MabSelect SuRe pcc

AFFINITY CHROMATOGRAPHY

MabSelect SuRe™ pcc affinity chromatography medium (resin) offers exceptional capacity at short residence time, making it well-suited for applications requiring fast mass transfer such as MAb capture in a continuous process.

The alkali-stabilized, protein A-derived ligand gives the medium greater stability than conventional protein A-based media under the alkaline conditions used in cleaning-in-place (CIP) protocols. Consequently, the possibility of cleaning with cost-effective reagents such as sodium hydroxide (NaOH) will improve process economy. In addition, the use of NaOH for cleaning efficiently inactivates bacteria, mold, and yeast, with improved product quality as a result.

The base matrix of MabSelect SuRe pcc combines a porosity optimized for MAbs with a rigidity that delivers good pressure/flow properties. These features provide the ability to run the medium at high flow rates, thereby increasing productivity of continuous capture steps. The small bead size also creates possibilities for high-resolution purification, for example, for purification of bispecific antibodies.

MabSelect SuRe pcc is part of Cytiva's program for custom designed media and is available in bulk as well as in prepacked columns (Fig 1).

Key benefits of MabSelect SuRe pcc include:

- Exceptional binding capacity (e.g., ~ 60 g IgG/L medium at 2.4 min residence time)
- High productivity through capture of large mass of MAbs in a short period of time
- Highly concentrated elution pools for operating flexibility and small unit operations
- Allows cost-effective cleaning with 0.1–0.5 M NaOH over hundreds of purification cycles
- High ligand stability reduces ligand leakage
- Generic elution conditions for different MAbs, enabling platform purifications

Fig 1. MabSelect SuRe pcc affinity chromatography medium offers the rapid mass transfer required for high-productivity MAb capture in continuous chromatography.

Medium characteristics

Optimized for continuous chromatography applications

In continuous chromatography, cost of goods sold (COGS) can be reduced by improved utilization of the chromatography medium (1). However, successful implementation of continuous chromatography puts special demands on the medium.

The MabSelect™ family, comprising MabSelect, MabSelect Xtra™, MabSelect SuRe, and MabSelect SuRe LX chromatography media, was developed for high-capacity MAb capture at process scale. To enable the high mass transfer required for continuous chromatography, MabSelect SuRe pcc was further developed from MabSelect SuRe LX. The high dynamic binding capacity (DBC) for MAbs at short residence time contributes to increased productivity by reducing total time required for column load operations. Comparative studies with MabSelect SuRe LX show that higher capacities can be achieved at shorter residence times with MabSelect SuRe pcc (Fig 2).

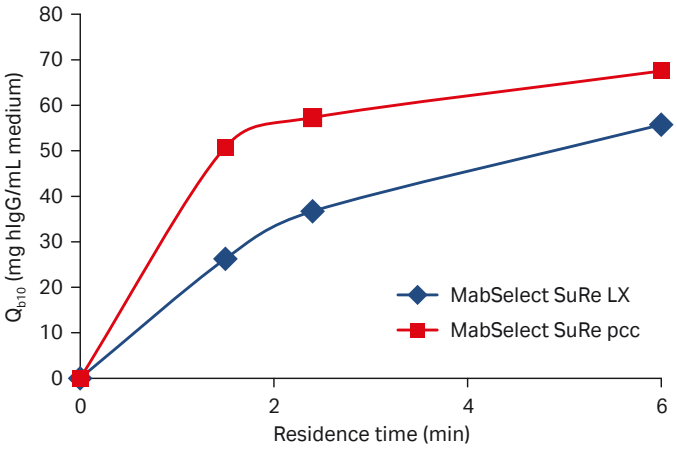

Fig 2. DBC at 10% breakthrough (Q_{b10}) for a human polyclonal IgG antibody (hIgG) as a function of residence time.

Table 1 lists the main characteristics of MabSelect SuRe pcc medium.

Table 1. Main characteristics of MabSelect SuRe pcc

Matrix	High-flow agarose
Ligand	Alkali-stabilized protein A-derived (*E. coli*)
Ligand coupling	Single point
Coupling chemistry	Epoxy
Average particle size ($d_{50,volume}$)*	50 µm
Dynamic binding capacity†	Approximately 60 mg human IgG/mL medium at 2.4 min residence time
Mobile phase velocity‡	Minimum 250 cm/h at 3 bar
pH stability (operational)§	3–12
CIP stability (short term)¶	2–13.7
Chemical stability	Stable in aqueous buffers commonly used in protein A chromatography
Cleaning-in-place stability	0.1–0.5 M NaOH
Delivery conditions	20% ethanol

* $d_{50,volume}$ is the median particle size of the cumulative volume distribution.
† Typical value. Determined at 10% breakthrough by frontal analysis at a mobile phase velocity of 250 cm/h in a column with a bed height of 10 cm, residence time is 2.4 min. Residence time is equal to bed height (cm) divided by nominal fluid velocity (cm/h) during sample loading. Nominal fluid velocity is equal to volumetric flow rate (mL/h) divided by column cross sectional area (cm^2).
‡ Determined in an AxiChrom™ 1000 column, bed height 20 cm, operating pressure less than 3 bar. Measured using process buffers with the same viscosity as water at 20°C.
§ pH interval where the medium can be operated and stored for longer periods of time without significant change in function.
¶ pH interval where the medium can be subjected to CIP or sanitization-in-place without significant change in function.

High flow rates at bed heights typical for continuous chromatography

MabSelect SuRe pcc medium is based on a high-flow agarose base matrix with average bead size of 50 µm. Good pressure/flow properties, combined with an optimized porosity, make the medium well-suited for MAb capture steps in continuous processes. The smaller bead size compared with MabSelect SuRe LX (85 µm) increases system pressures and limits the window of operation of MabSelect SuRe pcc (Fig 3). In continuous chromatography, however, the lower flow velocity of MabSelect SuRe pcc is counterbalanced by the use of smaller columns with shorter bed heights.

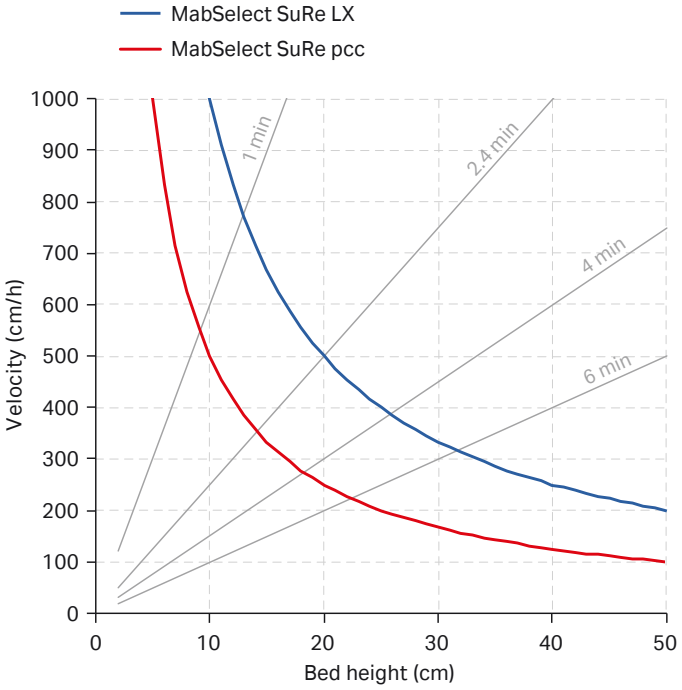

Fig 3. Window of operation for MabSelect SuRe pcc versus MabSelect SuRe LX. Pressure limits are assumed to be 3 bar. At 2.4 min residence time, for example, MabSelect SuRe pcc can be packed in beds up to 14 cm in height, whereas MabSelect SuRe LX can be packed at 20 cm bed height.

High alkaline stability extends working lifetime

Affinity media based on native or recombinant protein A ligands are highly sensitive to NaOH, which prevents its use in CIP procedures. Resistance to higher NaOH concentrations (e.g., 0.5 M NaOH) can be critical to the successful implementation of the medium in continuous chromatography, where consistent cycle-to-cycle performance and low contamination risk are important. Table 2 shows the effectiveness of 0.5 M NaOH for controlling bioburden.

In MabSelect SuRe products, the ligand is alkali-stabilized to allow for cost-effective CIP using 0.1 to 0.5 M NaOH over many purification cycles. The MabSelect SuRe ligand was developed by engineering one of the IgG-binding domains of protein A. Particularly alkali-sensitive amino acids were identified and substituted with more stable ones. The final construct is a tetramer of the engineered domain with a C-terminal cysteine, which enables single-point attachment to the base matrix. The resulting highly pure ligand is immobilized to the agarose matrix via a chemically stable thio-ether linkage. Figure 4 shows DBC over repeated CIP cycles for MabSelect SuRe LX, which employs the same ligand coupling chemistry and ligand density as MabSelect SuRe pcc.

Compared with protein A, the MabSelect SuRe ligand shows improved stability to proteases, which minimizes ligand leakage and further contributes to an extended working lifetime of the medium. In addition, the reduced affinity of the protein A-derived ligand of MabSelect SuRe pcc for the Fab region of antibodies enables more generic elution conditions to be employed in MAb processes (4).

Table 2. Bioburden control with 0.5 M NaOH to effectively inactivate bacteria, mold, and yeast (3)

Organism	Type	Time*
C. albicans	yeast	1 h
A. niger	mold	1 h
P. aeruginosa	bacteria gram -	1 h
S. aureus	bacteria gram +	1 h

* For reduction to below detection limit of < 3 organisms/mL

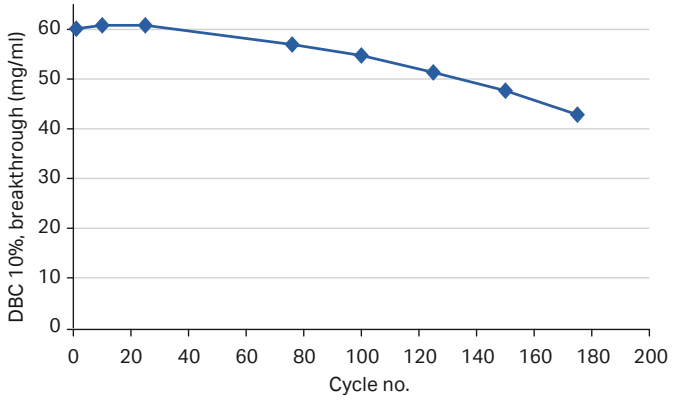

Fig 4. DBC results obtained with MabSelect SuRe LX over 175 CIP cycles using 0.5 M NaOH (15 min per cycle). As shown, > 90% of the initial DBC is retained after more than 100 cycles (2).

Application

Basic principles of periodic countercurrent chromatography (PCC)

PCC employs three or more chromatography columns to create a continuous capture step. In a PCC setup, columns are switched between the loading step and non-loading steps comprising column wash, elution, cleaning in place (CIP), and equilibration (Fig 5). At a predefined level of breakthrough, the primary column in the loading zone is disconnected from the loading zone and the load is redirected to the next column. In parallel, the disconnected, saturated column will be washed, eluted, and regenerated. Figure 5 gives an overview of a three-column PCC (3C PCC) operation.

The use of PCC maximizes utilization of the available capacity of the chromatography medium by enabling loading to much higher breakthrough levels (Fig 6).

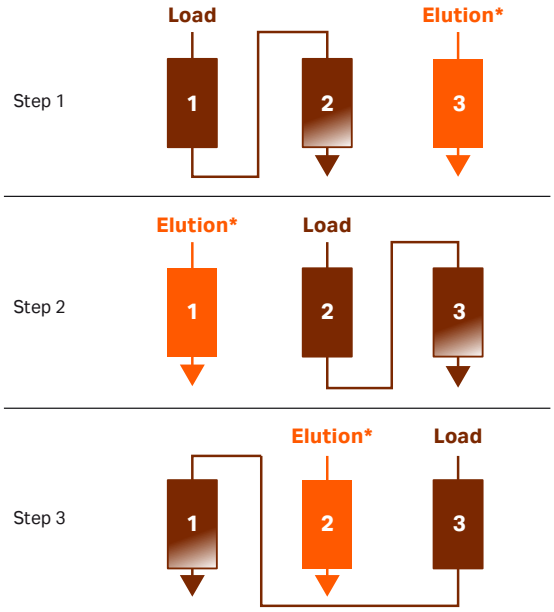

Fig 5. The principle of 3C PCC. Step 1: column 1 and 2 are loaded with clarified cell culture supernatant (brown). Step 2: column 1 has reached the determined level of breakthrough and column 2 becomes the first column in the loading zone. In step 2, column 3 becomes the second column in the loading zone, while column 1 is subjected to wash, elution, strip, CIP, and re-equilibration (orange). Step 3: column 2 has been loaded to the determined level of breakthrough and is disconnected from the loading zone and column 3 becomes the first column in the loading zone. In step 3, column 1 is now ready for the next cycle and becomes the second column in the loading zone. This procedure is repeated in a cyclic manner to achieve a continuous operation.

* Elution phase in this figure includes wash, elution, strip, CIP, and re-equilibration.

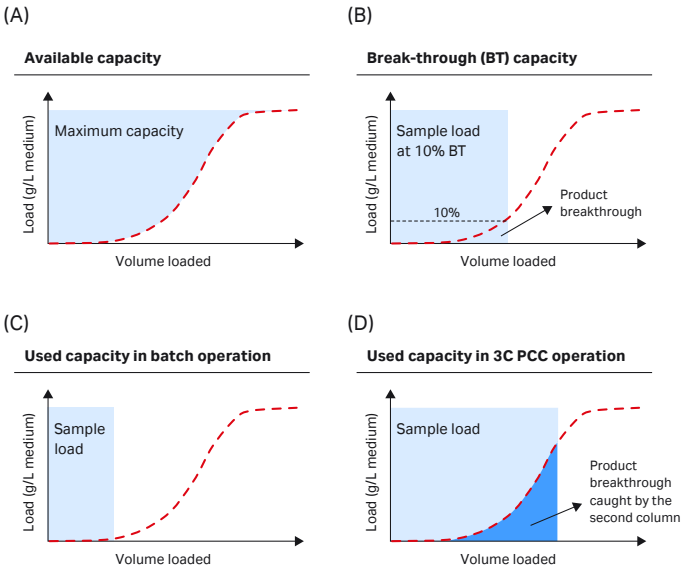

Fig 6. Capacity utilization for standard vs PCC. (A) Total available capacity of a chromatography medium. (B) The capacity typically measured during process development experiments. (C) The capacity typically utilized in manufacturing after adding safety factors. (D) Typical capacity utilized when implementing PCC. Note that product breakthrough is captured by the next column in the loading zone.

MabSelect SuRe pcc in MAb capture

Cell culture harvest containing MAb at a titer of 4.0 g/L was filtered through an ULTA™ Pure HC 0.22 µm capsule filter.

MabSelect SuRe pcc medium was packed in 5 mL HiTrap™ columns. An overview of the process conditions for the MabSelect SuRe pcc capture step is given in Table 3. Breakthrough capacities (Q_{b10}, Q_{b50}, and Q_{b80}) were determined at 1.5, 2.4, and 6.0 min residence time. Results of the scouting experiment are shown in Figure 7. Residence time selected for the process was 2.5 min. The MAb capture was performed using the ÄKTA™ pcc system run in 3C PCC mode. Eight cycles (corresponding to 24 sample loads) were performed and the results are shown in Figure 8.

Table 3. Process conditions for the MabSelect SuRe pcc capture step

Step	Buffer	Column volumes
Equilibration	10 mM phosphate, 27 mM KCl, 140 mM NaCl, pH 7.4	5
Sample load	4 g MAb /L medium	$Q_{b100\%}$
Wash 1	10 mM phosphate, 27 mM KCl, 140 mM NaCl, pH 7.4	5
Wash 2	50 mM acetate buffer, pH 6.0	1
Elution	50 mM acetate buffer, pH 3.5	4
Strip	50 mM acetate buffer, pH 2.9	2
CIP	0.1 M sodium hydroxide (5 min)	3

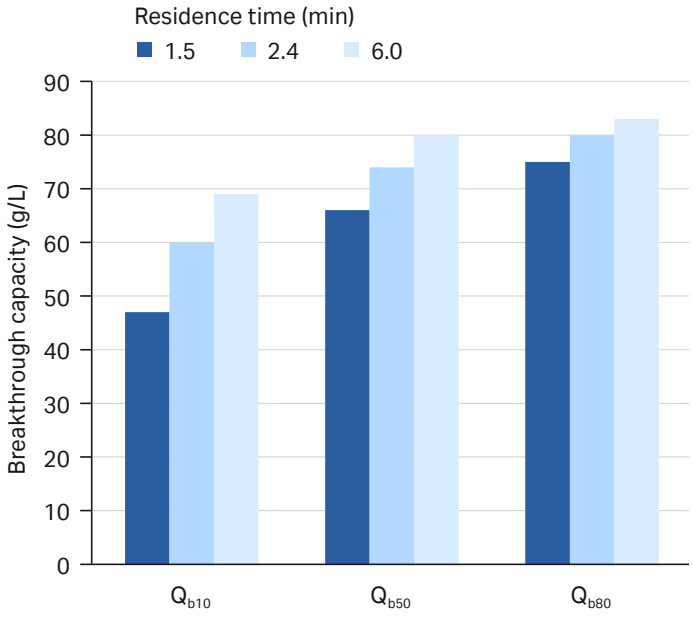

Fig 7. Results from breakthrough capacity experiments performed using MAb-containing cell culture feed. Note that capacity increases with increasing residence time, regardless of the breakthrough level. The effect of residence time is strongest at lower levels of breakthrough (e.g., Q_{b10}).

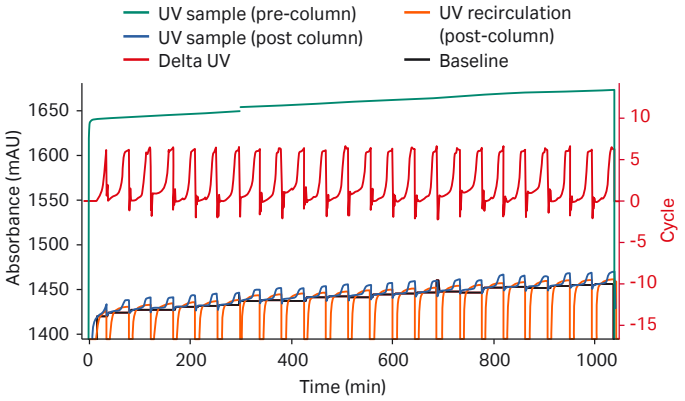

Fig 8. MabSelect SuRe pcc medium used in a 3C PCC setup on the ÄKTA pcc 75 system.

Elution pool was evaluated for MAb yield, aggregates, and host cell protein (HCP) content. The results show a yield between 95% and 100% at an aggregate content between 1.4% and 1.6% and an HCP reduction of approximately 1.4 log reduction value (LRV).

Gain in productivity

In the experiment described above, MabSelect SuRe pcc medium was evaluated in HiTrap column format using a bed height of 2.5 cm per column. In 3C PCC applications, the productivity is optimized by matching the load time and the regeneration time. To fully utilize the potential of this setup, the non-loading steps should be shorter or equal to the time for loading of a column.

Comparing MabSelect SuRe pcc and MabSelect SuRe LX at a load of 4.0 g MAb/L medium and a Q_{b50} of 65 g/L at 1.55 min and 5 min residence time, respectively, the productivity of the two media can be calculated using the following equation:

$$productivity = \frac{Q_{b50}}{t_{cycle} \cdot n}$$

Where

t_{cycle} = the longer of the load or the regeneration time

n = the number of columns in the process

In our example, MabSelect SuRe pcc has a load time of 24 min and a regeneration time of 31 min, while MabSelect SuRe LX has a load time of 81 min and a regeneration time of 31 min. As the dynamic binding capacity is the same for both media, the difference in productivity between the media is governed by the differences in cycle time (Table 4).

Table 4. Productivity calculations for MabSelect SuRe pcc and MabSelect SuRe LX media

	MabSelect SuRe pcc	MabSelect SuRe LX
Residence time	1.55 min	5.0 min
Q_{b50}	65 mg/mL	65 mg/mL
Load time	24 min	81 min
Regeneration time	31 min	31 min
Productivity	0.71 g/L/min	0.24 g/L/min

With MabSelect SuRe pcc, productivity can be increased approximately three-fold compared with MabSelect SuRe LX. This productivity gain can be used to reduce the amount of medium required for a given process or to increase the overall process output.

Storage

MabSelect SuRe pcc medium is delivered in 20% ethanol.

Store unused medium in its container at a temperature of 2°C to 8°C. Ensure that the screw-top is fully tightened. Equilibrate packed columns in buffer containing 20% ethanol or 2% benzyl alcohol to prevent microbial growth.

After storage, equilibrate with starting buffer and perform a blank run, including CIP, before use.

References

1. Walthe *et al*. The business impact of an integrated continuous biomanufacturing platform for recombinant protein production. *Journal of Biotechnology,* Epub May 23 (2015).
2. Application note: Use of sodium hydroxide for cleaning and sanitization of chromatography media and systems. Cytiva, 18-1124-57, Edition AI (2014).
3. Application note: Lifetime performance study of MabSelect SuRe LX during repeated cleaning-in-place. Cytiva, 28-9872-96, Edition AA (2011).
4. Ghose, G. *et al*. Antibody variable region interactions with protein A: Implications for the development of generic purification processes. *Biotechnol. Bioeng.,* ***92***, 665-673 (2005)

Ordering information

Product	Quantity	Product code
MabSelect SuRe pcc	25 mL	17549101
MabSelect SuRe pcc	200 mL	17549102
MabSelect SuRe pcc	1 L	17549103
MabSelect SuRe pcc	5 L	17549104
MabSelect SuRe pcc	10 L	17549105
HiTrap MabSelect SuRe pcc	5 × 1 mL	17549111
HiTrap MabSelect SuRe pcc	1 × 5 mL	17549112

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