Application note

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

The expanding range of therapeutic modalities, including monoclonal antibodies (mAbs) and their derivatives, present purification challenges due to the diversity of their physical properties and expression systems. This application note demonstrates the potential of Fabsorbent™ F1P HF for antibody fragment capture using an E. coli–derived variable kappa light chain (Vk) fragment as a model feedstock.

Purification of mAbs and their derivatives typically starts with a capture step using either Protein A, which binds the antibody Fc region, or Protein L ligands, which have specificity for the kappa light chain. The performance of these resins can be limited, however, by a lack of base stability and, for Protein L, its narrow specificity.

Fabsorbent™ F1P HF is a mixed-mode chromatography resin with a synthetic ligand that has broad specificity for both antibody kappa and lambda chains. The synthetic ligand allows for robust base stability, and the PuraBead® 6HF base matrix offers stable flow properties even for large-scale manufacturing. These attributes make it an appealing alternative to Protein L as a platform technology.

This application note 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. The optimized workflow showed superior host cell protein (HCP) clearance for Vk fragments compared to a Protein L adsorbent and serves as a guide for purifying antibody fragments using Fabsorbent™ F1P HF.

Optimization of Fabsorbent™ F1P HF load step

The Fabsorbent™ F1P HF load step was optimized using a design of experiments (DOE) screen to scout binding conditions for capture of the Vk fragment from an E. coli-derived feedstock. 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. Unoptimized Fabsorbent™ F1P HF purification buffers are shown in Table 1.

Screening was performed using a high-throughput system (Biomek i7) with a Captiva Plate® containing 16 x 0.25 mL Fabsorbent™ F1P HF adsorbent. Vk capacity, recovery and purity were assessed by densitometry. The DOE showed that binding capacity, recovery, and purity were optimized at high pH and low-NaCl concentration in the binding buffer (Figure 1).

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

Table 1: Unoptimized Fabsorbent™ F1P HF purification buffers.

The capacity, recovery, and purity results for best-performing screening conditions and the predicted results for the in-silico model optimal conditions for Vk fragment purification are shown in Table 2. These two load conditions were selected for further verification in 1 mL column mode.

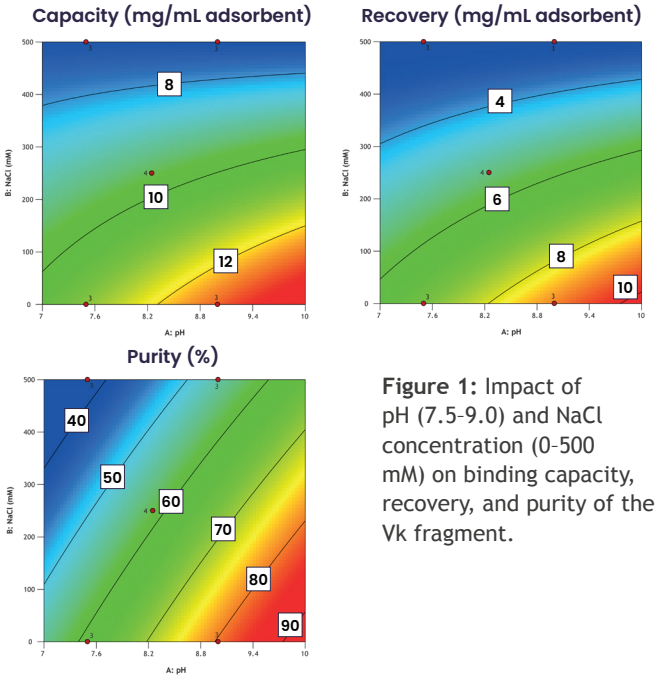


Figure 1: Impact of pH (7.5-9.0) and NaCl concentration (0-500 mM) on binding capacity, recovery, and purity of the Vk fragment.

Analysis	Predicted mean (pH 9.78 / 11.72 mM)	Screen mean (pH 9.0 / 0 mM NaCl)
Capacity (mg/mL adsorbent)	13.63	12.99
Recovery (mg/mL adsorbent)	9.86	9.56
Purity (%)	89.76	83.89

Table 2: Optimized load screen purification results for Fabsorbent™ F1P HF for the in-silico predicted condition and top performing screen condition.

		Reverse-phase HPLC			Densitometry	
Condition	Sample	Vk amount (mg/mL ads)	Vk recovery (%)	10% Breakthrough (mg/mL adsorbent)	Vk purity (%)	Elution purity fold-change
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	
pH 9.8	Load	16.38		14.14	17.71	4.79
	Non-bound	0.38	2.31		3.01	
	Elution	12.90	78.77		84.87	

Table 3: Comparison of Vk amount, recovery, breakthrough, and purity using loading buffers at either pH 9.0 or 9.8. Despite higher purity at pH 9.8, the pH 9.0 condition was selected for further development to maximize Vk capacity, with further optimization focusing on improving purity while maintaining high capacity.

To verify the optimized screen results in column mode, a 1 mL pre-packed Fabsorbent™ F1P HF column was loaded with two buffers meeting the pH requirements of the optimized model (25 mM Tris, pH 9.0, and 25 mM Glycine, 12 mM NaCl, pH 9.8). Fractions were collected during the load phase for Vk breakthrough analysis using reverse-phase HPLC, while densitometry was used to assess fraction purity.

At pH 9.0, 10% breakthrough occurred later, indicating a stronger interaction with the stationary phase (Figure 2). Despite higher Vk purity in the elution following the load at pH 9.8 (Table 3), the pH 9.0 condition was selected for further development to maximize Vk capacity, with additional optimization focusing on improving purity while maintaining high capacity.

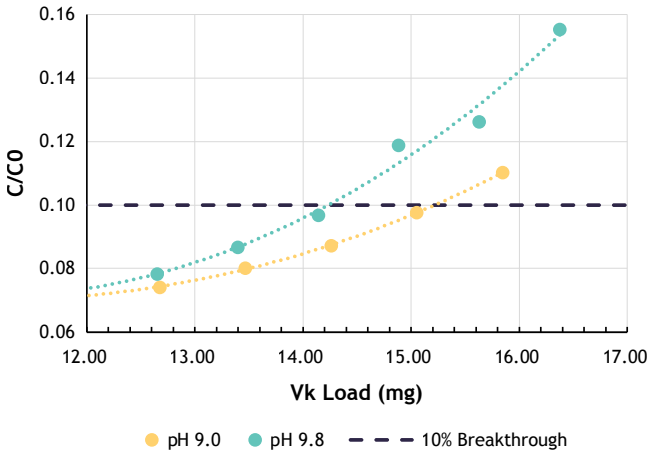


Figure 2: Vk breakthrough at pH 9.0 and pH 9.8

Optimization of Fabsorbent™ F1P HF elution step

To further 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 (Figure 3). All three gradients effectively separated Vk fragments from non-target proteins, with Vk fragments eluting at a higher pH in the pre-elution wash and elution gradient fractions, while the bound non-target protein was recovered in the strip fractions at pH 3.0 (Figure 4). However, both the citrate and McIlvaines gradients showed gradual recovery of Vk fragments from pH 7.5 to pH 4.0, while the acetate pH 5.5 wash condition demonstrated significantly improved recovery and separation.

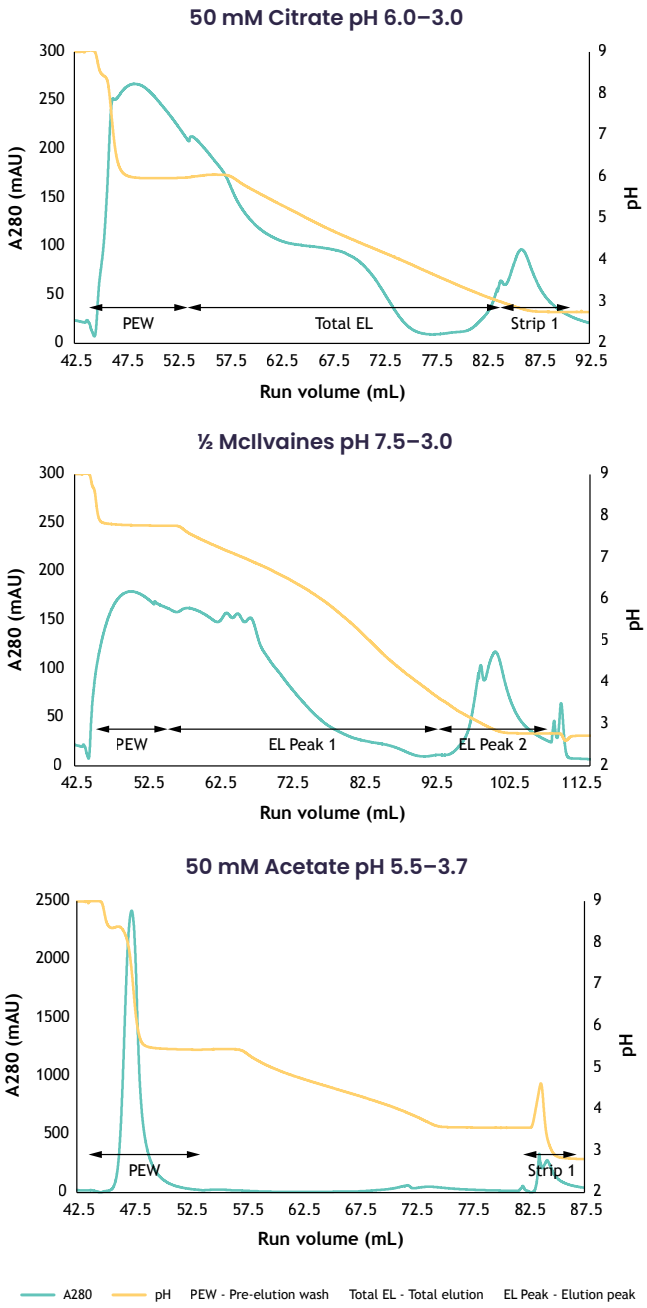
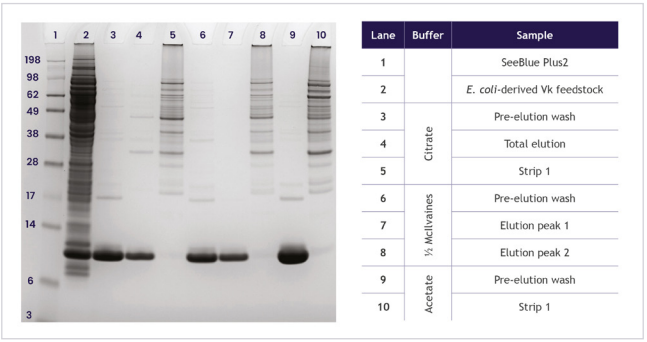


Figure 3: Separation of Vk fragments from non-target proteins using three different pH gradients.



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

Figure 4: SDS-PAGE analysis of fractions collected from the separations using the three different gradients.

To directly compare the effectiveness of the acetate buffer to the other two buffer systems, a 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 in each buffer, based on the Vk recovery profile from the gradient elution results. Vk fragment recovery was analyzed via reverse-phase HPLC, and purity was assessed by densitometry and a generic *E. coli* HCP ELISA.

The acetate pH 5.5 buffer outperformed the citrate and ½ McIlvaines buffers in terms of mild Vk elution pH and overall purity (Table 4). While all buffers reached the sensitivity limit of the densitometry assay and differences in purity were detectable only through HCP ELISA, the HCP clearance results confirmed the acetate buffer's superior performance for high-purity Vk fragments (Figure 5).

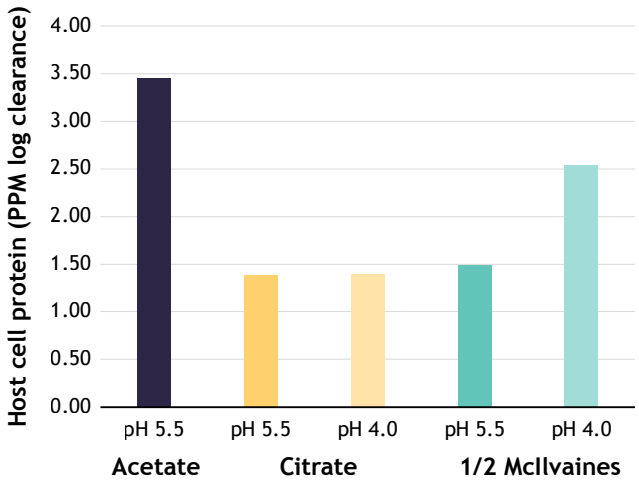


Figure 5: Impact of the different elution conditions on HCP removal.

			Reverse-phase HPLC		Densitometry		
Elution condition	Sample	pH	Vk amount (mg/mL adsorbent)	Vk recovery (%)	Combined Vk elution recovery (%)	Vk purity (%)	Elution purity fold-change
Acetate	Load		13.50		88.44	17	
	Elution	**5.5**	11.94	88.44		99	5.99
Citrate	Load		12.88		75.70	16	
	Elution	**5.5**	4.60	35.70		98	6.20
		4.0	5.15	40.00		100	6.36
½ McIlvaines	Load		12.99		94.35	16	
	Elution	**5.5**	6.12	47.14		98	6.22
		4.0	6.13	47.22		100	6.36

Table 4: Comparison of Vk amount, recovery, breakthrough, and purity using the three different elution conditions.

Verification of Fabsorbent™ F1P HF performance

The final buffer conditions selected for Vk fragment purification were a pH 9.0 equilibration followed by an elution step in acetate buffer at pH 5.0 (Table 5). The pH 5.0 condition was selected to improve the efficiency of the Vk fragment elution observed at pH 5.5, without significantly compromising the mild elution pH and Vk purity.

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

Table 5: Optimized Fabsorbent™ F1P HF purification buffers

The performance of a 1 mL pre-packed Fabsorbent™ F1P HF column was assessed and compared to a Protein L product, also in a 1 mL pre-packed column of equivalent dimensions. Optimization of the elution pH was performed for the competitor product, leading to selection of a pH 3.5 wash condition followed by a pH 2.5 elution step, both in 50 mM citrate buffer (data not shown). Column performance was assessed by Vk recovery (reverse-phase HPLC) and purity (strain-specific BL21(DE3) *E. coli* HCP ELISA). Non-reduced SDS-PAGE was also used to compare the Vk elution profiles.

The results showed that, when loaded to approximately 15 mg/mL adsorbent, elution recovery of Vk fragments was comparable between the two adsorbents (Table 6). SDS-PAGE comparison of the elution fractions showed both adsorbents recovered a significantly purified Vk fraction in comparison to the load but that the non-target protein profiles differed between adsorbents (Figure 6). The HCP clearance results demonstrated that the elution purity for Fabsorbent™ F1P HF was higher than that of the Protein L resin at 3.67 PPM log clearance compared to 1.84 PPM log clearance (Figure 7).

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

Table 6: Comparison of Vk load and recovery on Fabsorbent™ F1P HF and a Protein L resin.

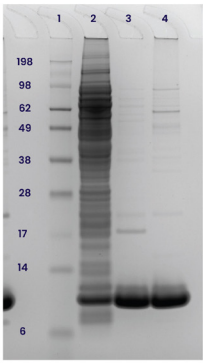


Figure 6: SDS-PAGE analysis of fractions collected from the Fabsorbent™ F1P HF resin and a Protein L resin.

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

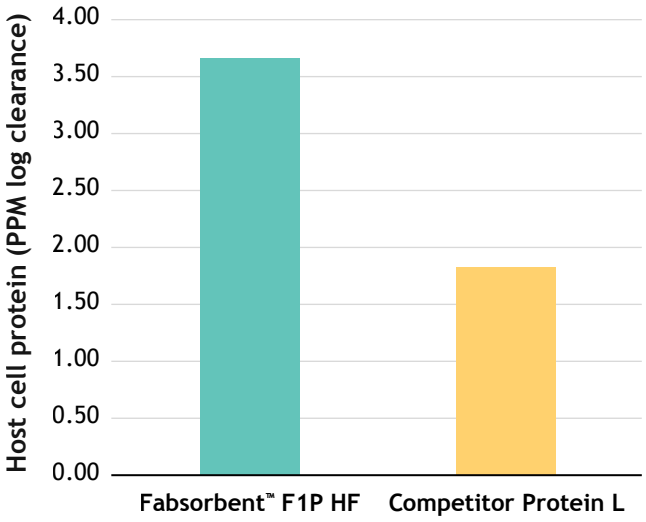


Figure 7: Clearance of HCP from the Vk feedstock using Fabsorbent™ F1P HF and a Protein L resin.

Conclusion

The optimized workflow for purifying *E. coli*-derived Vk fragments using Fabsorbent™ F1P HF demonstrated significant improvements in binding capacity, purity, and HCP clearance. Optimal load conditions were achieved at pH 9.0 with low NaCl for maximal binding capacity and purity. The best elution conditions were using acetate buffer at pH 5.0, ensuring mild conditions while maintaining high purity. Fabsorbent™ F1P HF outperformed Protein L in terms of HCP clearance, with a 3.67 PPM log clearance compared to Protein L's 1.84 PPM log clearance.

It is also worth noting that Fabsorbent™ F1P HF elution steps were performed at milder pH conditions than the competitor Protein L (pH 2.5), which will reduce the likelihood of protein denaturing and aggregation.

This workflow not only serves as a valuable template for optimizing Vk fragment purification but also provides insights into the potential of Fabsorbent™ F1P HF for the purification of other antibody fragments, such as Fab, scFv, or bispecific antibodies, highlighting its broad applicability across a range of therapeutic modalities.