Cellufine™ MAX IB

A mixed mode chromatography resin for polishing of monoclonal antibodies after rProtein A affinity capture

Mixed mode chromatography resins are well-known to have unique selectivity differences from traditional IEX or HIC resins. JNC Corporation has developed a new mix-mode resin, Cellufine™ MAX IB, for monoclonal antibody (Mab) purification after initial Protein A capture. This resin has a salt tolerant poly primary amine surface (see Figure 1) modification that has been partially derivatized with butyl groups. The basic chromatographic properties and chemical stability of Cellufine MAX IB are summarized in Table 1.

The resulting ligand structure exhibits a mixed mode functionality with a hybrid poly primary amine + a controlled hydrophobic surface chemistry. In addition to the Mab polishing application this resin can also be used in other downstream processes, such as plasma fractionation, exploiting the unique selectivity of this mixed-mode ligand. This mixed mode resin is built on the highly cross-linked Cellufine cellulose base bead that is very stable, resistant to base CIP and can be operated under high flow modes with minimal back pressure. The 90 µm (average) highly cross-linked cellulose beads is ideally suited for large scale bio-pharma manufacturing processes.

Figure 1, Mixed-mode resin ligand structure

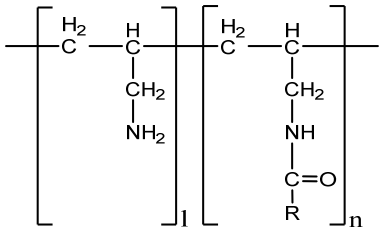


Table 1. Properties of Cellufine MAX IB

Properties	Value
Base bead matrix	Highly cross-linked cellulose
Particle size	90 µM average (40-130 µM)
Microscopic test (%)*	< 5
Ligand	Polyallyl primary amine partially derivatized with a butyl group
Ion exchange capacity	> 80 µequiv./mL
CIP	0.5 M NaOH
Sterilization	Autoclave at 121°C for 20 min
Recommended cleaning conditions**	Ethanol (70%), Isopropanol (30%), Guanidine HCL (6M) and Urea (6M)

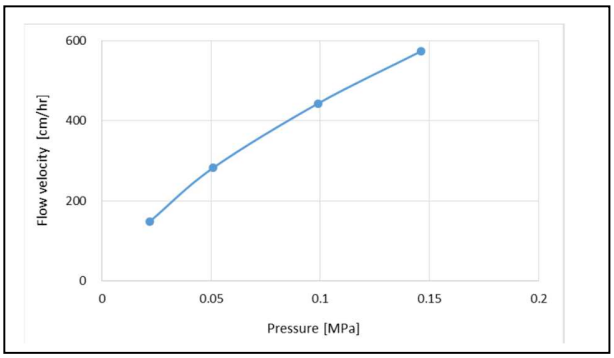
* % of broken particles under microscopic evaluation

** The listed cleaning conditions can be used if the column backpressure becomes too high or “carry over” occurs between runs. It is recommended to run this cleaning cycle in a reverse flow or up-flow direction to dislodge material that may be obstructing the inlet frit or the top layers of resin in the column.

Cellufine MAX IB Pressure-flow Curve

Cellufine MAX IB's highly cross-linked cellulose base beads shows superior flow properties as summarized in Figure 2 for a 10 cm ID x 13 cm L column with a pure water mobile phase at 24±1℃. Flow rates up to 500 cm/h are possible with < 0.3 MPa backpressure.

Figure.2. Pressure flow curve



Cellufine MAX IB Salt Tolerance

Cellufine MAX IB exhibits salt tolerance property derived from poly primary amine ligand with only an 8% loss of BSA binding capacity at 2M salt (see Table 2 above). The density of butyl groups in Cellufine MAX IB ligand can be easily controlled to optimize for target molecules other than Mab's. This ligand design concept will allow for flexibility in applying Cellufine MAX IB to a wide range of fields in bio-pharmaceutical purification.

Table 2, "Salt Tolerant" BSA Binding Capacity

Properties	Value
BSA adsorption capacity (saturation binding)	64 mg/mL (low salt) *** 59 mg/mL (high salt) ****
IgG Recovery in flow through ode	> 95%

*** 50 mM Tris-HCl (pH 8.5)
**** 50 mM Tris-HCl (pH 8.5) + 0.2 M NaCl

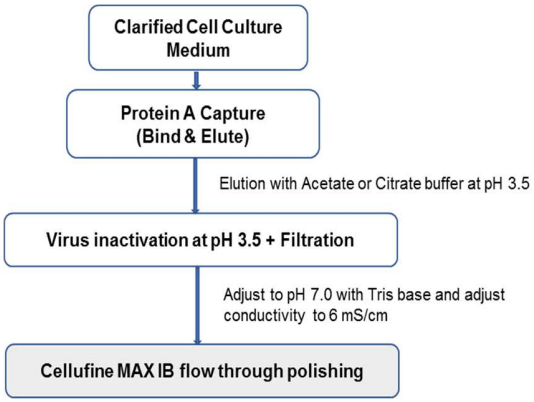
Cleaning-In-Place Recommendations

Cleaning in place (CIP) of Cellufine media can be carried out with up to 0.5 M NaOH solution. Used media should be stored in 20 % ethanol at 25 ℃ after cleaning with the recommended reagents listed in Table 1.

Cellufine MAX IB Polishing of Mab after rProtein A capture step

Cellufine MAX IB in flow-through mode was used to remove HCP from a CHO culture supernatant after capture of a Mab with a rProtein A resin in a two-step format (see Figure 2 below)

Figure 2, Two-step purification workflow



Loading buffer conductivities and elution buffer species (citrate and acetate) were evaluated in this study. Dextran modified anion IEX resins such as Cellufine MAX Q-h and a polymer modified Agarose-Q were used as reference. Samples for these resins were adjusted to the pH that matches the recommended flow through conditions for these resins. The results are summarized in Figure 3 below.

Figure 3. HCP removal by Cellufine MAX IB and Cellufine MAX Q-h (polymer modified Q) after Protein A Capture step

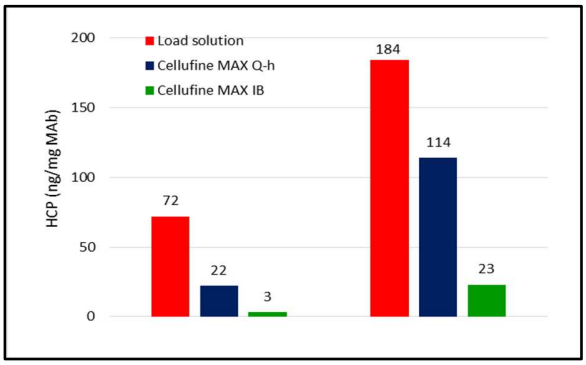


Table 3. Two-step Mab purification with Cellufine MAX IB and two different polymer modified Q IEX resins

	Elution Buffer from ProA	HCP (ng/mg mAb)	Leak ProA (ng/mg_mAb)	Aggregate (%)	HCD (pg/mg mAb)	Recovery (%)
Loading solution	60 mM Acetate Buffer (pH 3.5)	72	3.0	1.7	10	100
Cellufine MAX Q-h		22	2.1	1.9	< 10	97
Polymer modified Agarose Q		27	2.1	1.8		96
Cellufine MAX IB		3	0.0	1.0		95

Cellufine MAX IB was packed in a 5 mmID x 3 cmL column and equilibrated in 60 mM Tris Acetate buffer pH 7.0. Mab was eluted from Protein A with 60 mM Acetic acid pH 3.5 and after viral inactivation was adjusted to pH 7.0 with Tris base and a conductivity of 6 mS/cm with NaCl or dilution with column loading buffer. The sample was then filtered (0.22 micron) to remove any insoluble material and then loaded onto the Cellufine MAX IB column at 190-200 mg Mab /mL of resin at a flow rate of 0.75 mL/min (4 min residence time). The flow through fraction was collected and tested for the above contaminants in the Protein A elution fraction.

The results showed that Cellufine MAX IB was more efficient at HCP removal (< 5 ppm HCP in the flow through fraction with > 95% IgG recovery) from a rProtein A elution fraction in comparison with the anion IEX reference resin quaternary amine chemistries. Monovalent acetate buffer showed the best clearance of CHO HCP from the Protein A elution fraction. This study was then extended to look at clearance of leached protein A, residual dsDNA (HCD) and reduction of Mab aggregates in the final flow through fraction. These results are summarized in Table 3 below.

Conclusion

It has been reported (Ref 1) that the Zeta potential of primary amino or quaternary amino IEX surfaces is reduced by multivalent buffers such as sodium citrate leading to reduced protein adsorption. Cellufine MAX IB was not impacted by citrate buffer but gave superior CHO HCP, leached Protein A, residual HCD removal and reduced Mab aggregates with monovalent acetate buffer in a flow through polishing format (see Table 3). Furthermore, Cellufine MAX IB showed good HCP removal under high salt loading conditions (see Figure 2) such as 14 mS/cm (~120 mM NaCL), which

means that rProtein A elution samples after viral inactivation can be loaded directly after pH adjustment to 7.0, onto the mixed mode resin. A two-step purification workflow (see Figure 2) of rProtein A capture followed by Cellufine MAX IB flow through polishing is a highly efficient purification strategy for Mab isolation after cell culture.

References

1) Douglas B. Burns, Andrew L. Zydney. Buffer effects on the zeta potential of ultrafiltration membranes. Journal of Membrane Science Volume 172, Issues 1–2, 1 July 2000, Pages 39–48

Ordering Information

Description	Quantity	Catalogue No.
Cellufine MAX IB	5 x 1 ml cartridge	21-600-51
	1 x 5 mL cartridge	21-600-15
	10 mL	21600
	50 mL	21601
	100 mL	21602
	500 mL	21603
	5 L	21604
	10 L	21605

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