Simplifying Progress

Efficient Purification of Diverse Single-Domain Antibodies Using HyperCel Mixed Mode Resins

Performed in collaboration with VALIDOGEN

Lukas Eidenberger[1], Iskandar Dib[1], Marius Lechner[2], Julia von der Hardt[2]

[1] Validogen GmbH, Grambach, Austria
[2] Sartorius Stedim Biotech GmbH, August-Spindler-Straße 11, 37079 Göttingen, Germany

1. Introduction

Single-domain antibodies (sdAbs, VHH fragments, or NANOBODY®) are camelid-derived heavy-chain antibodies with unique features beneficial for research and medicine. They offer high thermal stability, specificity, and affinity in antigen binding, and their small size makes them suitable for hidden targets and tissue penetration. Their simplified structure allows versatile design, engineering, and manufacturing, enabling high-titer expression in various host cells. While full-length mAbs are easily purified using Protein A affinity chromatography, sdAb purification is complex due to the lack of such a universal capture chromatography method.

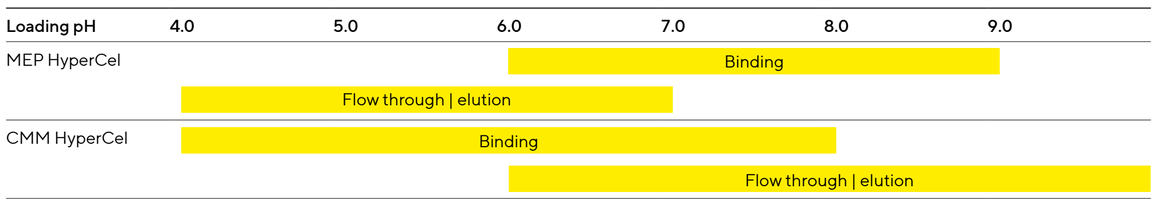
This study was performed in collaboration with VALIDOGEN, a leading contract research and development organization specializing in recombinant protein expression using its exclusive UNLOCK PICHIA® protein expression technology. VALIDOGEN provides advanced Pichia strain generation as well as fermentation and protein purification process development for the manufacture of biopharmaceuticals, enzymes, and other recombinant proteins.

Note. NANOBODY® is a registered trademark of Ablynx N.V.

HyperCel Mixed Mode Resins

HyperCel Mixed Mode resins are versatile chromatography consumables designed for purifying antibodies and recombinant proteins. They feature a rigid cellulose matrix offering high porosity, chemical stability, and low non-specific interaction. HyperCel resins ensure excellent flow properties at low backpressures, ideal for sdAb capture at high flow rates. HyperCel Mixed Mode resins use hydrophobic charge induction chromatography to separate proteins based on their pH-dependent behavior.

Figure 1: *pH Conditions for Hydrophobic Charge Induction Chromatography Purification on MEP & CMM HyperCel*



Advantages of MEP & CMM HyperCel Over Traditional Resins in sdAb Capture

- High dynamic binding capacities
- Protein binding in low-salt conditions
- Differentiated selectivity for separating host cell proteins (HCPs), DNA, aggregates, and misfolds
- Mild elution conditions minimizing aggregate formation

2. Materials & Methods

Table 1: *List of Different sdAbs Purified on MEP & CMM HyperCel*

Name	Molecular Weight [kDa]	Isoelectric Point
dV_HH1	28.0	6.8
dV_HH2	27.0	7.1
MV_HH	13.0	5.6

Table 2: *Buffers Used to Purify Different sdAbs on MEP & CMM HyperCel*

Target Molecule	Resin	Phase	Ingredients	pH	CV	Contact Time [s]
dV_HH1, dV_HH2	MEP HyperCel	Equilibration	50 mM Bis-Tris, 500 mM NaCl	6.0	5.0	90.0
		Load	Supernatant		Variable	90.0
		Wash	50 mM Bis-Tris, 500 mM NaCl	6.0	5.0	90.0
		Elution	50 mM Sodium acetate	4.5	5.0	90.0
		Regeneration	50 mM Acetate	3.0	5.0	90.0
MV_HH	MEP HyperCel	Equilibration	50 mM Bis-Tris, 300 mM NaCl	6.0	5.0	90.0
		Load	Supernatant		Variable	90.0
		Wash	50 mM Bis-Tris, 300 mM NaCl	6.0	5.0	90.0
		Elution	50 mM Sodium acetate	2.5	5.0	90.0
	CMM HyperCel	Equilibration	50 mM Sodium acetate, 250 mM NaCl	4.5		90.0
		Load	Supernatant		Variable	90.0
		Wash	50 mM Sodium acetate, 250 mM NaCl	4.5	5.0	90.0
		Elution 1	50 mM Tris	7.0	5.0	90.0
		Elution 2	50 mM Tris	8.0	5.0	90.0
		Elution 3	50 mM Tris	9.0	5.0	90.0

eShop MEP HyperCel

Capture of Dimeric sdAbs With Neutral pI on MEP HyperCel

Figure 2: *Recovery, HCP, and Host Cell DNA (hcDNA) Eluate Content After dV_HH1 & dV_HH2 Capture With MEP HyperCel*

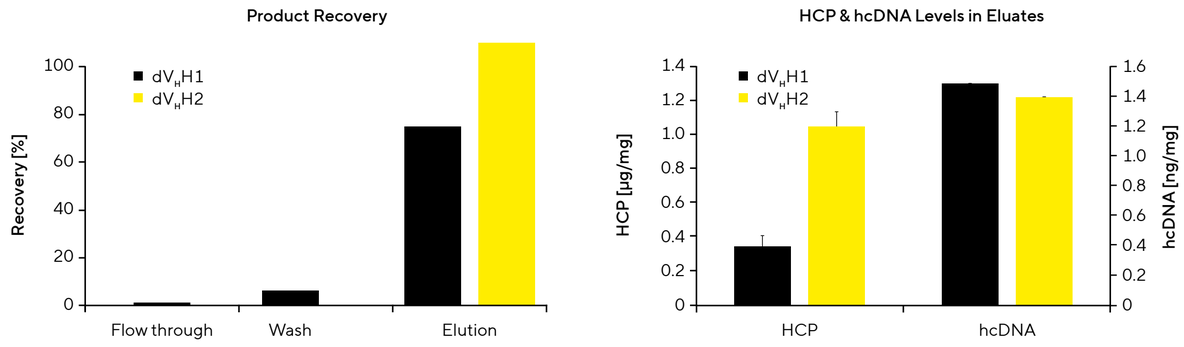
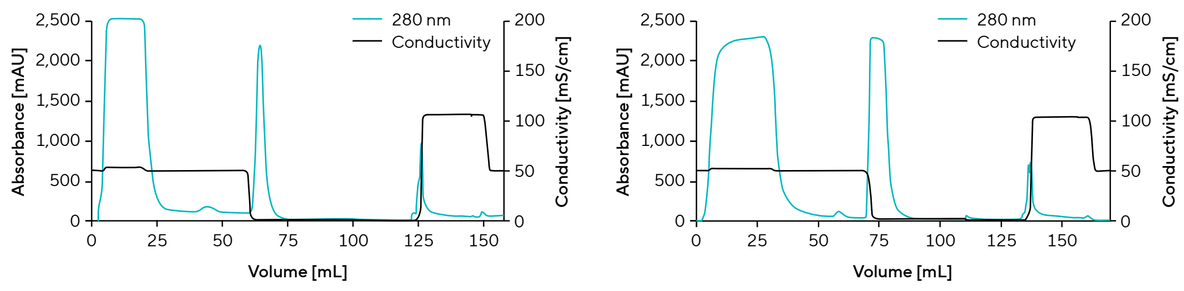


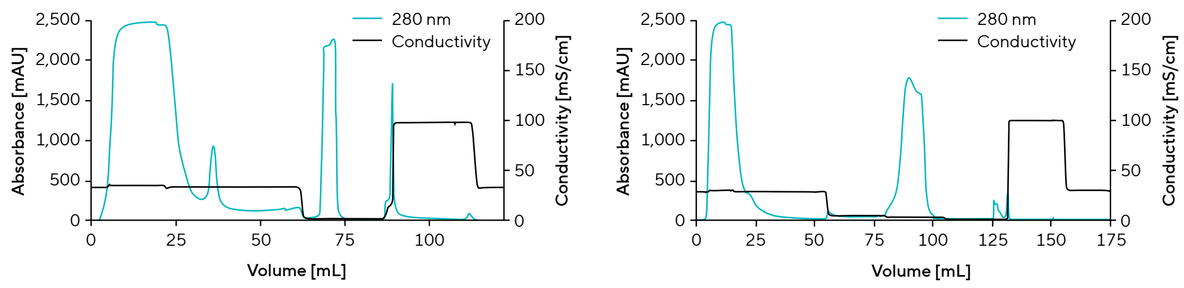
Figure 3: *Chromatograms of dV_HH1 (Left) & dV_HH2 (Right) on MEP HyperCel*



- Dynamic binding capacities (DBC10%): 23.4 g/L for dV_HH1 and 44.4 g/L for dV_HH2, surpassing alternative resins.
- Product recovery: Estimated at 75% for dV_HH1 and 110% for dV_HH2.
- HCP: log reduction of 1.2 – 1.65.
- hcDNA: log reduction of 1.9 – 2.8.

Capturing an Acidic sdAb on MEP & CMM HyperCel

Figure 4: *Chromatograms of mV_HH1 on MEP HyperCel (Left) & CMM HyperCel (Right)*



- Proof of principle for the capture of an acidic sdAb with MEP HyperCel with low pH elution
- 98% recovery showcasing the versatility of MEP HyperCel
- Alternative capture of an acidic sdAb with CMM HyperCel with near-neutral pH elution
- Almost 100% recovery was achieved with near-neutral pH elution

3. Conclusion

The study demonstrates the efficacy and versatility of MEP HyperCel as a capture platform for diverse sdAbs:

- Suitable for capture of a wide range of sdAbs with neutral to acidic pI and different size
- High dynamic binding capacity and recovery
- High HCP & hcDNA removal

Additionally, CMM HyperCel represents a viable alternative, with high recovery for acidic sdAbs requiring near-neutral pH elution, offering flexibility in optimizing purification processes.

The MEP & CMM HyperCel capture platform could be streamlined by further refining binding conditions at low salt concentrations, contact times, and dynamic binding capacities. Purification of sdAbs is complex due to the absence of a universal binding method like Protein A affinity chromatography used for full-length monoclonal antibodies (mAbs).

Last updated: 04 | 2025