

A Method for Rapid, Large-Scale Removal of Albumin and IgG From Human Serum Using the BioLogic DuoFlow™ Chromatography System

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Introduction

In human serum, albumin contributes more than 60% of total protein, and immunoglobulins, predominantly IgG, contribute 10–25%. The high concentrations of both albumin and IgG obscure low-abundance serum proteins and limit the amount of total serum protein that can be resolved by two-dimensional (2-D) electrophoresis.

The use of a chromatography system for the simultaneous removal of albumin and IgG from human serum requires the use of media that specifically remove these proteins. Affi-Gel® Blue support is a crosslinked agarose bead with covalently attached Cibacron Blue F3GA dye and efficiently removes albumin from human serum. Affi-Gel protein A support is an agarose bead coupled with protein A, which is well known for its specific binding to the Fc region of IgG molecules; it yields highly purified IgG and is also used to selectively remove IgG from human serum prior to electrophoretic analysis.

To remove both albumin and IgG from a large amount of human serum in a single, rapid process, we have developed a chromatographic method using the BioLogic DuoFlow high-resolution chromatography system. Coupled with BioLogic DuoFlow software, the system automates the processing of a biological sample on more than one column.

In this study, we removed albumin and IgG from 10 ml of human serum using one AVR7-3 injection valve, one SV5-4 buffer select valve, and one SVT3-2 diverter valve to link an Affi-Gel Blue and Affi-Gel protein A column in series (Figure 1). With this configuration, albumin and IgG were removed in a single automated operation, and the BioLogic DuoFlow system was extremely productive — it showed consistent performance with virtually no user intervention. As an added benefit, this method is flexible since both the Affi-Gel Blue and the Affi-Gel protein A columns can be proportionally scaled up or down to handle varying amounts of human serum.

Methods

System Components and Buffers

The BioLogic DuoFlow chromatography system was used in this study. Components were connected to the F10 workstation and controller as illustrated in Figure 1 and included two columns (details below), a UV detector, a conductivity monitor, a BioFrac™ fraction collector, an AVR7-3 injection valve, an SV5-4 buffer select valve, and an SVT3-2 flow-diversion valve. All three buffers were filtered, degassed, and connected to the SV5-4 valve as follows: A1, equilibration buffer (50 mM sodium phosphate, 150 mM NaCl, pH 7.1); B1, elution buffer 1 (100 mM citric acid, pH 3.0); B2, elution buffer 2 (100 mM citric acid, 2 M guanidine, pH 3.0).

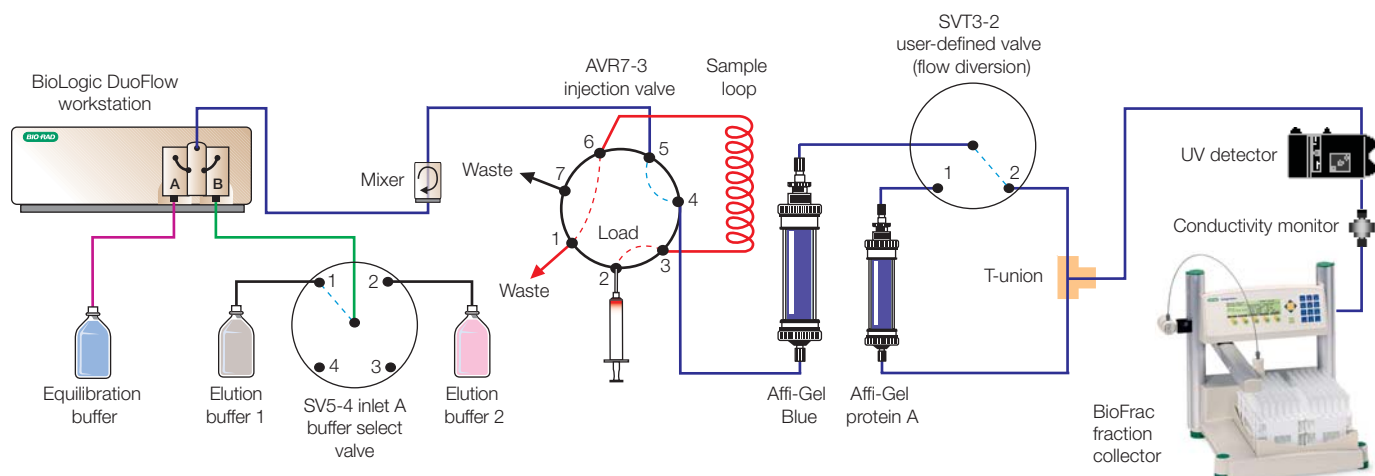


Fig. 1. Plumbing diagram of the BioLogic DuoFlow system used in this study.

Purification Protocol

A 40 ml (3.3 cm x 4.7 cm) and a 10 ml (2.3 cm x 2.4 cm) column were manually packed with Affi-Gel Blue and Affi-Gel protein A media, respectively. The AVR7-3 valve was set to load position, and the SVT3-2 valve was set to position 1 (Figure 1). Both columns were then equilibrated in series with 120 ml equilibration buffer, which corresponded to 3 column volumes (CV) of the Affi-Gel Blue column, at a flow rate of 3 ml/min. Next, 10 ml human male serum (Sigma-Aldrich Corp.) was loaded on a 10 ml injection loop and injected into the Affi-Gel Blue column through the AVR7-3 valve; flowthrough from the Affi-Gel Blue column flowed directly to the Affi-Gel protein A column. Unbound low-abundance proteins were then removed by washing both columns with 320 ml (8 CV) equilibration buffer. IgG was then eluted with 240 ml (6 CV) elution buffer 1. Once IgG was removed from the Affi-Gel protein A column, the SVT3-2 valve was switched to position 2, and albumin was eluted with 400 ml (10 CV) elution buffer 2. Following elution, the Affi-Gel Blue column was reequilibrated with 160 ml (4 CV) equilibration buffer. Then, the SVT3-2 valve was switched back to position 1, and the Affi-Gel protein A column was reequilibrated with 160 ml equilibration buffer.

In a second experiment, the 10 ml Affi-Gel protein A column was disconnected from the plumbing line and replaced with a 5 ml (1.8 cm x 2.0 cm) Affi-Gel protein A column. The same procedures described above were used except, due to the higher system pressure resulting from the narrower diameter of the smaller column, the flow rate throughout the entire procedure was decreased from 3 ml/min to 2 ml/min using the Edit All function of BioLogic DuoFlow software.

Fraction Collection and Analysis

The elution profiles from both experiments were monitored at 280 nm, and a series of 25 ml fractions was collected. To evaluate albumin and IgG removal, peak-containing fractions were analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) using both reducing and nonreducing conditions and Criterion™ 4–20% gradient Tris-HCl gels. Prior to SDS-PAGE analysis, 75 µl from each fraction was desalted using a Bio-Spin® 6 Tris column. The gel resulting from separation under nonreducing conditions was analyzed using a GS-800™ densitometer and Quantity One® 1-D analysis software.

Results

A human serum sample was loaded onto a BioLogic DuoFlow chromatography system configured to use both an Affi-Gel Blue and an Affi-Gel protein A column for removal of albumin and IgG, respectively (Figure 1). Since protein A media can be costly, the relative purification efficiency achieved using different amounts of this support was also examined. The chromatograms obtained following separation on a 40 ml Affi-Gel Blue column coupled with either a 10 ml or 5 ml Affi-Gel protein A column, as plotted by BioLogic DuoFlow software, are shown in Figure 2.

SDS-PAGE analysis of the peak-containing fractions is shown in Figure 3 and indicates that both albumin and IgG were substantially removed after the human serum sample was subjected to processing by the Affi-Gel Blue and Affi-Gel protein A columns. For quantitation, fractions were analyzed by SDS-PAGE using nonreducing conditions, which resulted in the migration of IgG as a single band (Figure 4). Analysis of the gel in Figure 4 revealed 94% and 90% removal of albumin and IgG by the 40 ml Affi-Gel Blue and 10 ml Affi-Gel protein A columns, respectively; substitution of the 10 ml Affi-Gel protein A column with the 5 ml column resulted in 95% and 83% removal of albumin and IgG, respectively (Table 1). These results confirm that the majority of albumin and IgG were successfully removed from the human serum sample.

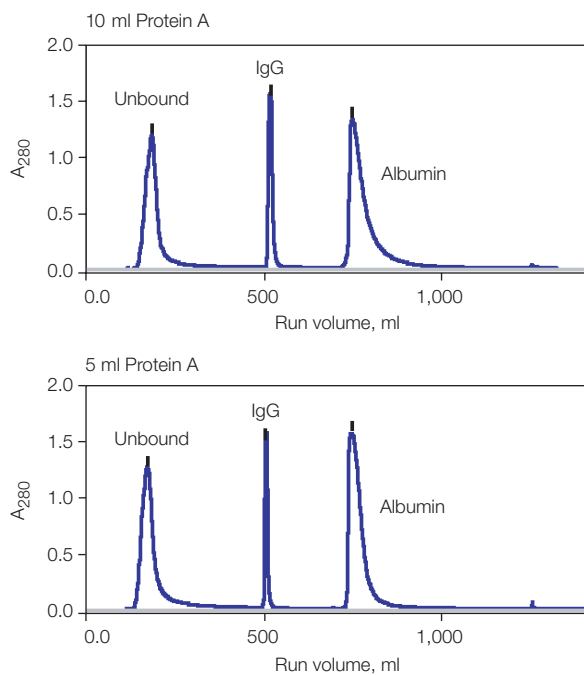


Fig. 2. Elution profiles showing removal of albumin and IgG from a human serum sample. Chromatograms plotted with BioLogic DuoFlow software are shown of separation on a 40 ml Affi-Gel Blue and a 10 ml (top panel) or 5 ml (bottom panel) Affi-Gel protein A column.

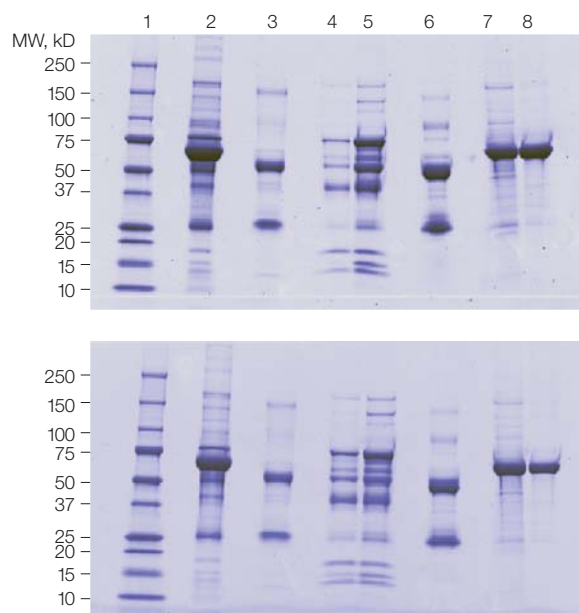


Fig. 3. SDS-PAGE analysis (reducing conditions) of fractions from Affi-Gel Blue and Affi-Gel protein A columns. Upper panel, results with 10 ml Affi-Gel protein A column; lower panel, 5 ml Affi-Gel protein A column. Both experiments were performed with a 40 ml Affi-Gel Blue column. Lane 1, Precision Plus Protein™ standards; lane 2, crude serum; lane 3, IgG standard; lanes 4 and 5, unbound proteins; lane 6, IgG elution; lanes 7 and 8, albumin elution.

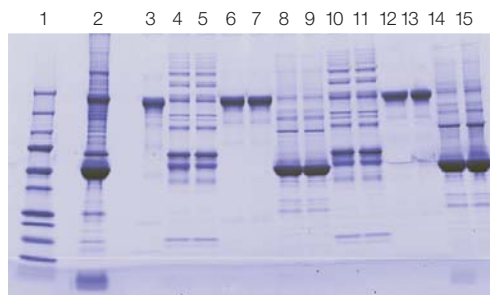


Fig. 4. SDS-PAGE analysis (nonreducing conditions) of fractions from Affi-Gel Blue and Affi-Gel protein A columns. Lane 1, marker; lane 2, crude serum; lane 3, IgG standard; lanes 4 and 5, unbound proteins from the 10 ml Affi-Gel protein A column; lanes 6 and 7, IgG elution from the 10 ml Affi-Gel protein A column; lanes 8 and 9, albumin elution from the 10 ml Affi-Gel protein A column; lanes 10 and 11, unbound proteins from the 5 ml Affi-Gel protein A column; lanes 12 and 13, IgG elution from the 5 ml Affi-Gel protein A column; lanes 14 and 15, albumin elution from the 5 ml Affi-Gel protein A column. This gel was analyzed by densitometry and Quantity One analysis software to generate the data shown in Table 1.

Table 1. Purification efficiency. Data were generated by densitometric analysis of the SDS-PAGE gel shown in Figure 4 (arbitrary units).

| | Unbound | Peak | Total Volume | % Removal |
|--|---------|---------|--------------|-----------|
| 40 ml Affi-Gel Blue, 10 ml Affi-Gel protein A columns | | | | |
| Albumin | 2.6664 | 39.3849 | 42.0513 | 93.66 |
| IgG | 1.2271 | 10.8973 | 12.1244 | 89.88 |
| 40 ml Affi-Gel Blue, 5 ml Affi-Gel protein A columns | | | | |
| Albumin | 2.5573 | 51.9654 | 54.5864 | 95.20 |
| IgG | 1.9732 | 9.8036 | 11.7890 | 83.16 |

Conclusions

The presence of high-abundance albumin and IgG in human serum masks many proteins of potential interest, especially those analyzed by 2-D electrophoresis. Affi-Gel Blue and Affi-Gel protein A media provide the high specificity and high degree of efficiency needed to meet the needs of large-scale removal of albumin and IgG from human serum. With the BioLogic DuoFlow chromatography system, separation with two media becomes a simultaneous, robust, and automated single-step process.

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