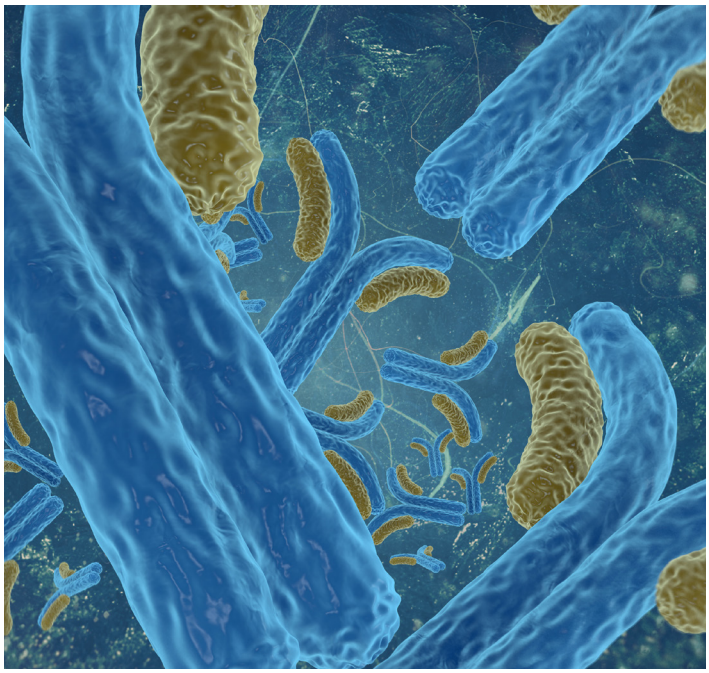
ExpiCHO Expression System

A guide to optimizing protein A purification of monoclonal antibodies

Introduction

The Gibco™ ExpiCHO™ Expression System enables high-titer production of a broad range of recombinant proteins. Since the ExpiCHO kit components have been optimized for high-yield expression in CHO cells, some differences in supernatant clarification and protein purification are to be expected compared to existing transient expression systems utilizing HEK 293 cells (e.g., Gibco™ Expi293™ Expression System). In some instances, vendor-recommended protein A purification protocols have been shown to be suboptimal for use with ExpiCHO supernatant, leading to a faint yellow discoloration of protein A columns over time. Here, an example of an optimized protocol for supernatant clarification and protein A purification is presented for the ExpiCHO Expression System.



Materials

- Thermo Scientific™ Nalgene™ Rapid-Flow™ Sterile Disposable Filter Units with PES Membrane, Cat. No. 166-0045 (0.45 µm filter)
- Thermo Scientific™ Nalgene™ Rapid-Flow™ Sterile Disposable Filter Units with Nylon Membrane, Cat. No. 150-0020 (0.22 µm filter)
- Applied Biosystems™ POROS™ MabCapture™ A Media, Cat. No. 4374731; or MabSelect™ SuRe resin, Cat. No. 11-0034-93 (GE Healthcare)
- ÄKTA pure L system, Cat. No. 29-0182-24 (GE Healthcare)

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Methods

Supernatant clarification

Note: For best results, clarification by centrifugation and filtration should be performed prior to freezing ExpiCHO supernatants.
Clarify ExpiCHO supernatants as follows:

- Centrifuge supernatant at ≥3,000 x *g* for 30 minutes.
- If using standard bottle-top vacuum filters, filtration of the supernatant using a 0.45 µm filter followed by a 0.22 µm filter is recommended (Cat. No. 166-0045 and 150-0020).
- Other filters types (e.g., depth filters) may provide improved filtration compared to standard bottle-top vacuum filters.
- For larger-volume filtration, one Pall Seltz PDK5 dual-layered depth filter capsule with 0.0022 m^2 surface area (Supracap™ 50 Depth Filter Capsules, Pall Cat. No. SC050PDK5) is recommended. For instance, 1 L of clarified supernatant can be processed at 8 mL/min with a processing time of 2 hr. The use of multiple capsules (e.g., two connected in parallel) will reduce processing times further.

Column loading conditions

It is critical to load the optimal amount of monoclonal antibody (mAb) to achieve maximal purity and to maintain long-term consistency of resin performance. Because the ExpiCHO Expression System expresses many proteins at significantly higher levels than other expression systems, the amount of protein load per volume of resin should first be determined as follows:

- Determine the antibody titer in the crude cell culture supernatant
- Verify the dynamic binding capacity (DBC) of the resin at 5% breakthrough volume (C5)
- Load 80% of the C5 of the resin

Purification parameters

For all experimental conditions, the ÄKTA pure L chromatography system was used with linear velocity set to 150 cm/hr, and a residence time of 4 min. Protein A resin was equilibrated with 10 column volumes (CVs), and 28 mg of mAb per mL of resin was loaded. The column was then washed with 10 CVs of equilibration buffer, and elution was performed with 5 CVs of elution buffer. Finally, the column was re-equilibrated to return it to neutral conditions before sanitization for 10 min with 0.1 N sodium hydroxide. Before storage, the column was returned to neutral pH with equilibration buffer and then stored in 20% ethanol at 4°C.

Experimental design

Vendor-recommended (suboptimal) conditions for protein A purification are shown in Table 1.

A custom design of experiment (DoE) model was made to determine factor significance and interactions during protein A purification of a human IgG1 (pI 8.68), with the end goal to maximize both monoclonal antibody recovery and impurity removal (Table 2).

Table 1. Vendor-recommended protein A purification protocol.

Test condition	Factors
Binding buffer type	Sodium phosphate
Binding buffer concentration	25 mM
Binding buffer pH	7.4
Elution buffer type	Citrate, pH 3.0
Elution buffer concentration	100 mM
Addition of NaCl to binding or elution buffer	Binding buffer only
NaCl concentration	150 mM

Table 2. DoE test condition ranges.

Test condition	Factors
Binding buffer type	Tris or sodium phosphate
Binding buffer concentration	25–100 mM
Binding buffer pH	7.1–7.4
Elution buffer type	Citrate or acetate, pH 3.0
Elution buffer concentration	25–100 mM
Addition of NaCl to binding or elution buffer	Binding or elution buffer
NaCl concentration	25–150 mM

Results

Individual DoE models were generated to obtain optimized conditions for both monoclonal antibody recovery and impurity removal. These two models were then merged to generate a final recommended purification protocol that maximized both recovery and impurity removal (Table 3). The data indicated that switching from the vendor-recommended condition of 25 mM sodium phosphate/150 mM NaCl for the binding buffer and 100 mM sodium citrate, pH 3.0 for the elution buffer, to the DoE-optimized conditions of 25 mM Tris/25 mM NaCl in the binding buffer and addition of at least 50–100 mM NaCl to the elution buffer (100 mM of either sodium citrate or sodium acetate, pH 3.0; Table 3), was sufficient to eliminate the impurity that led to the yellow discoloration of the protein A resin. Specifically, resolution of the impurity leading to the discoloration was achieved in the optimized condition (Figure 1, arrow), whereas the distinct resolution of the impurity was not observed under the vendor-recommended condition (Figure 2).

Table 3. Optimized conditions for protein A purification.

Test condition	Optimized factors
Binding buffer type	Tris
Binding buffer concentration	25 mM
Binding buffer pH	7.1 or 7.4
Elution buffer type	Acetate or citrate, pH 3.0
Elution buffer concentration	100 mM
Addition of NaCl to binding or elution buffer	Binding or elution buffer
NaCl concentration	25 mM for binding buffer and 50–100 mM for elution buffer

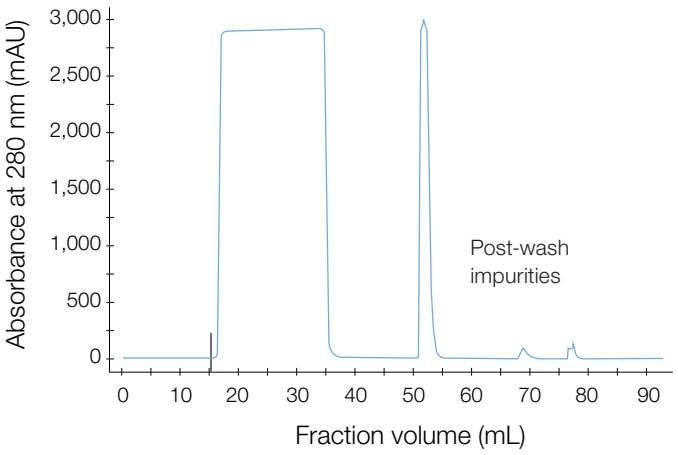

Figure 1. ExpiCHO protein A purification using optimized conditions.

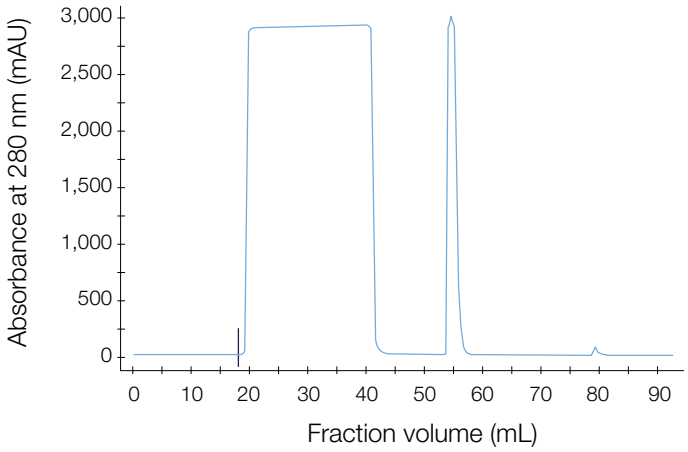

Figure 2. ExpiCHO protein A purification using vendor-recommended conditions.

Conclusion

This application note provides recommendations on supernatant clarification, loading conditions, and buffers for protein A purification of ExpiCHO supernatants. The conditions determined in this study provided optimal product recovery and impurity removal. These conditions may be used as guidance for further optimization of purification conditions specific to particular proteins generated in the ExpiCHO Expression System.

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