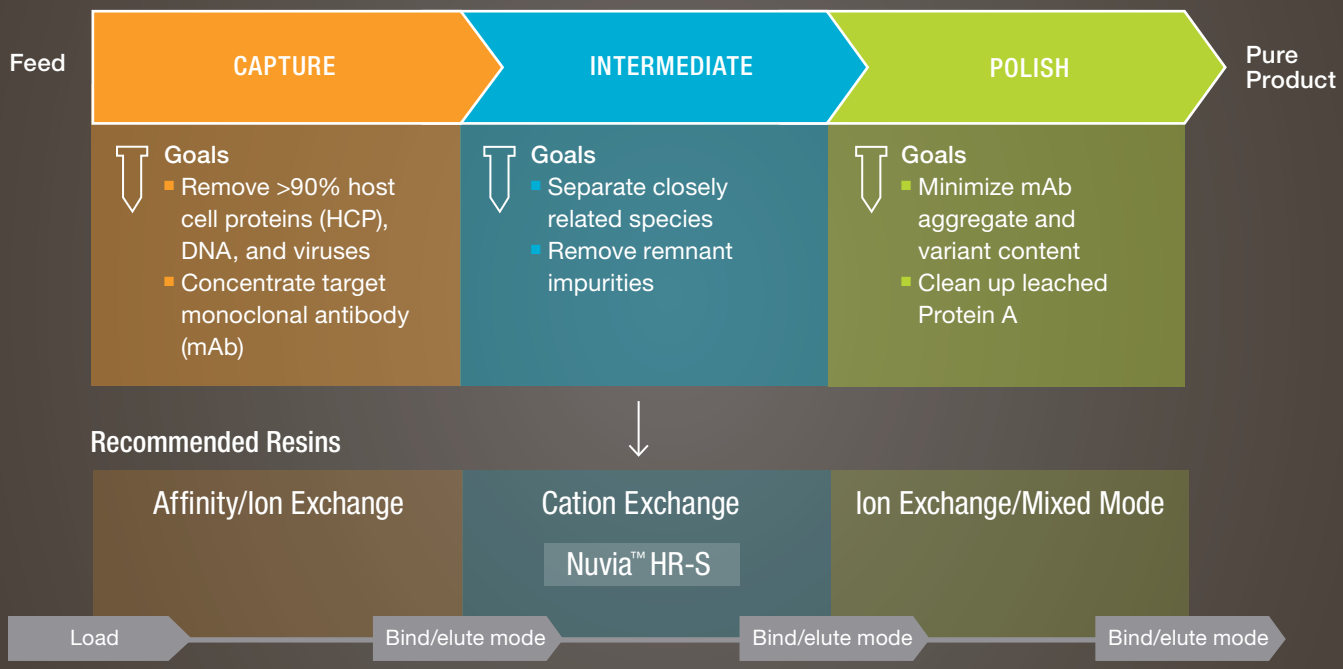
Quick Purification Strategy

Resolving Monoclonal Antibody Charge Variants



Protein deamidation at neutral or alkaline pH is of significant concern to manufacturers of biotherapeutic proteins.

Deamidation at asparagine and glutamine residues:

Caused by

- Enzymatic or alkaline hydrolysis

Affects

- Immunogenicity, stability, and activity of proteins

Results in

- Minimal changes in protein structure and charge
- Downstream purification challenges due to deaminated species
- Molecular heterogeneity

Refer to experimental details on page 2.

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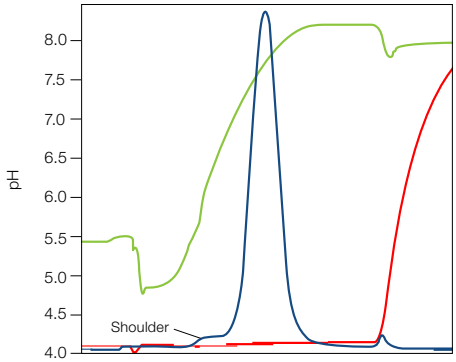
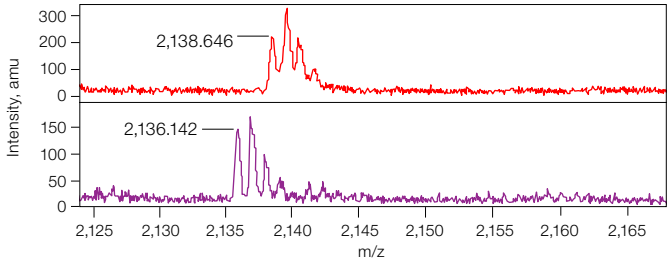
Monoclonal Antibody Purification Strategy Details

Intermediate purification to resolve charge variants with Nuvia HR-S

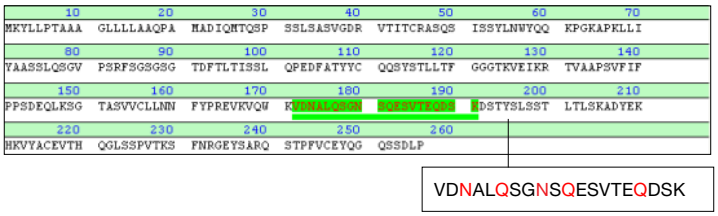
	Step	Buffer	Column Volume	Flow Rate, cm/hr	
Capture Affinity/ion exchange chromatography	Equilibration	10 mM sodium acetate, pH 4.5 (buffer A)	15	300	Polish Ion exchange/ mixed-mode chromatography
	Sample Loading	Eluate from the capture step diluted 1:4 in buffer A	–	300	
	Wash 1	Buffer A	15	300	
	Wash 2	10 mM sodium citrate, 10 mM sodium phosphate, pH 5.0 (buffer B)	15	300	
	Elution	Gradient of buffer B to 10 mM sodium citrate, 10 mM sodium phosphate, pH 8.0	15	300	



Purification of the mAb on Nuvia HR-S. The distinct shoulder on the front side of the main peak is a modified mAb fragment. A_{280} (—); conductivity (—); pH (—).



Mass spectrometry (MS) analysis of the mAb. MS analyses of the shouldered peak (top spectrum) and the main peak (lower spectrum) show a mass shift of about 2.5 atomic mass units between the two peaks, which is consistent with two or three deamidation events on a single peptide.



Shoulder fraction protein sequence. Examination of the shoulder fraction protein sequence confirms deamidation events on a single mAb peptide.

Resources

Visit bio-rad.com/web/NuviaHRS to get more information.

Visit bio-rad.com/web/ProcessResins to get technical details about Nuvia HR-S Resin.

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

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