

# Downstream Purification Solutions for Viral Vectors: Enabling Platform Approaches to Advance Cell and Gene Therapies

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## Introduction

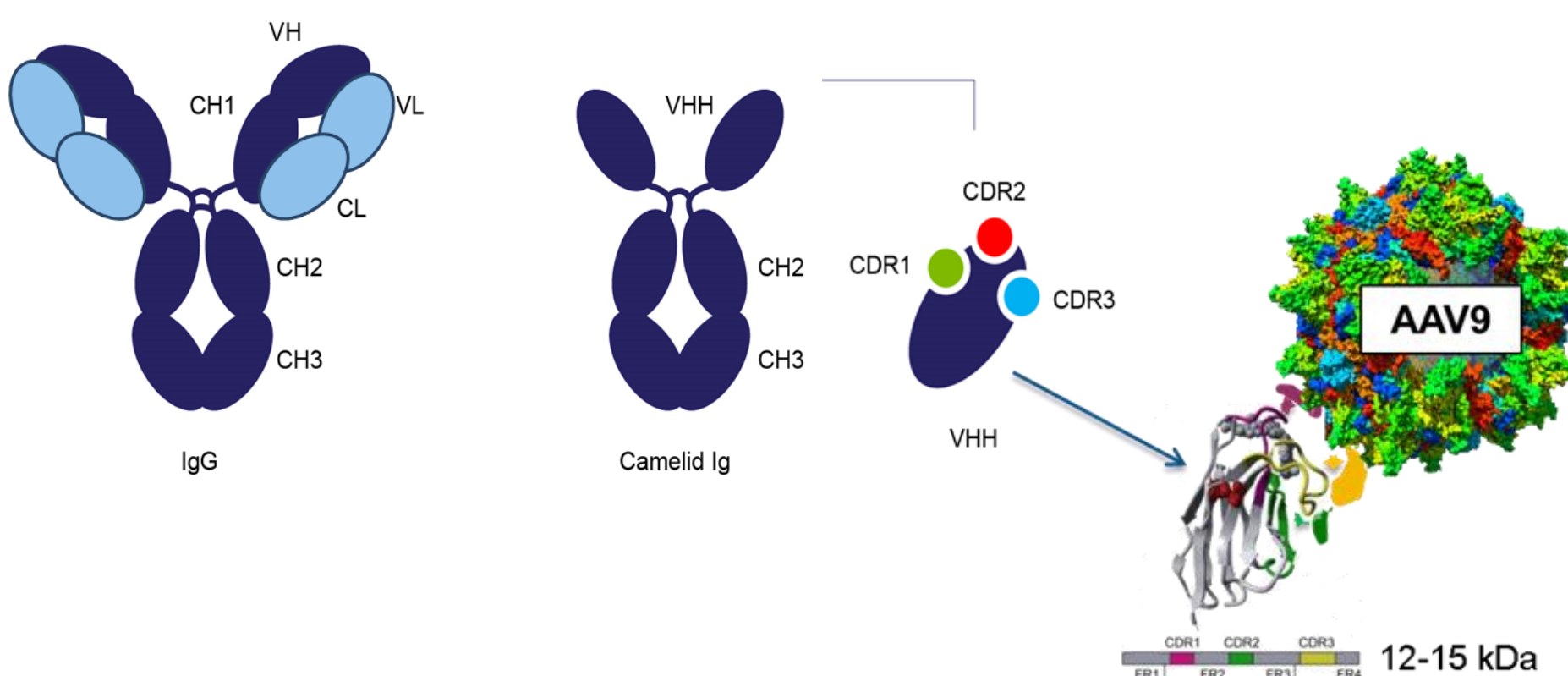
Over the past decade, gene therapy applications and their importance in the biopharmaceutical industry have been increasing. Vectors centered on the nonpathogenic adeno-associated virus (AAV) have emerged as the vector of choice for many therapies. Although the biopharmaceutical industry is primed to purify viral vectors on a scale suitable for clinical supply or orphan indications, it is still working toward generating industrialized platform technologies that will maximize productivity and enable efficient, simple, and inexpensive purification of biologically active viral vectors for large-scale commercial manufacturing. Thermo Fisher Scientific has developed downstream purification solutions enabling the scale-up of these viral vectors.

## CaptureSelect™ Affinity Technology

Thermo Scientific™ CaptureSelect™ purification and affinity separation products are developed for the discovery and manufacturing of therapeutic proteins, including various antibody formats and viral vectors. These products help enable a reduced time-to-market and an overall lower cost of goods for biopharmaceutical development via increased product recovery and purity.

### CaptureSelect Ligands

- Affinity to 3 complementarity defining regions (CDR) provides unique, tunable specificity
- Ligand candidates are screened for optimal elution & stability to help ensure process robustness
- Produced in an animal origin free yeast expression process



- ✓ **Single-domain [VHH] antibody fragments combining antibody-based selectivity and process robustness**

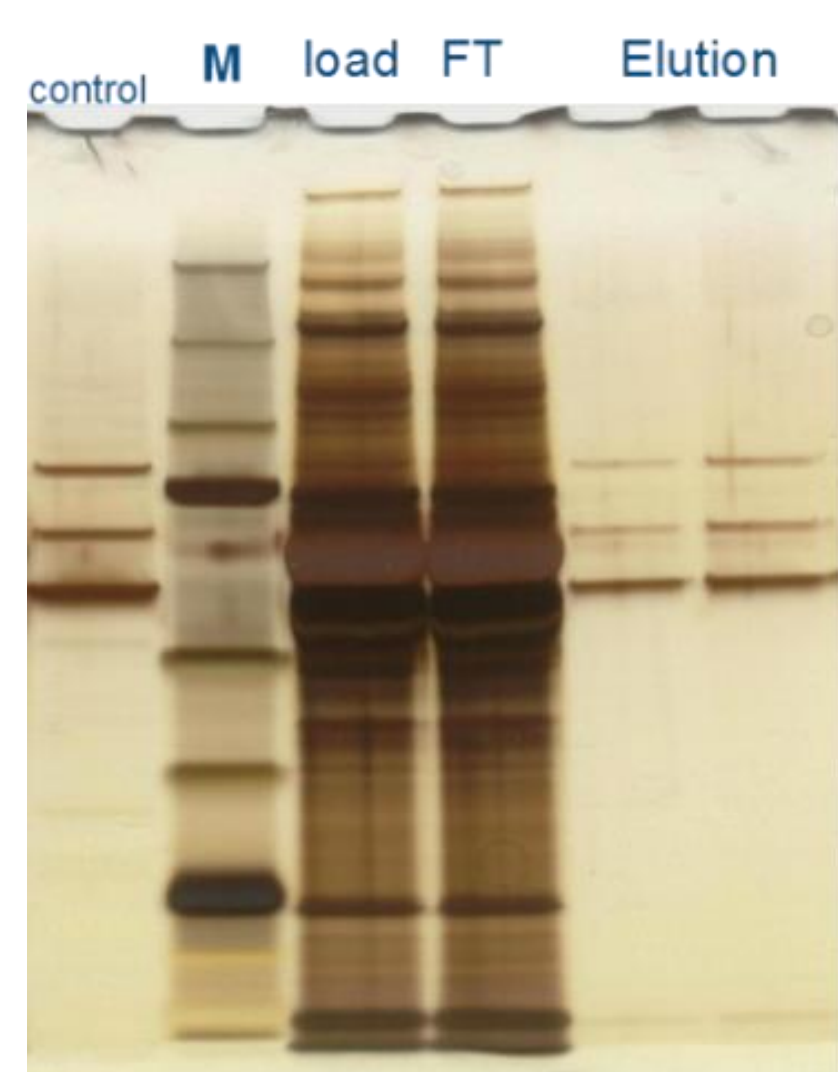
## Affinity Purification of Adeno-Associated Virus

By using the CaptureSelect™ technology a unique affinity ligand was developed for the highly specific capture of Adeno-associated virus particles (AAVs), which enabled the successful introduction of a first antibody-based affinity chromatography medium (i.e. AVB Sepharose™ High Performance, GE Healthcare), now widely applied in purification processes of AAV vectors, capturing AAV1, 2, 3 and AAV5. High target purity and yield can be obtained in a single capture step thereby circumventing the need of standard methods like cesium chloride gradient centrifugation that can be challenging for scale-up.



## POROS™ CaptureSelect™ AAV8 and AAV9 Affinity Matrix

To meet the large-scale manufacturing demands in gene therapy applications for emerging AAV vectors, two new CaptureSelect™ affinity ligands have been developed and coupled to a rigid POROS™ backbone for the affinity purification of AAV vectors of serotype AAV8 and AAV9, respectively. The POROS™ CaptureSelect™ AAV8 and AAV9 affinity matrices have a unique pore structure that is ideal for large-sized macromolecules like viral particles and support purification at considerably faster flow rates than conventional liquid chromatography separations while maintaining high dynamic binding capacity.

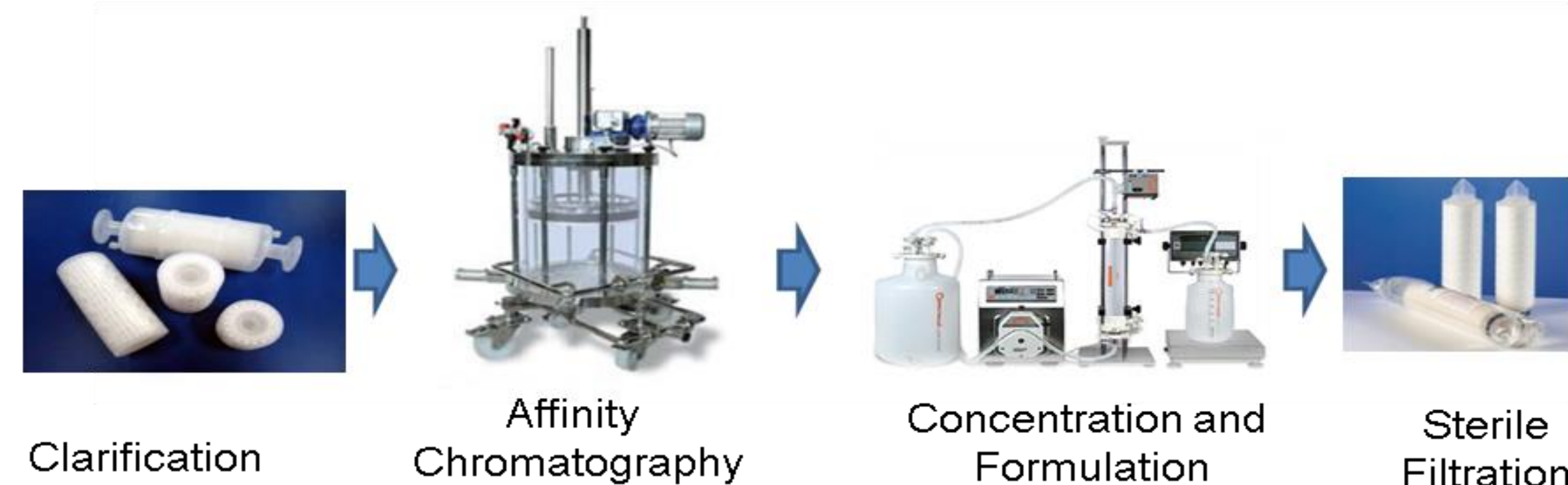


Thermo Scientific™ resin	Binding capacity (vg/mL)
POROS™ CaptureSelect™ AAV9	>10 <sup>14</sup>
POROS™ CaptureSelect™ AAV8	>10 <sup>13</sup>

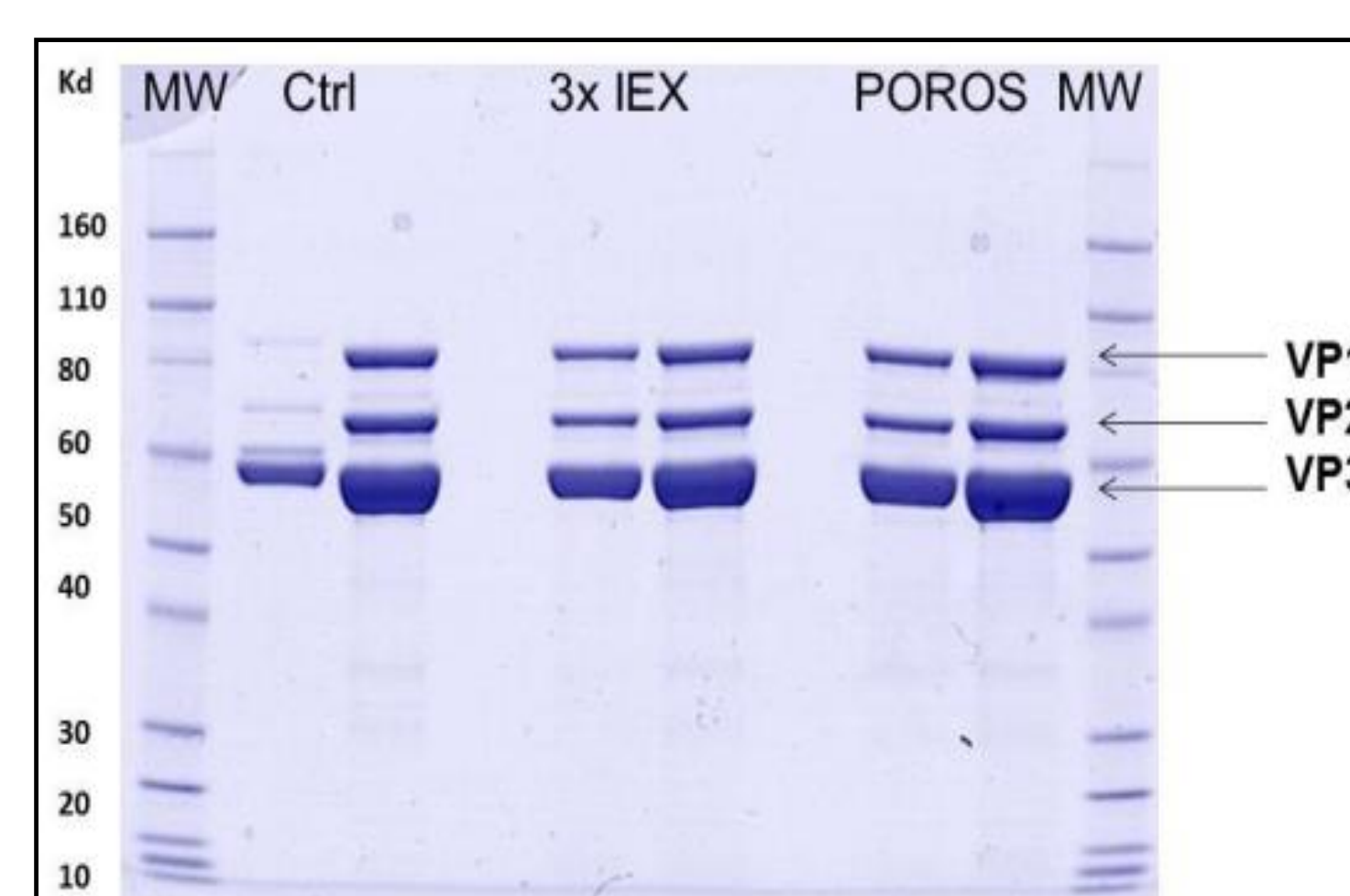
- ✓ **Affinity purification of AAV in a single step with high capacity**

## Proven Affinity Solution for Viral Vector Purification

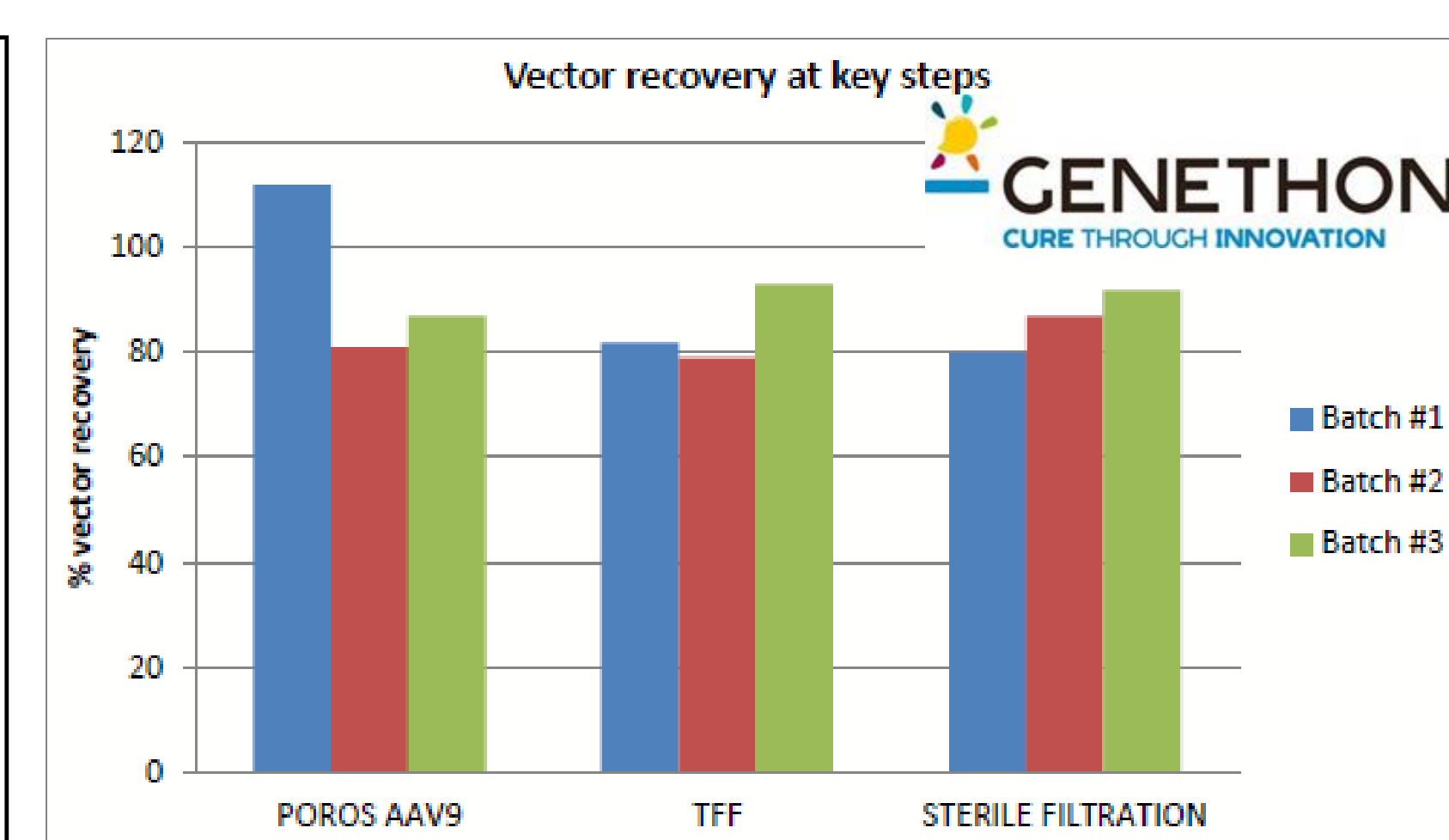
POROS CaptureSelect™ AAV8 and AAV9 resins combine the resolving and flow properties of POROS™ beads with the tunable specificity of CaptureSelect™ ligands to provide high capacity, scalable solutions for AAV capture. This combination enables a more efficient, lower cost and higher yielding purification process than traditional methods.



### Purity



### Process yield



POROS Capture Select AAV9 resin in a single step affinity process achieved an equivalent purity profile to a 3-step IEX process. Vector recovery throughout the process was greater than 80%. The recovery stayed consistent throughout scale up attesting to the scalability of POROS CaptureSelect™ AAV resins.

- ✓ **POROS Capture Select AAV resin simplifies processes and reportedly reduced costs by a factor of 6 and increased the process recovery from 20% to 60%**

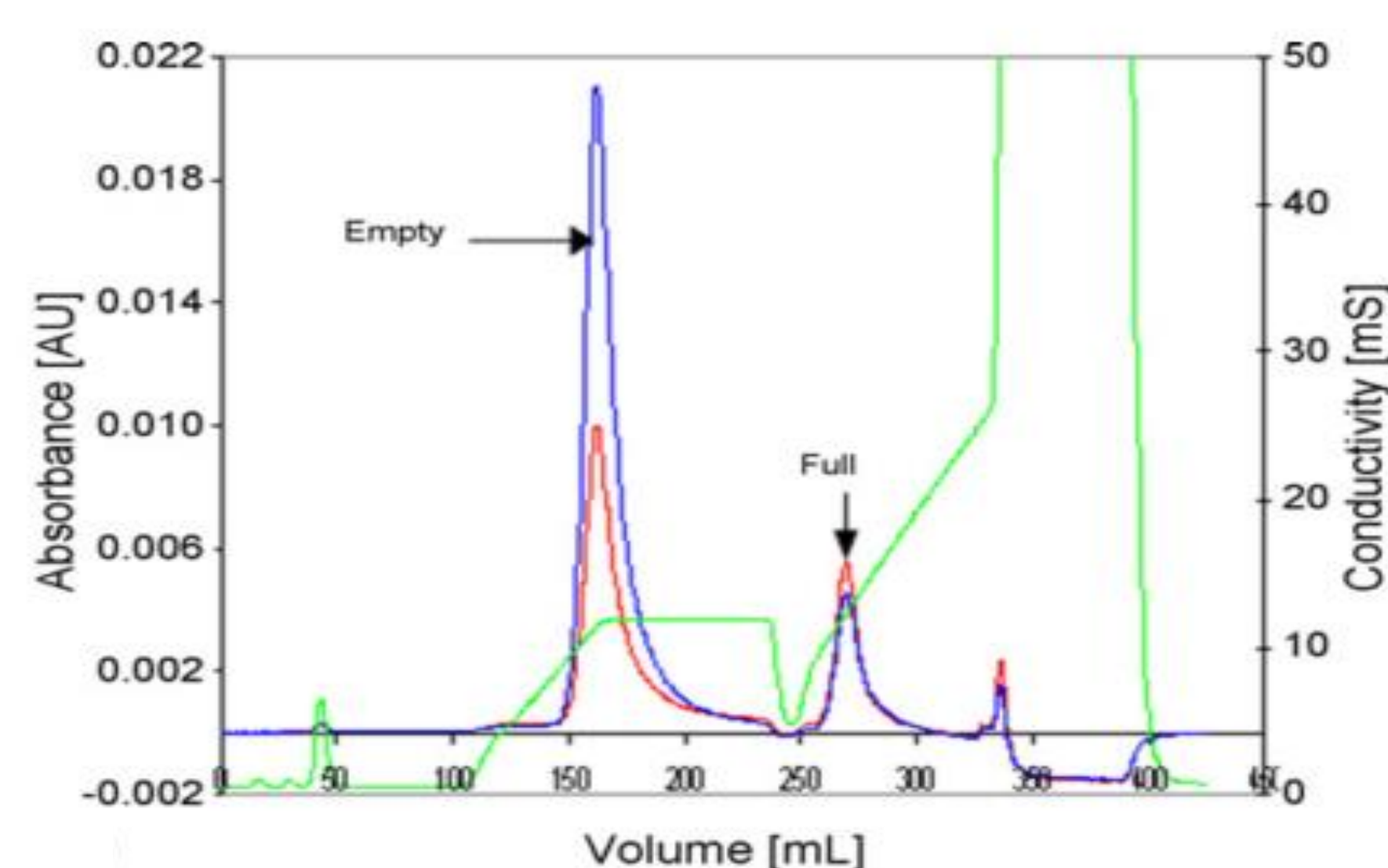
## POROS Anion Exchange (AEX) for Empty/Full Separation

The separation of AAV capsids lacking genetic material (empty capsids) from those containing genetic material is a common consideration in the purification process. POROS ion exchange resins have been proven to provide very effective resolution in this application (POROS HQ, PI, D, and XQ).

	Full	Empty
Size	20 nM (200 Å)	20 nM (200 Å)
Density	1.40 g/cm <sup>3</sup>	1.32 g/cm <sup>3</sup>
Average pI	5.9	6.3

Qu W, Wang M, Wu Y, Xu R. *Curr Pharm Biotechnol.* 2015;16(8):684-95.

Density and Isoelectric point are two differences that can be exploited when attempting to separate empty and full capsids. Centrifugation can be utilized to separate by density, but has scalability limitations.



G. Qu et al. *Journal of Vir Methods.* 2007;140: 183-192.

- ✓ **POROS CaptureSelect AAV8 and AAV9 affinity and POROS AEX resins provide an industrialized purification platform for efficient scale up and commercial manufacturing of gene therapies designed to ultimately meet market demands.**