

## HPLC columns and accessories

# Adeno-associated viruses (AAVs)

## complete workflow solutions

Adeno-associated viruses (AAVs) have gained significant attention in the field of gene therapy due to their ability to efficiently and safely deliver genetic material to target cells in the body. AAVs are important because they offer a potential treatment option for genetic disorders and other diseases caused by faulty genes. By delivering a functional copy of a gene to the affected cells, AAV-based gene therapy can potentially correct the underlying cause of the disease. AAVs have already been used in clinical trials for a range of diseases, including inherited retinal disorders, hemophilia, spinal muscular atrophy, and for vaccine production. An AAV is composed of an icosahedral protein shell with a single-stranded genome of approximately 4.7 kb. The intact AAVs act as a vehicle to protect and deliver oligonucleotide therapeutics.

AAV based gene therapeutics are more complex than many traditional biotherapeutics. Aside from the full capsid containing the desired gene material, the final product could contain many different types of process- and product-related impurities.

Full characterization, including sequence and post-translational modification (PTM) identification of viral proteins is required to mitigate immunogenicity and ensure the safety, quality, and efficacy of AAV products.

- The full capsid needs to be carefully characterized to ensure product efficacy
- Impurities such as host cell DNA and proteins need to be accurately characterized and controlled to ensure product quality and safety

A successful viral manufacturing pipeline must deliver a consistent, pure, and high-titer product that exhibits good safety and efficacy to meet regulatory expectations.

### There are different solutions for AAV analysis, depending on what your needs are:

- If you need to separate proteins and glycoproteins, then a strong anion-exchange column is recommended based on their surface charge, allowing for rapid and efficient separation of full and empty capsids
- For peptide mapping use reversed phase columns. They offer exceptional performance for a wide variety of peptides, delivering high resolution and critical quality attribute analysis while minimizing retention times for hydrophobic peptides
- Use a size exclusion column to efficiently assess AAV product titers after affinity purification, lowering molecular weight substances, facilitating precise quantification of viral capsid concentration

## Workflow solution for determination of full and empty AAV using SAX

The Thermo Scientific™ ProPac™ 3R SAX column enables rapid, efficient, and high-resolution separation of proteins and glycoproteins based on surface charge. Its 3 μm, non-porous particles, made of a compatible polymer resin, offer exceptional resolving power. With improved column packing and reproducibility, thanks to consistent size distribution, this column effectively separates AAV empty and full capsids and other impurities using linear salt gradients. The unique design ensures the high resolution, robust performance, and lot-to-lot reproducibility required for AAV analysis. [Application note](#)

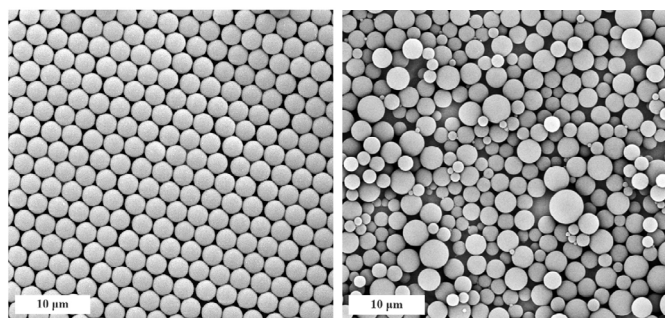


Figure 1. SEM image of 3 μm monodisperse particles (left) vs. 3 μm polydisperse particles (right). White scale bars are 10 μm in length.



### Workflow solution for determination of full and empty AAV using SAX

Description	Quantity	Cat. No.
Thermo Scientific Columns		
ProPac 3R SAX Column, 3 μm, 2 × 50mm	Each	17807903
Thermo Scientific Vials and Caps		
SureSTART™ 0.3mL GOLD-Grade Clear Glass Insert	100/pack	17393973
SureSTART™ 2mL Vial Clear Glass (for holding insert)	100/pack	17343893
SureSTART™ 9mm Screw Cap	100/pack	17334063
Thermo Scientific Instruments		
Vanquish™ Flex Quaternary UHPLC System*	Each	IQLAAAGABHFAPUMBHV
Vanquish™ Flex System Base*	Each	VF-S01-A
Vanquish™ Quaternary Pump*	Each	VF-P20-A
Vanquish™ Column Compartment H*	Each	VH-C10-A
Vanquish™ Split Sampler FT*	Each	VF-A10-A
Vanquish™ Fluorescence Detectors*	Each	VF-D51-A
Vanquish™ Fluorescence Detector F Flow Cells*	Each	6079.4230

\*Please request a quotation

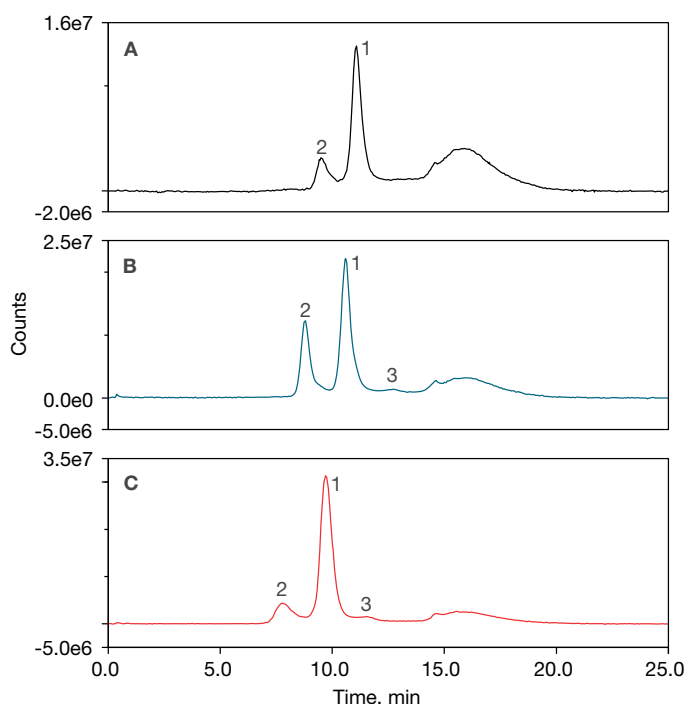


Figure 2. Linear salt gradient separation of full capsid AAV samples spiked with empty capsid to give a 1:10 Empty: Full ratio. (A): AAV1 sample, (B): AAV6 sample and (C) AAV8 sample

## Workflow solution for determination of AAV titers using SEC

The Thermo Scientific™ MAbPac™ SEC-1 column can be used to provide a cost-efficient and precise method to rapidly determine AAV product titers following a single-step affinity purification. Using the MAbPac SEC-1 HPLC column efficiently separates AAV capsids from lower molecular weight compounds and therefore enables precise analysis of the viral capsid concentration.



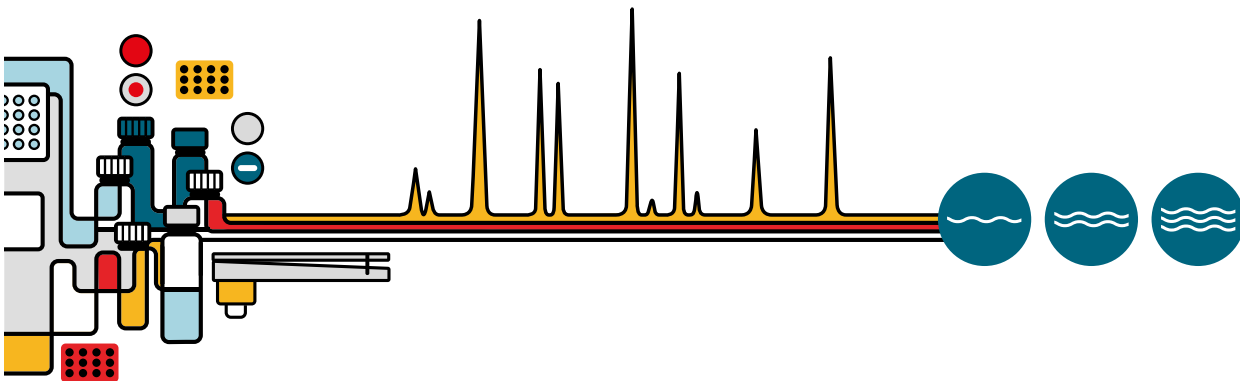
### Workflow solution for determination of AAV titers using SEC

Description	Quantity	Cat. No.
Thermo Scientific Columns and Consumables		
MAbPac SEC-1 Column, 2.1 × 150mm	Each	15590244
Thermo Scientific vials and caps		
SureSTART™ 2mL Polypropylene Vial	100/pack	17303923
SureSTART™ 9mm Screw Cap	100/pack	17334043
Thermo Scientific Instruments		
Vanquish™ Horizon UHPLC System*	Each	QLAAAGABHFAPUMBHV
Orbitrap Exploris™ 480 Mass Spectrometer*	Each	BRE725535

\*Please request a quotation

## Recommended vials and caps

We recommend our Thermo Scientific™ SureSTART™ Performance Level 3 products as they contain the lowest amounts/levels of metals, extractables and leachables, ensuring that you can obtain the sensitivity and selectivity for your most challenging analytes consistently and with confidence.



## Workflow solution for AAV protein analysis and host cell protein profiling

The Thermo Scientific™ Hypersil GOLD™ C18 UHPLC column is an excellent choice for a broad range of peptides, offering high resolution for all critical quality attributes without extremely long retention times for more hydrophobic peptides. The column offers sub-2µm particles providing ultra-short diffusion paths that result in extremely efficient separations.



### Workflow solution for AAV protein analysis and host cell protein profiling

Description	Quantity	Cat. No.
Thermo Scientific columns		
Hypersil GOLD™ C18 column, 1.9 µm, 1 × 100 mm	Each	10715004
Thermo Scientific vials and caps		
SureSTART™ 0.3mL GOLD-grade clear glass insert	100/pack	17393973
SureSTART™ 2mL vial clear glass (for holding insert)	100/pack	17343893
SureSTART™ 9mm screw cap	100/pack	17334063
Thermo Scientific instruments		
Vanquish™ Flex Quaternary UHPLC system	Each	IQLAAAGABHFAPUMBHV
Quaternary Pumps	Each	VF-P20-A
Vanquish™ Split Samplers	Each	VF-A10-A-02
Vanquish™ fluorescence detector	Each	VF-D50-A

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