MAb capture step development using MabSelect SuRe™ LX

Introduction

In monoclonal antibody (MAb) purification two or three chromatography steps are commonly used to obtain the desired quality of pure antibody. Typically, the chromatography media (resins) used for the three-step process are protein A followed by cation and anion exchangers, and for the two-step process, protein A and Capto™ adhere (multimodal). All MAbs have some similar properties that allow a platform approach. Please note that platform does not mean identical processes, it just means that some aspects of the process do not need to be developed from scratch, as past experience with other MAbs can be leveraged.

MabSelect SuRe LX is a protein A-based medium that has outstanding capacity at longer residence times, is designed for high titer processes, and withstands rigorous and cost-effective cleaning-in-place (CIP) protocols (i.e., 0.1-0.5 M NaOH). This quick start guide provides a starting point that may be employed using MabSelect SuRe LX for purification of the majority of MAbs produced in mammalian cells.

Simplified process development

Table 1 provides a summary of a general guide for process development with MabSelect SuRe LX.

Load preparation—The load sample must be clarified prior to loading onto the column. A sterile filter should be used and an adsorptive depth filter is typically placed before the sterile filter. The chromatography run should be carried out as soon as possible after the cell culture harvest. If the cell culture harvest needs to be stored before the run, it should be sterile filtered and kept at 4˚C or frozen if feasible.

Table 1. Outline of typical methods employed in a capture step using MabSelect SuRe LX

Basic set up

System	System control	Medium	Column	Bed height (cm)	CV (mL)
ÄKTA™ avant 25 or 150	UNICORN™ 6 or higher	MabSelect SuRe LX	Tricorn™ 10/200	20	15.71*

Chromatography method for the capture step with MabSelect SuRe LX

Step	Volume or time	Buffer composition	Residence time, min (Linear flow rate, cm/h)
0. ONLY after storage—Equilibration	3 CV	20 mM Na-phosphate, 0.15 M NaCl, pH 7.4	7.5 (160)
1. Equilibration	0.25 CV	20 mM Na-phosphate, 0.15 M NaCl, pH 7.4	3.4 (350)
2. Sample loading	70% to 80% of 10% breakthrough (BT)	As required	6.0 (200 cm/hr) Longer residence time → Higher binding capacity
3. Wash 1	1.5 CV 3.5 CV	20 mM Na-phosphate, 0.5 M NaCl, pH 7.4	6.0 (200) 3.4 (350)
4. Wash 2	1 CV	50 mM Na-phosphate, pH 6.0	3.4 (350)
5. Elution	Controlled by a UV watch or at predetermined volumes	50 mM acetate, pH 3.5	3.4 (350)†
6. Strip	2 CV	100 mM acetic acid, pH 2.9	3.4 (350)
7. CIP	3 CV = 15 min	0.1-0.5 M NaOH	5 (240)
8. Re-equilibration	3 CV	20 mM Na-phosphate, 0.15 M NaCl, pH 7.4	3.4 (350)
9. ONLY after last run—Storage	4 CV	20% ethanol	7.5 (160)

Note: can increase flow rate to as high as 500 cm/h for steps other than the load step

* Column packing should be done correctly—see MabSelect SuRe LX product instructions for more details (packing factor 1.15).

† Increased flow rate during elution will lead to increased pool volume.

Simplified process development (cont.)

Run 0—Blank run - Should be performed prior to the first cycle on new MabSelect SuRe LX medium to remove non-covalently immobilized ligand, and thus decrease the ligand leakage during chromatography. All phases in the chromatography method listed in Table 1 should be used, with two alterations: (1) the equilibration buffer should be used during the load phase (i.e., no protein load); and (2) the elution phase in the blank run should be set to 3 CV and not controlled by a watch function.

Run 1—Load conditions - The next run should be done to determine the dynamic binding capacity (DBC) of the column for your specific MAb at 6.0 min residence time. Follow the procedure in Table 1 and overload the column up to 65 g/L, collect the fractions in flowthrough, then determine the MAb concentration in the flowthrough pools and calculate the 10% breakthrough.

Run 2—Set load conditions to 70% to 80% of 10% breakthrough (BT) and follow all steps in Table 1. The process used here may be locked if this run leads to acceptable purity, quality, and yield levels.

Step durations—All the step durations mentioned here are only indicative - the actual step durations may be shortened if the chromatograms and fraction data in the specific step warrant it.

Elution conditions—Various elution conditions, instead of acetate, may be considered, such as citrate buffer (10–100 mM), or glycine. When optimizing elution conditions for better impurity clearance, determine the highest pH that allows efficient desorption of antibody (this may, however increase pool volumes). Alternatively, design the elution condition to match the pH required for virus inactivation, as discussed below.

Analysis—Purity and quality must be analyzed for the protein A step after titrating up to load conditions for the next step and filtering through a sterile filter. Additionally, purity and quality must be analyzed after the two or three chromatographic steps based on the platform process. If this process leads to low yield or high host cell protein (HCP) in the elution pool, further development must be done as the wash and elution conditions will need to be optimized.

Virus inactivation—The pH in the elution pool should be kept at a pH of 3.6 or lower for at least 30 min for appropriate virus inactivation. If the elution pool has a higher pH it needs to be titrated by addition of acid or decreased through further optimization of the elution buffer volume. After virus inactivation, the pH must be immediately adjusted to match loading conditions for the next step (e.g., pH 5-6 for cation ion exchangers or pH 6-8 for Capto adhere) by addition of 0.1 M NaOH. Precipitation may occur during elution and commonly occurs after pH titration and subsequent to the low pH virus-inactivation. It may be assumed that the precipitate contains mainly lipids and only trace-amounts of MAb, HCP, and protein A. This precipitate can be safely removed by filtration using a sterile filter but note that correct filter sizing must be done; filters typically used in manufacturing are usually large enough for this step.

Further optimization

To optimize the process conditions for scale up, we recommend using high-throughput process development (HTPD). During HTPD, a large number of conditions (for binding, elution, and wash steps) can be evaluated in a short amount of time using PreDictor™ 96-well filter plates (1,2). Information gained during HTPD may be used to set the parameters for a robustness study at lab scale before scaling up to pilot plant and manufacturing. This method is especially useful for the quality by design (QbD) approach.

Conclusions

This quick start guide provides a starting point that may be employed using MabSelect SuRe LX for purification of the majority of MAbs produced in mammalian cells. The information provided is based on previous experience.

For further assistance with step or process development, please contact your local GE Healthcare sales representative or GE Healthcare's Fast Trak organization.

References

1. Handbook: High-thoughput Process Development with PreDictor Plates, GE Healthcare, 28-9403-58 (2009).
2. Application note: High-throughput screening and optimization of a Protein A capture step in a monoclonal antibody purification process, GE Healthcare, 28-9468-58 (2009).

Additional reading

Instruction: MabSelect SuRe LX, GE Healthcare, 28-9765-00 (2011).

Data file: MabSelect SuRe LX, GE Healthcare 28-9870-62 (2011).

Application note: MabSelect SuRe—studies on ligand toxicity, leakage, removal of leached ligand, and sanitization, GE Healthcare, 11-0011-64 (2004).

Application note: High-throughput process development for design of cleaning-in-place protocols, GE Healthcare, 28-9845-64 (2010).

Application note: Dynamic binding capacity study on MabSelect SuRe LX for capturing high-titer MAbs, GE Healthcare, 28-9875-25 (2011).

Application note: Lifetime performance study of MabSelect SuRe LX during repeated cleaning in place, GE Healthcare 28-9872-96 (2011).

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