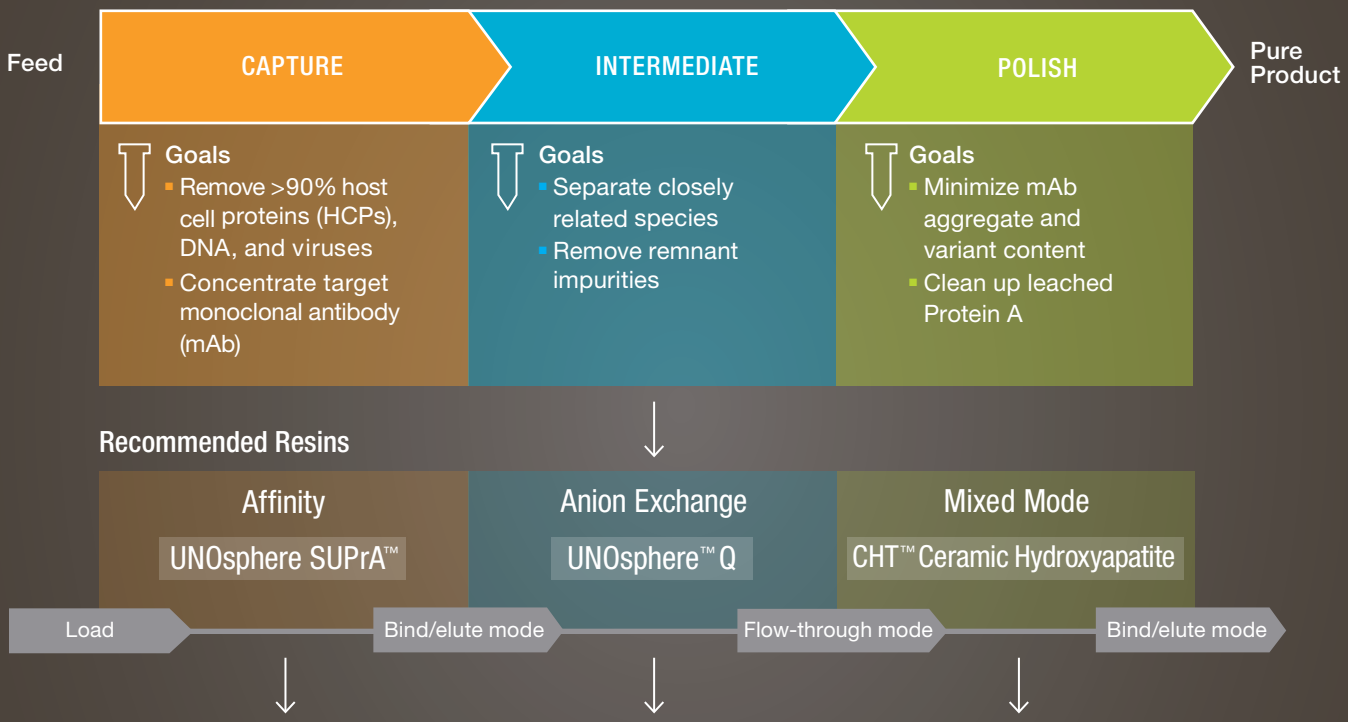
Quick Purification Strategy
Affinity-Based Capture



Advantages

- Same set of buffers works for multiple mAbs (reduced optimization)
- Target molecule is in flowthrough, therefore less optimization needed relative to using cation exchange in bind/elute mode
- Best aggregate, endotoxin, and Protein A removal

Results

	Feed	Capture	Intermediate	Polish
Protein A, ng/mg	NA	ND	112	<0.4
HCP, ng/mg	1.4 x 10^6	197	86	48
DNA, ng/mg	>1.6 x 10^5	19	1.9	3
Aggregate %	ND	42	40	<0.1

NA, not applicable. ND, not determined.

Refer to experimental details on page 2.

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Monoclonal Antibody Purification Strategy Details

Capture Conditions

Step	Buffer	Column Volume	Flow Rate, cm/hr
Equilibration	1x PBS	10	300
Sample Loading	CHO cell supernatant	–	300
Wash	1x PBS	20	300
Elution	100 mM glycine, pH 3.0	–	300

Pooled elution fractions incubated for 1 hr at pH 3.0 to mimic virus deactivation.

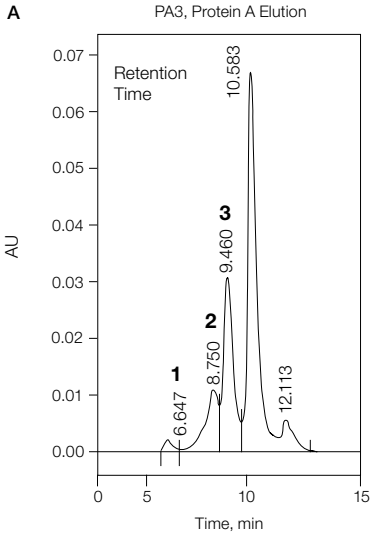
Intermediate Conditions

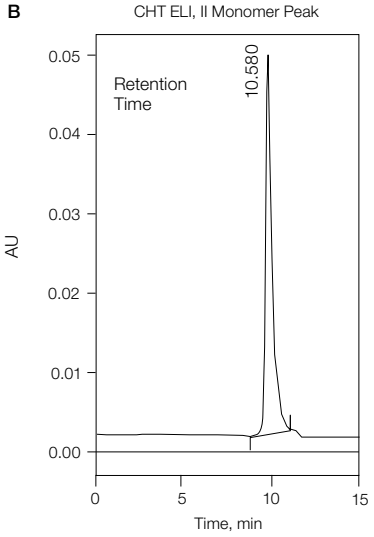
Step	Buffer	Column Volume	Flow Rate, cm/hr
Equilibration	10 mM Na_3PO_4, pH 7.8 (buffer A)	10	300
Sample Loading	Eluate from UNOsphere SUPrA column adjusted to pH 7.8	–	300
Flow-Through Wash	Buffer A	10	300

Flow-through fractions collected.

Polish Conditions

Step	Buffer	Column Volume	Flow Rate, cm/hr
Equilibration	10 mM Na_3PO_4, pH 6.8 (buffer A)	10	300
Sample Loading	UNOsphere Q flow-through pooled fractions adjusted to pH 6.8	–	300
Wash	Buffer A	15	300
Elution	Salt gradient between buffer A and 10 mM Na_3PO_4, 1 M NaCl, pH 6.8	25	300





Removal of impurities by CHT chromatography. Elution fractions from the capture (**A**) and final polishing (**B**) chromatographic steps were analyzed by HPLC-SEC using a Bio-Sil® SEC 400-5 Column to evaluate levels of contamination. The peaks corresponding to aggregates (peaks 1 and 2) and dimers (peak 3) are removed by CHT to yield a clean sample. AU, absorbance units.

Resources

Download bulletin 5728 to get details about this workflow.

Visit **bio-rad.com/web/ProcessResins** to get technical details about the resins used in this workflow.

Visit **bio-rad.com/web/ProcessApplications** to see other applications in which these resins can be used.

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