

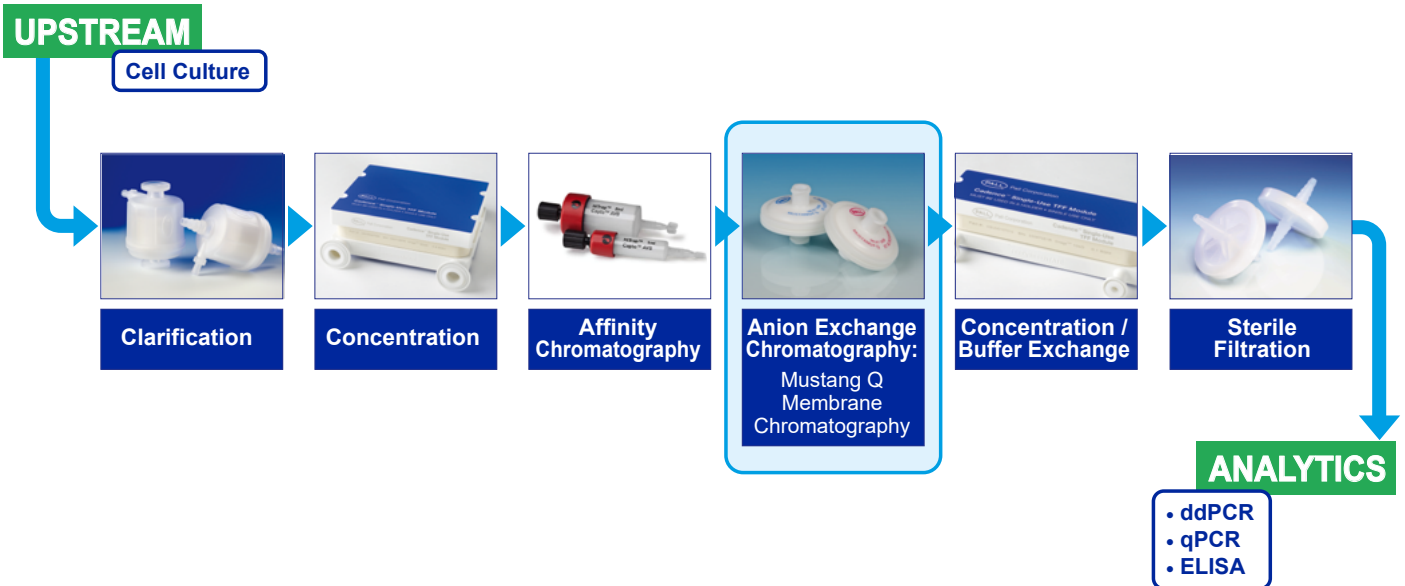
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Anion Exchange Chromatography Membrane for Full Adeno-Associated Virus (AAV) Capsid Enrichment

Kurt Boenning, Adam Hejmowski, Julio Huato, Aydin Kavara and Mark Schofield
Pall Corporation, 20 Walkup Drive, Westborough, MA 01581, USA

INTRODUCTION

- The production of adeno-associated viruses inherently generates a significant population of empty capsids that can illicit an immune response without delivering the therapeutic payload. Increased levels of AAV antigen can lead to dose-limiting side effects and overall reduced transduction efficiency¹. However, empty capsids may act as a decoy for antibodies present due to natural infection of wild-type virus. Therefore, an increased AAV dose may help overcome neutralizing antibodies that prevent pathogens from entering cells², but promote AAV capsid-specific CD8+ T cell destruction of cells that were successfully transduced³. The effect of empty capsids on clinical outcome is unclear, but a controlled ratio of capsids is crucial.
- Conventional AAV purification suffers from high cost, limited scalability, and technical barriers. Ion exchange chromatography can address the current process deficiencies, but the small difference in isoelectric point difference makes separation challenging. Utilizing a novel 1 mS/cm step gradient approach, we are able to demonstrate enrichment of full AAV capsids of serotypes 5 and 8 with the Mustang® Q XT membrane following affinity purification.



MUSTANG Q MEMBRANE PRODUCT RANGE

- Mustang Q membrane devices are ready-to-use chromatography adsorbers with bed volumes ranging from 0.86 mL to 5 L
- Scalable range covers screening to large scale applications
- These ion exchange supports are polyethersulfone (PES)-based membranes, with 0.8 μm pore size and a cross-linked polymeric coating of quaternary amine functional group



Mustang Q XT membrane in Acrodisc™ unit 0.86 mL



Mustang Q XT 5 capsule 5 mL

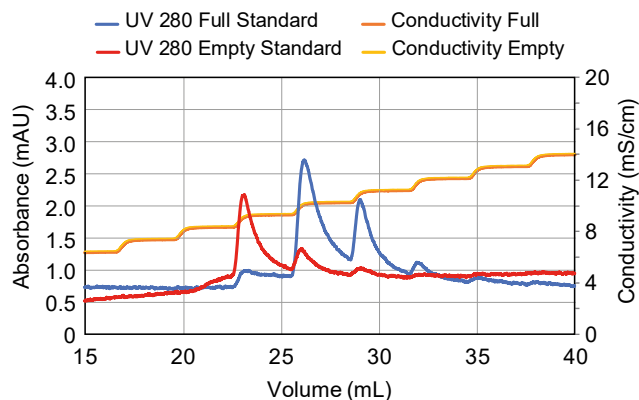
Mustang Q XT 5000 capsule – 5 L



Mustang Q XT 140 capsule 140 mL

MUSTANG Q MEMBRANE: AAV5 EMPTY AND FULL STANDARDS

- Empty and full AAV5 capsid standards were obtained by ultracentrifugation and individually eluted using Mustang Q membrane
- Individual chromatograms are overlaid, distinct elution patterns are observed with empty and full capsids
- Empty capsids elute at an earlier conductivity step relative to full capsids

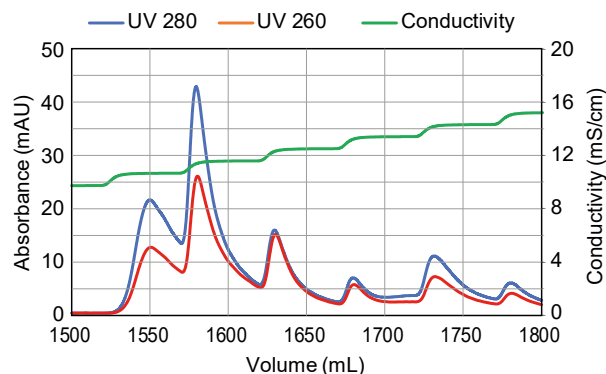


→ Empty/full standards verify separation

MUSTANG Q XT 5 mL CAPSULE

AAV5 Scale Up

- The 1 mS/cm step gradient elution strategy was scaled up from the 0.86 mL Mustang Q XT membrane in Acrodisc® unit to the Mustang Q XT 5 mL capsule
- Similar patterns in the UV 260/280 absorbance ratio are observed across the devices
- Each step elution fraction was analyzed using ELISA and ddPCR. An increase in total vector genomes (ddPCR) to total capsids (ELISA) is seen from peak 2 to 3, which corresponds to a full capsid population enrichment
- High overall yield of both capsids and vector genomes is achieved across the elution

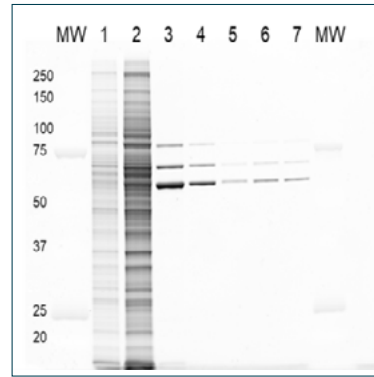


	Load	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Sum
Total capsids (1×10^{12})	695	140	245	59	30	55	31	---
Total vg (ddPCR, 1×10^{12})	35	0	11	17	4	1	1	---
vg/capsids	5%	0%	5%	29%	12%	2%	2%	---
ELISA yield	---	20%	35%	9%	4%	8%	4%	81%
ddPCR yield	---	0%	31%	49%	11%	3%	2%	97%

→ Successful scale up to 5 mL Mustang Q capsule

DNA and HCP Removal

- Contaminant levels were monitored during the 5 mL Mustang Q capsule scale up process. Individual capsid populations were eluted from Mustang Q capsule
- DNA and HCP were quantitated using PicoGreen[♦] and ELISA assays respectively
- An SDS-PAGE gel was used to visualize the reduction of contaminants through the entire downstream process platform
- VP1, VP2 and VP3 are clearly visible on lanes 3-7
- After the Mustang Q membrane polishing steps, the contaminant levels are reduced to the analytical assays detection limits of 0.2 ng/mL and 10 ng/mL for DNA and host cell protein (HCP) respectively



Gel Lane	Sample	DNA Concentration (ng/mL)	HCP Concentration (ng/mL)
1	Crude harvest	2.7×10^3	1.9×10^4
2	Post clarification/TFF*	6.9×10^3	9.4×10^4
3	Post affinity	60	109
4	Post Mustang Q empty capsids	< 0.2	< 10
5	Post Mustang Q full capsids	< 0.2	< 50
6	Post ILDF**/TFF	< 0.2	< 10
7	Post sterile filtration	< 0.2	< 10

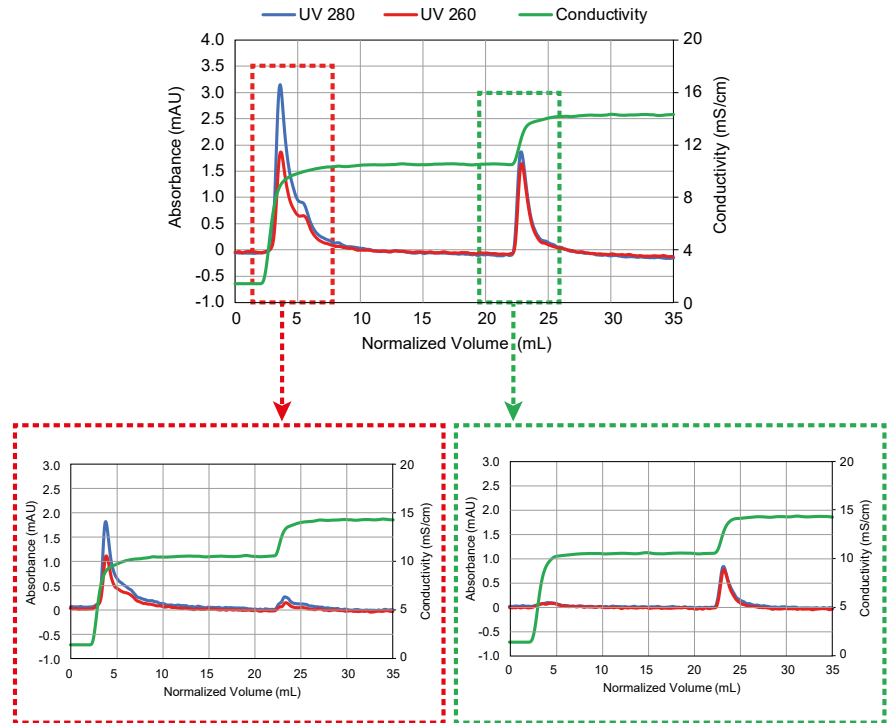
* Tangential flow filtration ** Inline diafiltration

➔ Mustang Q membrane effectively clears remaining contaminants post-affinity

MUSTANG Q XT 0.86 mL ACRODISC UNIT

AAV5 2-Step Elution Reload

- The 1 mS/cm step elution approach was developed into a 2-step elution to reduce the complexity in a manufacturing scenario
- Analytical assays were performed to quantitate total capsids and vector genomes. The relative proportion of full capsids is increased five-fold across steps
- To affirm true population separation, the eluted fractions were individually reloaded onto a Mustang Q membrane and eluted using the same strategy
- The reloaded empty and full fractions, boxed in red and green respectively, elute at the original conductivity step

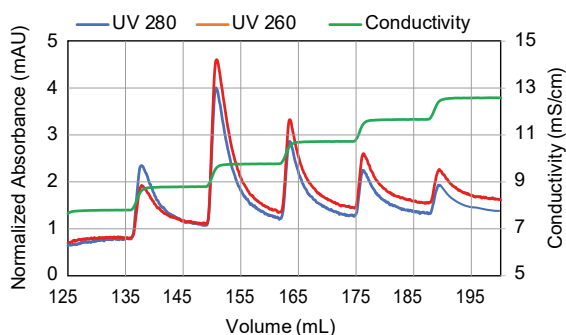


	Step 1	Step 2
Conductivity (mS/cm)	10	14
Total capsids (1×10^{11})	13	7
Total vg (1×10^{11})	1	3
vg/capsids	10%	48%

➔ Reloading reaffirms true separation

AAV8 Step Elution

- AAV8 was loaded and eluted from Mustang Q membrane following the 1 mS/cm step elution protocol developed for AAV5
- Process development suggested sodium acetate provided superior separation to NaCl (not shown)
- An increase in the 260/280 nm absorbance ratio and total vector genomes relative to the total number of capsids is observed from peak 1 to peak 2
- Chromatogram and analytic trends match those observed with AAV5 – enrichment achieved
- High recovery of full capsids



	Load	Peak 1	Peak 2	Peak 3	Peak 4	Sum
Total capsids (1x10 ¹²)	30	4	4	2	1	---
Total vg (1x10 ¹²)	7	1	4	2	0	---
vg/capsids	20%	20%	100%	100%	40%	---
ELISA yield	---	12%	12%	5%	3%	32%
ddPCR yield	---	11%	55%	32%	7%	105%

→ Full capsid enrichment of AAV8 has been achieved with Mustang Q membrane

CONCLUSION

- Mustang Q membrane can separate empty and full capsids of multiple serotypes
 - 1 mS/cm step elution generates distinct populations as observed by 260/280 ratio
 - Empty and full standards confirm separation
- Elution salt can impact separation
- 1 mS/cm steps can be used to determine larger steps
- Reloading of individual peaks confirms separation
- Results obtained on the 0.86 mL Mustang Q XT membrane in Acrodisc unit are scalable to the 5 mL Mustang Q capsule
- HCP and DNA removal is seen following Mustang Q membrane polishing

References

1. Gao, Kai, et al. "Empty Virions in AAV8 Vector Preparations Reduce Transduction Efficiency and May Cause Total Viral Particle Dose-Limiting Side Effects." *Molecular Therapy - Methods & Clinical Development*, vol. 1, 1 Jan. 2014, p. 9, doi:10.1038/mtrm.2013.9.
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3. Wang, Qizhao, et al. "Syngeneic AAV Pseudo-Particles Potentiate Gene Transduction of AAV Vectors." *Molecular Therapy - Methods & Clinical Development*, vol. 4, 17 Mar. 2017, pp. 149–158, doi:10.1016/j.omtm.2016.12.004.



Corporate Headquarters

Port Washington, NY, USA
 +1-800-717-7255 toll free (USA)
 +1-516-484-5400 phone

European Headquarters

Fribourg, Switzerland
 +41 (0)26 350 53 00 phone

Asia-Pacific Headquarters

Singapore
 +65 6389 6500 phone

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