

An Efficient Alternative for Purification of Antibodies Using a Synthetic Liquid

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ABSTRACT

Antibodies are a valuable research tool with a wide array of uses ranging from the basis of quantitative assays to targeting activity of specific proteins. Researchers typically use Protein A or Protein G based resins for IgG purification from a variety of sources. The purification of antibodies using Protein A or Protein G has limitations, such as ligand leaching, poor performance with some IgG isotypes from some species, and prohibitive costs. In addition, elution of antibodies from Protein A or G resins require a low pH buffer that can cause antibody aggregation and loss of activity. The challenge for developing an alternative purification method is to incorporate the performance benefits of Protein A or Protein G resins while overcoming the disadvantages of these ligands.

Pall has developed a novel method for efficient antibody purification from source material containing albumin as a growth additive. This cost effective method offers good antibody capture regardless of class and species. The method uses two simple chromatography steps for achieving > 90% pure immunoglobulin molecules. An additional advantage to this method is that it produces a relatively higher elution pH for minimal antibody aggregation.

This new purification method is demonstrated using cell culture supernatant (CCS) for monoclonal mouse IgG. To improve antibody purification with MEP HyperCel™ alone, Blue Trisacryl® M resin is used to remove albumin. Blue Trisacryl M treated plasma or CCS is subjected to MEP HyperCel resin and the bound IgG is eluted at pH 5.0 after two simple wash steps. These resins are available in pre-packed columns that are reusable without any compromise on performance. The method provides a cost effective approach for attaining high purity IgG that can be used for any downstream application and is especially good for those applications sensitive to low levels of leached Protein A.

MATERIALS AND METHODS

- ▶ Blue Trisacryl M resin packed in 16 mm x 100 mm column with a 10 mL packed bed.
- ▶ MEP HyperCel resin packed in 5 mm x 200 mm column with a 4 mL packed bed.
- ▶ Monoclonal antibody (mAb) in hybridoma culture supernatant containing 8% FBS.
- ▶ 20 mM phosphate buffer saline (PBS), pH 7.2.
- ▶ 20 mM sodium acetate buffers at pH 5.5, 5.0, 4.0 and 3.0.

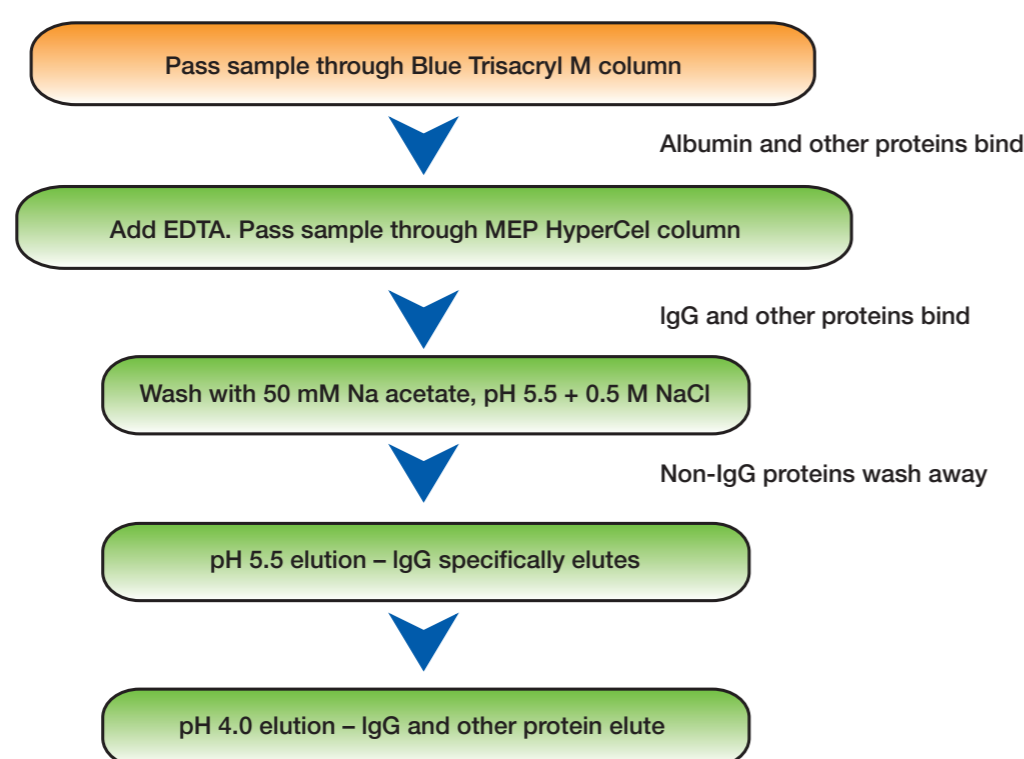
Depletion of Albumin using Blue Trisacryl M column

Approximately 25 mL of CCS is injected directly onto the Blue Trisacryl M column at a flow rate of 0.3 mL/min (residence time ~60 minutes). Flow through (FT) fraction depleted of albumin (BSA) containing IgG is collected. The column is stripped with PBS containing 3 M sodium chloride.

Purification of IgG Using MEP HyperCel Column

EDTA is added to the FT fraction from the Blue Trisacryl M column to a final concentration of 10 mM to prevent binding of transferrin resulting in higher purity of eluted IgG. The sample is then loaded on the MEP HyperCel column for IgG purification. The MEP load sample (~450 mg total protein) is injected at 0.2 mL/min (residence time = 4 minutes) on an MEP HyperCel column. FT fraction is collected for analysis. The column is washed first with 5 CV of 50 mM sodium acetate, pH 5.5 containing 0.5 M NaCl. Bound proteins are eluted with 20 mM sodium acetate buffer, first pH 5.5 and then with pH 5.0 and pH 4.0. The eluted fractions are immediately neutralized to pH 7.0 using 1.5 M Tris buffer. The column is stripped of remaining proteins with 5 CV of 20 mM sodium acetate, pH 3.0. A schematic representation of the entire procedure is shown in Figure 1.

Figure 1 Schematic of IgG Purification Blue Trisacryl M and MEP HyperCel Chromatography Resins



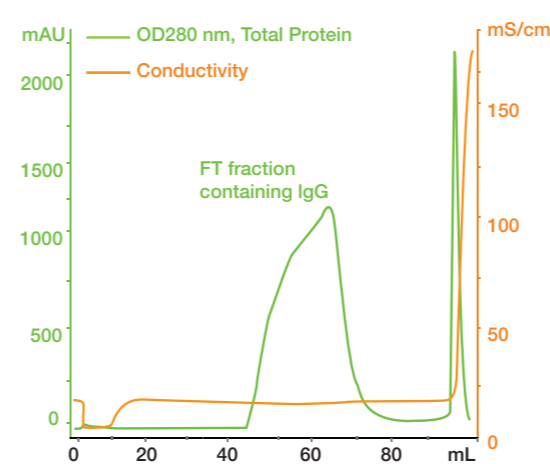
RESULTS

Human Antibody Purification Using Blue Trisacryl M and MEP HyperCel

Although MEP is selective for antibody, the high amount of BSA (8% FBS in cell culture medium) results in some co-purification with IgG. To significantly reduce the BSA, CCS is first passed through the Blue Trisacryl M column, which removes most of the BSA. The chromatogram from Blue Trisacryl M purification step (Figure 2a) shows removal of a significant amount of protein (blue trace, 2nd peak). The FT fraction from the Blue Trisacryl M column (Figure 2a, first peak), containing IgG and other plasma proteins, is used for IgG purification with MEP HyperCel resin.

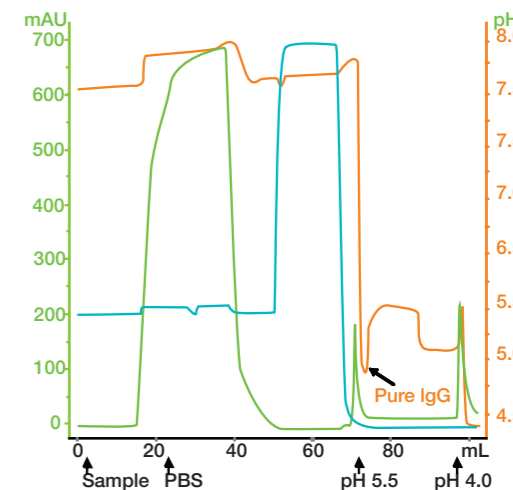
RESULTS (continued)

Figure 2a Albumin Depletion From Hybridoma CCS Using Blue Trisacryl M Resin



Chromatogram from Blue Trisacryl M column OD280 (total protein) in green and conductivity (salt) in orange. FT fraction (first peak) is collected for IgG purification. The column is stripped with PBS + 3M NaCl (second peak).

Figure 2b IgG Purification From Albumin Depleted Hybridoma CCS using MEP HyperCel Resin



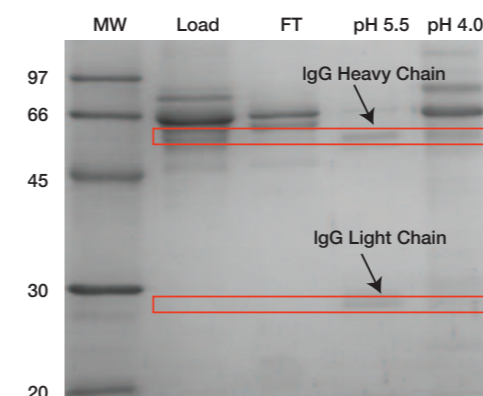
Chromatogram from MEP HyperCel column OD280 (total protein) in green, pH in orange, and conductivity (salt) in teal. FT fraction from Blue Trisacryl M column is loaded on MEP HyperCel column. Buffer changes are indicated with arrows.

The MEP HyperCel column chromatogram (Figure 2b) shows the bulk of protein in the FT fraction. This contains proteins other than IgG, mostly residual BSA (Figure 3, MEP FT). Some BSA weakly binds to MEP HyperCel resin via hydrophobic interaction and can be separated from bound mAb by differential pH elution depending on the mAb pI. Elution at pH 5.5 under low salt conditions removes most of the IgG (tall blue peak) from the column due to electrostatic repulsion (MEP becomes partially charged). Tightly bound proteins and residual IgG are eluted at pH 4.0 (last blue peak, Figure 2b).

Excellent IgG Purity Demonstrated by SDS-PAGE

SDS-PAGE analysis of MEP HyperCel load sample (Blue Trisacryl M FT fraction) and MEP HyperCel fractions is shown in Figure 3. Total protein information for these fractions is seen in Table 1. The mAb concentration in the hybridoma CCS is 25 µg/mL which makes the visualization by SDS-PAGE very difficult (Load). Residual BSA in the load and FT fractions is seen at ~66 kDa. Although MEP HyperCel resin is not as selective as Protein A or G, in combination with Blue Trisacryl M resin, the mAb purity is quite high (pH 5.5 lane). The eluate fraction at pH 4.0 contains other proteins and possibly some free IgG heavy chain, but not light chain, suggesting that intact IgG is only present in the pH 5.5 fraction. The protein analysis of the MEP HyperCel column fractions is consistent with OD280 (Figure 2b) and SDS-PAGE results. Most of the protein passes through the column, and the mAb is relatively more concentrated in the pH 5.5 eluate fraction as compared to load fraction.

Figure 3 SDS-PAGE Analysis of MEP HyperCel Chromatography Fractions



Reduced 15% SDS-PAGE (0.5-24 µg total protein). MW = molecular weight markers, Load = sample loaded onto MEP column, FT = FT fraction, pH 5.5 and 4.0 = sample from elution at indicated pH. Red boxes indicate location of IgG heavy and light chains.

Table 1 Protein Analysis of MEP HyperCel Column Fractions

MEP HyperCel Fraction	Total Volume	Protein Concentration	Total Protein
Load	25 mL	18 mg/mL	450 mg
Flow Through	32 mL	11 mg/mL	353 mg
pH 5.5 Eluate	5.0 mL	0.8 mg/mL	4 mg
pH 4.0 Eluate	4.0 mL	5.35 mg/mL	21.4 mg

Protein determinations done using the Bradford assay.

DISCUSSION

The affinity properties of Cibacron® Blue dye for albumin and other plasma proteins are well established. The Blue Trisacryl M column, which utilizes the Cibacron blue ligand, effectively removes albumin from the CCS, thereby improving MEP HyperCel resin performance. The combination of these resins has yielded the following results:

- ▶ A new two step method for the purification of IgG is fast and relatively simple.
 - In the first column run (Blue Trisacryl M resin) IgG merely passes through the column.
 - In the second column, the IgG is actually purified.
- ▶ Purity of IgG achieved is > 70%.
- ▶ Cost effective: The total cost of Blue Trisacryl M and MEP HyperCel resins is less than half the price of Protein A resin required to purify an equivalent amount of IgG.

MEP HyperCel resin binds antibody through hydrophobic and affinity interactions. Elution is accomplished by charge repulsion which is dependent on the pKa of the MEP ligand (4.8) and target molecule pI. Elution based on charge repulsion from MEP HyperCel resin is best accomplished at a pH of < 5.5. Additionally, a decrease in salt concentration in elution buffers reduces the hydrophobic interaction of mAb with the MEP ligand. MEP HyperCel resin has many advantages as compared to Protein A or G. Protein A leaching into the purified antibody can lead to problems for some downstream applications. Efficient capture of IgG even at lower concentrations often seen with CCS samples eliminates the need for the initial concentration step typically required for Protein A purification methods. Additionally some monoclonal antibodies are particularly sensitive to low pH elution conditions used with Protein A and G, typically ~3.0-2.5. Loss of antibody activity, often resulting from low pH induced aggregation, can be largely eliminated with the less harsh elution pH of 4.0-5.5. The structure of MEP ligand also allows for binding of all IgG isotypes from all species tested thus far, as well as some of the other Ig proteins. Taken together, these performance characteristics make MEP HyperCel resin a good choice for antibody purification. As specificity of MEP HyperCel is different than Protein A or G, an additional step of Blue Trisacryl M separation is recommended for antibody samples containing albumin.