Addressing AAV manufacturing challenges-
Innovative solutions across the viral vector production workflow

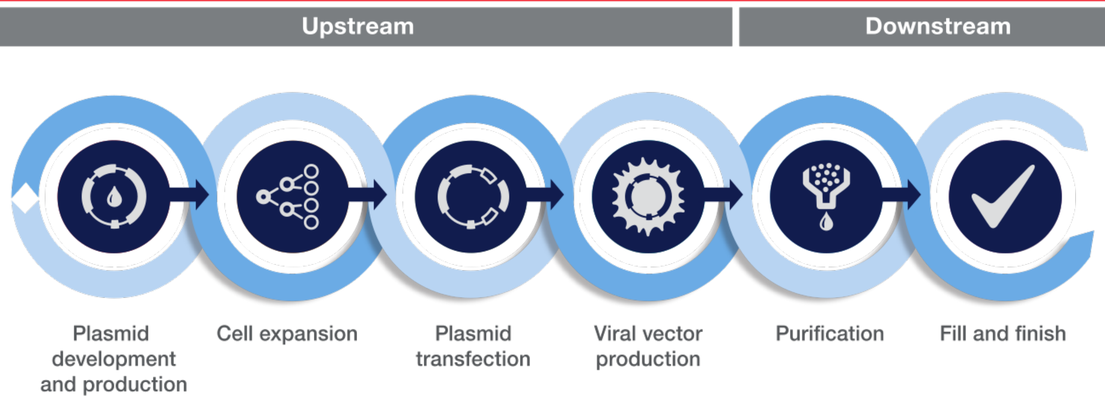
Jessica de Rooij, Orjana Terova, Pim Hermans, Frank Detmers, Florian Durst, Darren Bauer, Hetal Brahmbhatt & Megan Hardy
Thermo Fisher Scientific, Waltham, MA

ThermoFisher SCIENTIFIC

Bioprocessing

AAV workflow

Upstream | Downstream



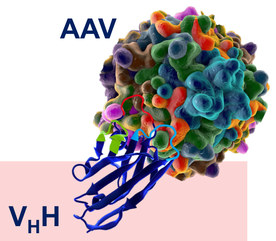
INTRODUCTION

Each production process for viral vectors has its own unique impediments and AAV is no exception. A successful production workflow must be consistent, reproducible, scalable and deliver high-titer product free from impurities so it can pass regulatory requirements.

Working together with leading gene therapy developers, Thermo Fisher Scientific has realized innovative solutions that span the entire viral vector production workflow. Here we present our technologies developed for improving viral vector manufacturing and outline new developments in the field of purification of AAV and other viral vectors.

DOWNSTREAM – SIMPLIFY AAV PURIFICATION

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



- ✓ Based on CaptureSelect camelid-derived single domain (V_HH) technology
- ✓ Broad selectivity to both natural and synthetic capsids
- ✓ High dynamic binding capacity
- ✓ High elution recovery at different flow rates
- ✓ Robust, with less process optimization steps
- ✓ Limits the number of steps in a purification procedure without compromising product purity and yield

A SCALABLE AAV PURIFICATION SOLUTION

The use of AAVX at various scales shows:

- ✓ The robustness of the resin during upscaling
- ✓ Comparable recoveries at various scales
- ✓ Consistency in resin performance

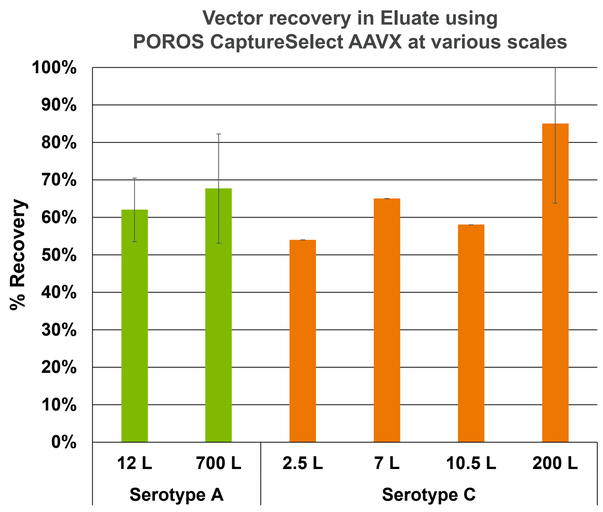


Fig. 1 Purification yield is comparable at various scales. Vector recovery in Eluate after using POROS CaptureSelect AAVX resin for the purification of two different serotypes at various production scales.
Purification was performed in dynamic mode. % of vector recovered determined using total vector in Eluate relative to total vector in load.

The use of AAVX at various total vector loading amounts shows minimal variation in recovery.

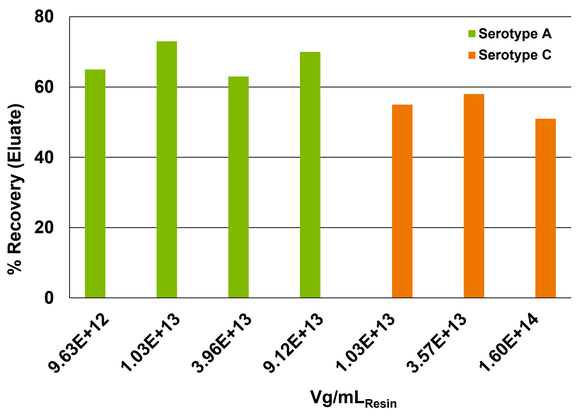
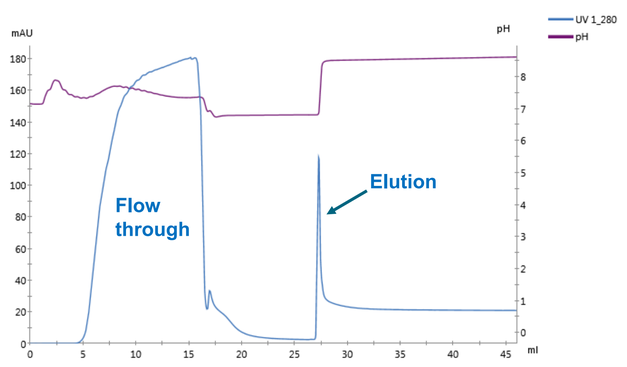


Fig. 2 Purification recoveries are comparable for at least 10-fold total vector load differences. Determine vector binding range to establish optimal purification conditions.

DOWNSTREAM – PURIFICATION OF OTHER VECTORS

ADENOVIRUS PURIFICATION* – MILD ELUTION



Sample	Viral Genomes	Recovery Rate [%]
Sample AdV	1.62 E+08	-
Flow Through	1.15 E+07	7.08
Wash	6.83 E+05	0.42
Elution	1.50 E+08	**92.66**

Experimental details	
Resin	POROS CaptureSelect AdV5
Column dimensions	0.5 cm x 5.0 cm
Sample	6.3 ml AdV5
Flow rate	0.7 ml/min
Equilibration buffer	20mM Tris pH 7
Wash buffer	20mM Tris pH 7, 0.1M NaCl
Elution buffer	1M Arginine, 1M NaCl – **pH 8.5**

Fig. 3 Purification of AdV5 showing high recovery mild elution.
After **e**lution optimization, performed by varying pH and salt concentration, a nearly 93% recovery of AdV5 is obtained by eluting at a very mild pH of 8.5

- ✓ **The POROS CaptureSelect AdV5 resin demonstrates efficient elution at mild pH, which helps to maintain activity of the intact enveloped virus (AdV5)**

***Data kindly provided by Labor Dr. Merk & Kollegen GMBH**

CELL & GENE THERAPY PURIFICATION RESINS DEVELOPMENT PIPELINE

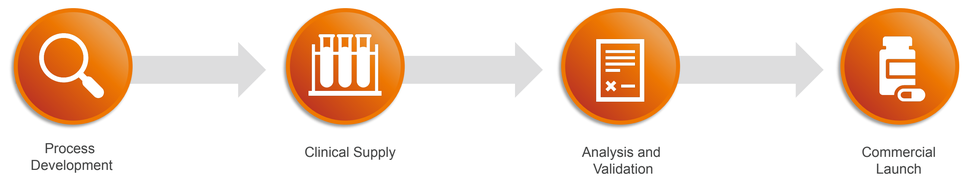
Together with leading industry partners in cell and gene therapy, new affinity resins are in development:

Prototype resin testing	Lead selection	RUO resin	Bioprocess resin
• Lentivirus (VSV-G) • Exosomes (CD81)	• Influenza (HA) • Baculovirus (depletion resin)	• AdV5	• AAV8 • AAV9 • AAVX

ACROSS THE WORKFLOW- VIRAL VECTOR CONTRACT DEVELOPMENT

brammer bio
part of Thermo Fisher Scientific

Thermo Fisher Scientific's Viral Vector CDMO Services (Brammer Bio) support clients from drug development, through clinical trials to full scale manufacturing.



A PLATFORM FOR MANUFACTURING OF VIRAL VECTORS

- 10 viral vector manufacturing platforms
  - *AAV, Adenoviral, Herpesviral, Lentiviral, Retroviral*
  - *Suspension: 200 L to 1000 L*
  - *Adherent: Up to 48 HYPERstack® - 36 layer or iCELLis® 500 ***
- State of art manufacturing capabilities – from preclinical to commercial development
- Custom and platform process development options suitable for manufacturing offered

**Hyperstack is a registered trademark of Corning Incorporated. iCELLis is a registered trademark of Pall Corporation

- ✓ **Brammer Bio has the capability the help customers deliver innovative gene therapies**

ACROSS THE WORKFLOW - ANALYTICS

To help ensure regulatory approval of the gene therapy, QC testing must be implemented. Thermo Fisher Scientific has developed rapid molecular methods for contaminant and impurity testing across the viral production workflow, such as mycoplasma screening during cell culture and residual DNA testing following virus harvest and purification.

- Rapid mycoplasma screening with the MycoSEQ Mycoplasma detection system
- Host cell residual DNA testing with the resDNASEQ quantitation system

TRADEMARKS/LICENSING

Learn more at thermofisher.com/bioprocessing

thermo scientific