Optimization of process conditions for Vk fragment capture and purification using Fabsorbent™ F1P HF

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Abstract

The growth of biotherapeutics in the industry has been fuelled by the increasing diversity of monoclonal antibodies (mAbs) and their derivatives, including bispecific antibodies and various fragments like Fab, scFv, and individual light (VL) or heavy (VH) chain fragments. These molecules' varying physical properties and expression systems create significant purification challenges. Purification typically relies on Protein A or Protein L ligands, depending on the domain composition. However, Protein A and Protein L can be limited by base instability and, in the case of Protein L, its specificity for kappa light chain species.

Fabsorbent™ F1P HF is a mixed-mode chromatography resin with a synthetic ligand that demonstrates broad specificity for both kappa and lambda chains. Its robust base-stability and the stable flow properties of the PuraBead® 6HF matrix make it an attractive alternative to Protein L for antibody fragment purification, even at large scale.

This work demonstrates the potential of Fabsorbent™ F1P HF for capturing and purifying antibody fragments using an *E. coli*-derived variable kappa light chain (Vk) fragment as a model feedstock. An optimization workflow is outlined, showing superior host cell protein clearance for Vk fragments compared to a competitor Protein L adsorbent. This workflow serves as a guide for optimizing the purification of other antibody fragment targets on Fabsorbent™ F1P HF.

Optimization of Fabsorbent™ F1P HF load step

1 Scouting binding conditions

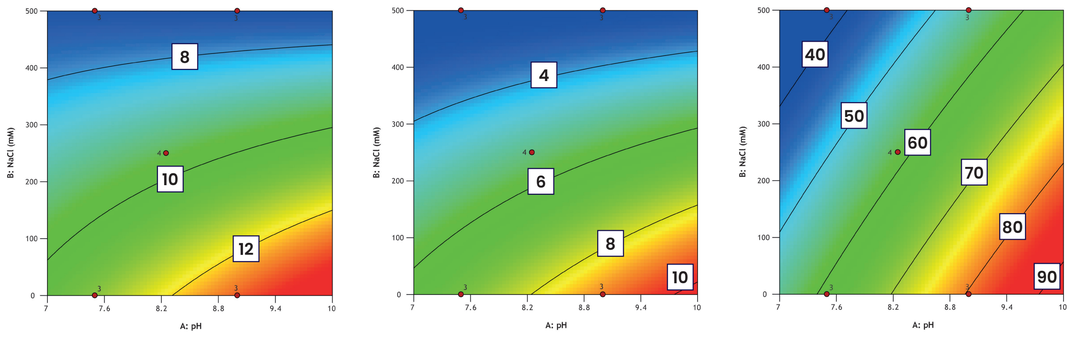
A design of experiments (DOE) screen was conducted to optimize Vk fragment capture from an *E. coli*-derived feedstock using Fabsorbent™ F1P HF adsorbent. The full factorial design assessed the impact of pH (7.5-9.0) and NaCl concentration (0-500 mM) on binding capacity, recovery, and purity.

Screening was performed using a high-throughput system (Biomek i7) with a Captiva Plate® containing 16 x 0.25 mL Fabsorbent™ F1P HF adsorbent. The DOE showed that binding capacity, recovery, and purity were optimized at high pH and low NaCl concentration.

Unoptimized Fabsorbent™ F1P HF purification buffers	
Equilibration:	25 mM Tris
Elution:	50 mM sodium citrate, pH 3.0
Clean-in-place:	0.5 M NaOH

The best performing condition from screening was selected for further verification in 1 mL column mode, with fractions collected during the load phase for Vk breakthrough analysis using reverse-phase HPLC. The verification run showed comparable elution purity to the optimized screen result and a 10% breakthrough capacity of 15 mg/mL adsorbent.

Capacity (mg/mL adsorbent) **Recovery (mg/mL adsorbent)** **Purity (%)**

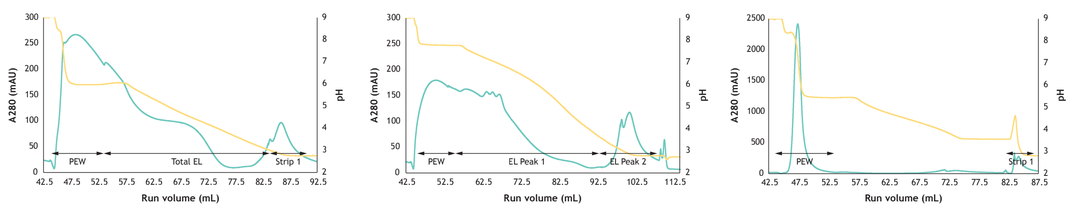


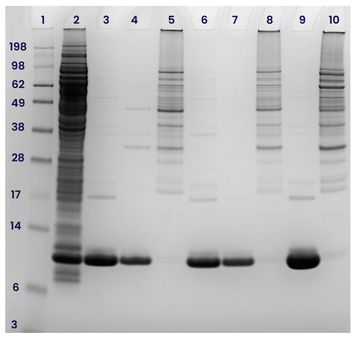
		Reverse-phase HPLC			Densitometry	
1 mL column verification condition	Sample	Vk amount (mg/mL ads)	Vk recovery (%)	10% Breakthrough (mg/mL adsorbent)	Vk purity (%)	Elution purity fold-change
25 mM Tris, pH 9.0	Load	15.85		15.05	19.52	4.14
	Non-bound	0.35	2.21		1.79	
	Elution	12.66	79.87		80.85	

Optimization of Fabsorbent™ F1P HF elution step

2 Scouting elution conditions

50 mM Citrate pH 6.0–3.0 **½ McIlvaines pH 7.5–3.0** **50 mM Acetate pH 5.5–3.7**





Lane	Buffer	Sample
1		SeeBlue Plus2
2		*E. coli*-derived Vk feedstock
3	Citrate	Pre-elution wash
4		Total elution
5		Strip 1
6	½ McIlvaines	Pre-elution wash
7		Elution peak 1
8		Elution peak 2
9	Acetate	Pre-elution wash
10		Strip 1

To enhance Vk fragment purity, various buffer systems were tested under optimized load conditions (25 mM Tris base, pH 9.0) using the 1 mL pre-packed Fabsorbent™ F1P HF column. Different pH gradients were created, and fractions were collected for SDS-PAGE analysis.

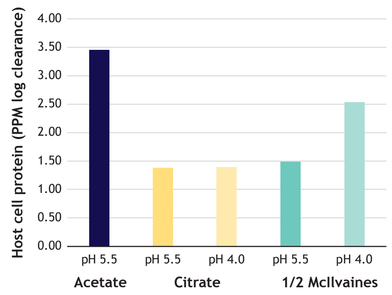
All gradients effectively separated Vk fragments from non-target proteins, with Vk fragments eluting at a higher pH.

While citrate and McIlvaines gradients showed gradual recovery of Vk fragments from pH 7.5–pH 4.0, the acetate pH 5.5 wash condition demonstrated significantly improved recovery and separation.

3 Comparison of step elution across buffer systems

To compare the effectiveness of the acetate buffer, pH 5.5 step elution was performed using citrate and ½ McIlvaines buffers on the 1 mL pre-packed Fabsorbent™ F1P HF column. After the pH 5.5 step, a pH 4.0 step was applied to each buffer. Vk fragment recovery was analyzed via reverse-phase HPLC, and purity was assessed by densitometry and *E. coli* HCP ELISA.

The acetate pH 5.5 buffer outperformed citrate and ½ McIlvaines buffers in terms of mild Vk elution pH and overall purity. While all buffers reached the densitometry assay's sensitivity limit, differences in purity were detectable only through HCP ELISA, confirming the acetate buffer's superior performance for high-purity Vk fragments.



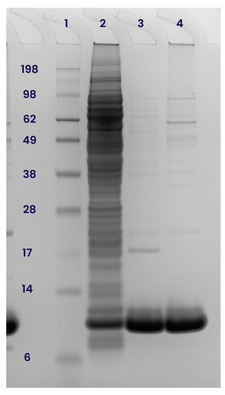
			Reverse-phase HPLC		Densitometry	
Elution Condition	Sample	pH	Vk amount (mg/mL adsorbent)	Combined Vk elution recovery (%)	Vk purity (%)	Elution purity fold-change
Acetate	Load		13.50	88.44	17	
	Elution	5.5	11.94		99	5.99
Citrate	Load		12.88	75.70	16	
	Elution	5.5	4.60		98	6.20
		4.0	5.15		100	6.36
½ McIlvaines	Load		12.99	94.35	16	
	Elution	5.5	6.12		98	6.22
		4.0	6.13		100	6.36

Verification of Fabsorbent™ F1P HF performance

4 Comparison to competitor product

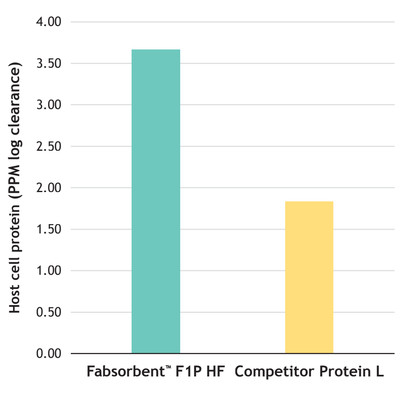
The final buffer conditions for Vk fragment purification included pH 9.0 equilibration, followed by acetate buffer elution at pH 5.0 to enhance elution efficiency while maintaining Vk purity. Performance was assessed using a 1 mL pre-packed Fabsorbent™ F1P HF column and compared to a competitor Protein L product under optimized conditions. Vk recovery and purity were evaluated via reverse-phase HPLC and BL21(DE3) *E. coli* HCP ELISA, with SDS-PAGE for comparison.

At 15 mg/mL adsorbent load, Vk elution recovery was comparable between the two adsorbents, with Fabsorbent™ F1P HF showing higher purity than the Protein L product.



Optimized Fabsorbent™ F1P HF buffers	
Equilibration:	25 mM Tris base, pH 9.0
Elution:	50 mM acetate, pH 5.0
Strip:	50 mM citrate, pH 3.0
Clean-in-place:	0.5 M NaOH

Lane	Sample
1	SeeBlue Plus2
2	*E. coli*-derived Vk feedstock
3	Fabsorbent™ F1P HF Elution
4	Competitor Protein L Elution



		Reverse-phase HPLC	
Adsorbent	Sample	Vk amount (mg/mL adsorbent)	Vk recovery (%)
Fabsorbent™ F1P HF	Load	14.58	
	Elution	11.26	77.28
Competitor Protein L	Load	16.56	
	Elution	12.94	78.14

Summary

An optimization workflow was developed using the Fabsorbent™ F1P HF adsorbent to purify a complex *E. coli*-derived Vk fragment feedstock. A 2-factor design of experiments (DOE) identified that low NaCl concentration and high pH (pH 9.0) maximized Vk binding and purity. Elution conditions were then tested with various buffer systems. While citrate, ½ McIlvaines, and acetate buffers effectively recovered purified Vk fragments, acetate at pH 5.5 provided a mild elution pH and higher host cell protein (HCP) clearance.

The final optimized conditions, using pH 9.0 for loading and acetate buffer at pH 5.0 for elution, achieved 15 mg/mL Vk binding capacity and a 3.67 PPM log clearance of HCP, outperforming a competitor Protein L product with only 1.84 PPM log clearance.

This workflow highlights the potential of Fabsorbent™ F1P HF as a purification platform, serving as a template for optimizing the purification of other antibody fragment targets.

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