Praesto® Protein A resins – a short user instruction

Introduction

Monoclonal antibodies (MAbs) are captured directly from clarified cell culture supernatant using Protein A affinity resins. Although there are a number of different buffers and process conditions mentioned in the scientific literature, the outcome with respect to yield and purity is, in general, acceptable for most protocols.

This short instruction should be considered as a starting point and will work well for the majority of CHO cell expressed human MAbs. However, it is important to acknowledge that all MAbs are unique and will differ both in chemical and physical characteristics. In particular, optimization of the intermediate wash step is a good opportunity to further reduce host cell protein (HCP) levels.

The Protein A step is followed by two (sometimes one), additional chromatography steps to achieve sufficient purity and virus clearance before final formulation.

Suggested purification protocol

The table below summarises suggested buffers and process steps in a "generic" MAb purification protocol. Ideally the elution buffer should be designed allowing a simple titration to condition the sample for the subsequent step. The suggested buffer volumes below are dimensioned for large columns. In a small-scale lab system, the column to system ratio is typically less optimal, hence why it is recommended to increase the wash and equilibration volumes.

Adsorption and desorption in a bead is mainly a diffusion driven process. Thus, a high flow rate would have to be compensated with larger buffer volumes to achieve the same contaminant clearance (HCP), compared to what would be the result at a lower flow rate. We recommend a flow rate corresponding to a residence time (Rt) of between 4-6 minutes for the Praesto® protein A resins - although they they have a wider operating window depending upon the application.

Step		
0	ONLY after storage or before first use. Run a blank run including CIP to wash out storage buffer, minimize risk for biological contamination, and elevated Protein A leakage levels.	
1	Equilibration buffer	3 CV 20 mM Na-phosphate, 0.15 M NaCl, pH 7.0-7.4
2	Sample Load	70-90% of the dynamic binding capacity (DBC)
3	Wash 1	2 CV Equilibration buffer
4	Wash 2	2 CV 20 mM Na-phosphate, 1.0 M NaCl, pH 8.0
5	Wash 3	2 CV 20 mM Na-phosphate, pH 6.5
6	Elution and regen.	5 CV 0.1 M acetic acid (By UV watch or pre-determined volume)
7	CIP	3 CV 0.1 M NaOH (at a flow rate resulting in 15-30 min. contact)
8	Repeat from step 1 or transfer to storage buffer (20 % ethanol, 50 mM sodium acetate, pH 5.0, alternatively 2 % benzyl alcohol)	

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Comments:

Elution and regeneration

There is no clear rational in choosing between acetate, citrate, or glycine. Acetate and citrate are the most commonly used buffers. Acetic acid has little buffering capacity at the elution pH, and can be easily titrated without increasing conductivity too much for the following step, which is commonly a cation exchange.

0.1 M acetic acid is a good starting point and typically results in an elution pool of around pH 3.6, which is sufficient for acid virus inactivation (but be aware, that the preceding buffer and the elution pool volume will significantly impact the pH of the elution pool).
If 0.1 M acetic acid is used for elution, extending the elution buffer with 2-3 CV's after the Mab is eluted, is sufficient for regeneration.

Citrate has buffering capacity over a wide pH range (3-7), and is preferable if it is important to have a very specific elution buffer pH - (but be aware that the preceding buffer and the elution pool volume will impact the pH of the elution pool).

Typical concentrations used are 10-100 mM. In both cases it is not uncommon to see precipitation after pH adjustment, however it is rarely the target MAb that precipitates. The precipitate is easily removed by a depth filter + 0.22 µm filter.

Intermediate wash (wash 2) - while the Mab is bound to the Protein A resin it is common to introduce an intermediate wash step. There are different strategies but in principle any shift from the sample loading conditions with respect to conductivity and pH will lower HCP levels in the elution pool. There are published methods including solvents or detergents, however such additives have to be assayed to show the removal, which can be difficult.

Praesto® resins are available in OPUS pre-packed formats, both to aid in optimization and verification during screening but also to support with manufacturing.

Cleaning In Place (CIP)

A basic protocol for CIP is 15 minutes, 0.1 M NaOH (adjust flow rate to match 15 minutes contact time). However, for maximizing life length one could consider a milder CIP between cycles, and a higher concentration between campaigns.

Conclusion

This short instruction provides a starting point for purification of most common MAbs. For subsequent polishing steps, Purolite® provides a wide range of high performance agarose-based ion exchange resins.

Ordering Information

To place your order, simply contact us via email, or telephone using the information at the base of this sheet.

If you wish to discuss your purification challenges with a specialist, we have dedicated experts on-hand, across the globe to provide knowledgeable, same day technical assistance.

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