Accelerating advancement in gene therapy by improving downstream purification of viral vectors

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Bioprocessing

INTRODUCTION

Adeno-Associated Virus (AAV) has become the vector of choice in many gene therapies. Recent advances in chromatography resin development for viral vectors have demonstrated that AAV purification can be scalable and efficient, achieving high purity and yield in a single step. With an extensively growing pipeline of gene therapy clinical trials, it is evident that scalable production solutions are needed. Here we outline the benefits of implementing affinity chromatography in the downstream purification of viral vectors.

AFFINITY SOLUTIONS FOR VIRAL VECTOR PURIFICATION

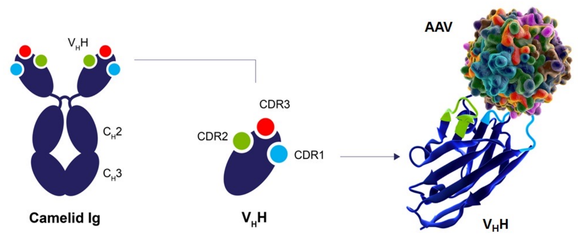
Thermo Scientific™ resin	Binding Capacity (vg/mL)	Serotype Affinity
POROS™ CaptureSelect™ AAV8	>10^{13}	AAV8
POROS™ CaptureSelect™ AAV9	>10^{14}	AAV9
POROS™ CaptureSelect™ AAVX	>10^{14*}	AAV1, AAV2, AAV3, AAV4, AAV5. AAV6, AAV7, AAV8, AAV9, recombinant & chimeric vectors

* viral genomes per millilitre (vg)/mL, binding capacity will vary based on serotype, feed stream, additives, and mutations to parent serotypes

- Affinity through antibody selectivity (CaptureSelect™ technology) : technology based on single domain [V_HH] antibody fragments.
- Animal origin free production process (*Saccharomyces cerevisiae)*
- Combined with the large through-pore POROS backbone

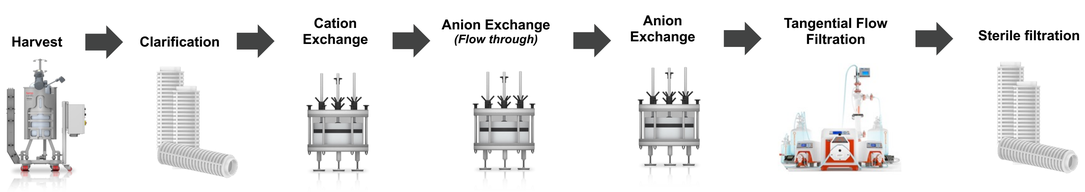
Fig.1 CaptureSelect™ ligands are V_HH fragments (single domain antibody fragments – sdAb), the smallest antigen binding molecule.

The small size of V_HH fragments (15kD) allows binding at difficult to reach epitopes. Overall, V_HH fragments offer high specificity, affinity and stability.



✓ **Combining antibody-based selectivity and process robustness in unique AAV affinity resins**

ENABLING INDUSTRIAL SCALE DEVELOPMENT OF AAV VECTORS



CaptureSelect™ Paradigm
- Affinity capture = fewer chromatography steps
- Simplified process
- Lower cost & speed to market
- Fewer steps = higher yield

Customer testimonial: "Process yield improvement from 20% to 60% & cost reduction by a factor of 6"

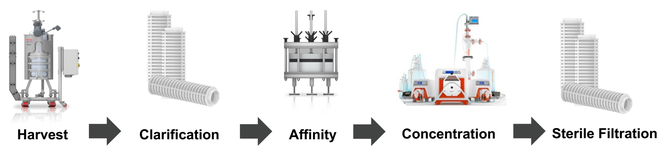
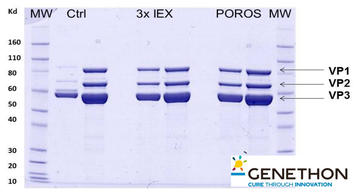


Fig. 2 Reduced number of process steps through AAV affinity chromatography resins

PURITY

SDS-PAGE purity profile of AAV9



YIELD

Vector recovery using POROS CaptureSelect AAV9 at various scales

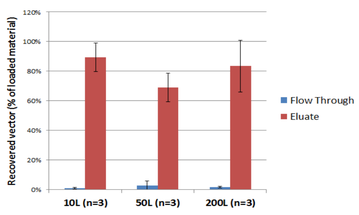


Fig. 3 Vector purity. A 1-step Comparible purity, as determined by SDS page, was obtained between a 1-step AAV9 affinity step en 3 IEX steps.

Fig. 4 Vector recovery. Yields > 80% were obtained from 10 L tot 200 L scale.

✓ **Reduce the number of steps in a purification procedure without compromising product purity and yield**

✓ **Increase process flexibility and throughput**

UNDERSTANDING THE VIRAL CLEARANCE POTENTIAL

Viral clearance data from an AAV8 clinical production process using the AAVX resin.

	RNA env	DNA non-env	RNA non-env		DNA env	
Run Description	**XMuLV**	**MVM**	**Reo-3**	**HAV**	**PRV**	**HSV-1**
Manufacturing process conditions	> 6.4	4.4	2.7	> 4.9	4.0	3.1
Higher load ratio + residence time *(worst case scenario)*	4.6	3.6	2.5	5.0	3.8	3.6
≥ 4 LRV Effective / 1 - 3 LRV Contributing / < 1 LRV Negligible — Clearance	Effective	Effective	Contributing	Effective	Effective	Contributing

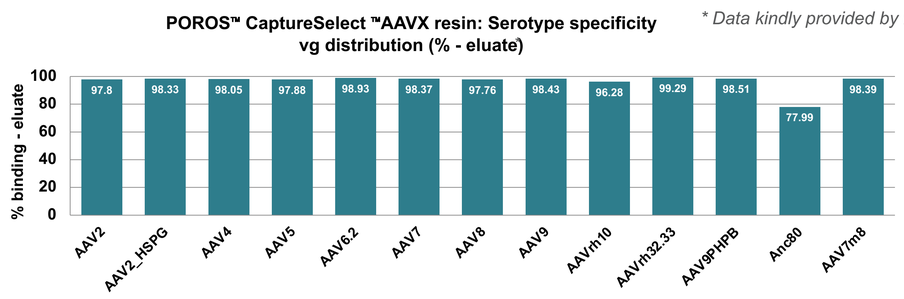
Table 1. Clearance of model viruses. Log reduction values (LRV) of enveloped (env) and non-enveloped (non-env) model viruses using the AAVX resin.

✓ **The AAVX resin can be an effective viral clearance step in the downstream process of AAV production**

POROS™ CAPTURESELECT™ AAVX RESIN: A TRUE PLATFORM FOR AAV PURIFICATION

- ✓ **Broad selectivity to both natural and synthetic capsids**
- ✓ **High dynamic binding capacity**
- ✓ **High elution recovery at different flow rates**
- ✓ **Robust, with less process optimization**

AAVX SEROTYPE SPECIFICITY



** Data kindly provided by Massachusetts Eye and Ear*

Fig.6 AAVX resin serotype specificity with a large variety of serotypes.

Experimental settings. Static binding mode experiment: resin was mixed with AAV serotype in tube – no flow properties performed. vg was determined by qPCR

✓ **To date, the AAVX ligand has shown affinity towards all serotypes tested**

BREAKTHROUGH ANALYSIS AND ELUTION RECOVERY

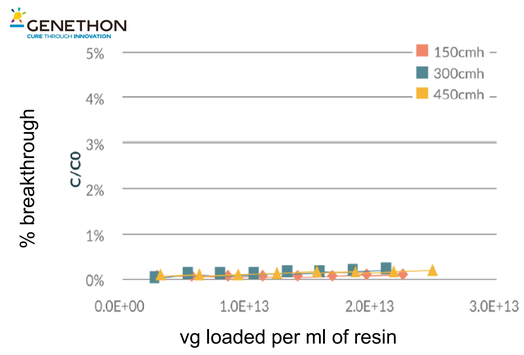


Fig. 7 AAVX Breakthrough analysis. Breakthrough as a function of vg loaded per ml of resin. The breakthrough stayed below 0.5% up to 2e13 VG/ml of resin.

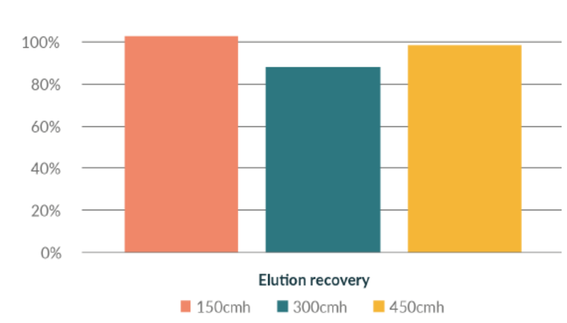


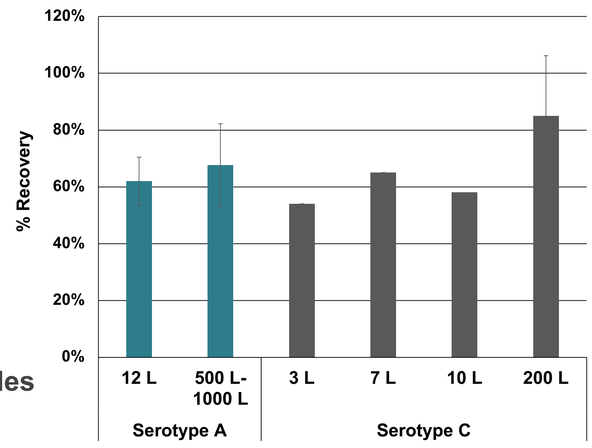
Fig. 8 Elution recovery. Elution recovery was above 80% at each of the three different flow rates used. Recovery was not influenced by flow rate or residence time.

✓ **High capacity and a high degree of process design flexibility**

✓ **High elution recovery at different flow rates**

DEMONSTRATING SCALABILITY

Fig. 9 Purification yield is comparable at various scales. Vector recovery in eluate after using POROS™ CaptureSelect™ AAVX resin for the purification of two different serotypes at various production scales.



Use of AAVX at various scales shows:

- ✓ **Robust resin scale up**
- ✓ **Comparable recoveries at various scales**
- ✓ **Consistency in resin performance**

CONCLUSIONS

POROS™ CaptureSelect™ AAV resins address the current challenges involved with viral vector purification. Use of these resins will:

- **Simplify your purification process and increase process design flexibility**
- **Increase purity and yield**
- **Allow for scalable purification of multiple AAV serotypes in a single platform**

In addition we have demonstrated that the AAVX resin can be an effective viral clearance step in the downstream process of AAV manufacturing

TRADEMARKS/LICENSING

Learn more at thermofisher.com/captureselect

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