proteins stack up at the low pH end of the strip. Depleted human serum samples utilizing salt-based kits typically require a precipitation step to remove the salt, which requires extra time and can lead to protein loss. The ProteoPrep Blue kit contains a Tris-buffered urea solution, therefore, the depleted serum does not require precipitation prior to 2DE.

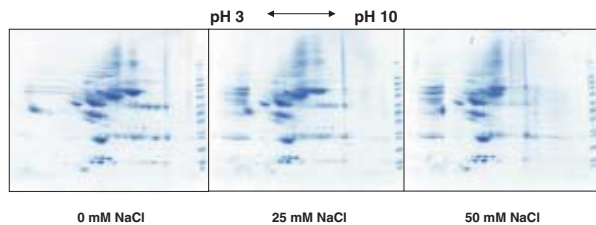


Figure 3. Negative effect of salts on the resolution of proteins on a 2DE gel. A 75- μ l sample of human serum was depleted of albumin and IgG using the ProteoPrep Blue Albumin Depletion Kit. Two-dimensional electrophoresis was carried out on a 5- μ l serum sample and the depleted serum using pH 4-7 IPG strips, the presence of no salt or with 25mM NaCl and 50 mM NaCl.

Salt and urea are effective at reducing non-specific binding of serum proteins to the resin (Figure 4). Replacing sodium chloride (Lanes 3) with urea (Lanes 2) maintained the albumin and IgG binding of the resin. However, the urea-based buffer (Lane 5) shows improved non-specific binding as can be seen by less bound proteins in the upper part of the gel than when using the salt-based buffer (Lane 6).

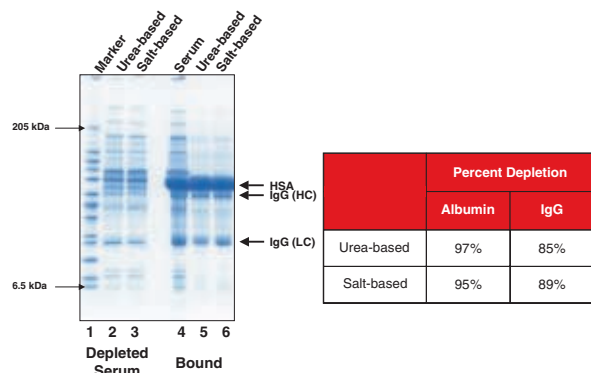


Figure 4. Improved depletion specificity with urea-based depletion method. Two 75- μ l samples of human serum were processed using the ProteoPrep Blue Albumin Depletion Kit equilibrated either with the supplied urea-based buffer (Lanes 2 and 5) or with 25 mM Tris-HCl, 150 mM NaCl pH 7.4 (Lanes 3 and 6). A 0.3- μ l serum sample (Lane 4) and an equivalent volume of normalized depleted serum and bound samples were run on 4-20% SDS-PAGE gels.

Superior specificity and capacity over other kits

As seen in Figure 5, two commercially available kits which use Cibacron Blue resins (Kits A and B) typically display relatively high non-specific binding for proteins other than albumin as seen in the large number of proteins bound to the resins (Lanes 7 and 8). Kit A also contains Protein A resin for removing the high abundance IgG proteins. ProteoPrep Blue, with urea-based buffers and a proprietary albumin-binding medium provides excellent albumin and IgG depletion (Lane 2) with low non-specific binding (Lane 6). Many of the other commercially available kits significantly increase the volume of the depleted serum. Since the ProteoPrep Blue kit does not require dilution of the serum prior to application onto a semi-dry medium, dilution of the depleted serum is minimized.

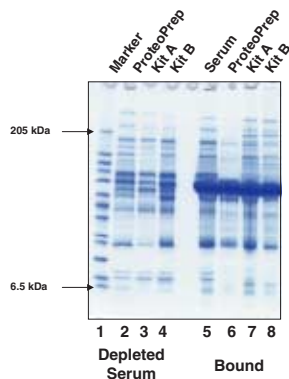


Figure 5. ProteoPrep Blue performance comparison. Each of the commercially available kits (Kits A and B) was used according to the protocols supplied with each kit. For the ProteoPrep Blue kit and Kits A and B, 75 μ l, 50 μ l, and 75 μ l of human serum were applied, respectively. The final volumes of depleted serum for the ProteoPrep Blue kit and Kits A and B, were 175 μ l, 400 μ l, and 175 μ l, respectively. The bound proteins were eluted from the columns using Laemmli Sample Buffer. A 0.375- μ l serum sample and an equivalent volume of normalized depleted serum and bound samples were run on 4-20% SDS-PAGE gels.

ProteoPrep Blue is the next generation of blue ligand-based albumin and IgG depletion kits. ProteoPrep Blue displays superior specificity, capacity and 2DE compatibility.

Ordering Information

Product	Description	Unit
PROT-BA	ProteoPrep™ Blue Albumin Depletion Kit	1 kit