LC columns

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

- AAV aggregates need to be monitored to ensure product efficacy

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 its surface charge, allowing for rapid and efficient separation of full and empty capsids

- Use a size exclusion column to efficiently monitor AAV aggregation, product titers after affinity purification, and lower molecular weight impurities

- Reversed-phase columns are highly recommended for peptide mapping due to their exceptional performance with a diverse range of peptides. These columns provide high resolution and enable critical quality attribute analysis.

thermo scientific

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 polymer resin, offer exceptional resolving power. With improved column packing and reproducibility, thanks to consistent particle size distribution, this column effectively separates AAV empty and full capsids and other impurities using linear salt gradients. The unique design ensures high resolution, robust performance, and lot-to-lot reproducibility required for AAV analysis.
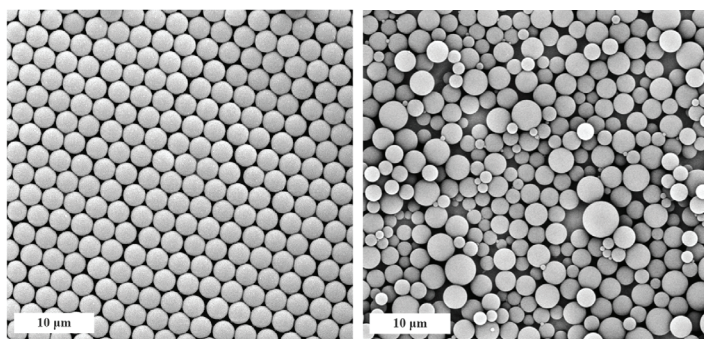

Figure 1. SEM image of 3 µm monodisperse particles (left) vs. 3 µm polydisperse particles (right). The homogeneous particle size enables consistent column-to-column reproducibility
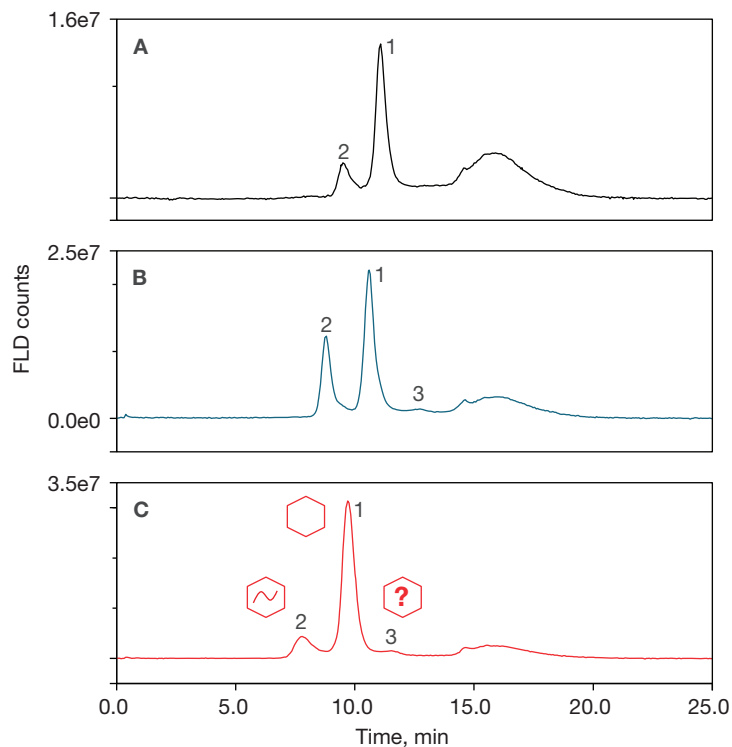

Figure 2. Linear salt gradient separation of full capsid AAV samples spiked with empty capsid. Fluorescence detection. Empty:Full ratio 1:10. (A): AAV1 sample, (B): AAV6 sample, and (C): AAV8 sample

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 × 50 mm	Each	43203-052068
Thermo Scientific vials and caps		
Thermo Scientific™ SureSTART™ 0.3 mL GOLD-Grade Clear Glass Insert	100/pack	6PME02CG
Thermo Scientific™ SureSTART™ 2 mL Clear Glass Vial, for holding insert	100/pack	6ASV9-1P
Thermo Scientific™ SureSTART™ 9 mm Screw Cap, PTFE/silicone/PTFE septa	100/pack	6PSC9TST
Thermo Scientific instruments		
Thermo Scientific™ Vanquish™ Flex Quaternary UHPLC System	Each	IQLAAAGABHFAPUMBHV
Thermo Scientific™ Vanquish™ Fluorescence Detector with standard flow cell	Each	VF-D51-A

Determination of AAV aggregates with SurePac Bio SEC MDi columns

Our groundbreaking MDi™ technology integrates our advanced monodisperse silica particle platform with biocompatible, inert hardware, ensuring superior reliability in size-exclusion chromatography. With our Thermo Scientific™ SurePac™ Bio 550 SEC MDi™ HPLC Column, leverage the power of 3 μm monodisperse silica particles for robust, reproducible, and highly efficient separations and higher throughput, outperforming traditional polydisperse 5 μm media.
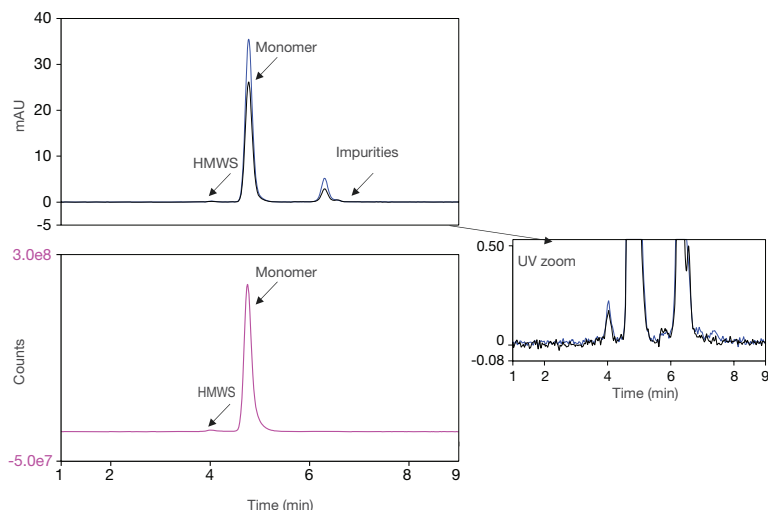

Figure 3. Separation of AAV8 monomer and high molecular weight species using both UV and FLD detection.

Workflow solution for determination of AAV titers using SEC

Description		Quantity	Cat. no
Thermo Scientific columns			
Thermo Scientific™ SurePac™ Bio 550 SEC MDi™ Analytical Column, 3 μm	2.1 x 150 mm		**43903-152131**
	4.6 x 150 mm		**43903-154631**
	7.8 x 150 mm		**43903-157831**
Thermo Scientific™ SurePac™ Bio 550 SEC MDi™ Guard Column, 3 μm	2.1 x 30 mm		**43903-032131**
	4.6 x 30 mm		**43903-034631**
	7.8 x 30 mm		**43903-037831**
Thermo Scientific vials and caps			
Thermo Scientific™ SureSTART™ 2 mL Polypropylene Screw Vial		100/pack	**6ESV9-04PP**
Thermo Scientific™ SureSTART™ 9 mm Screw Cap		100/pack	**6PSC9ST1**
Thermo Scientific instruments			
Vanquish Flex Quaternary UHPLC system		Each	**IQLAAAGABHFAPUMBHV**
Vanquish Fluorescence detector		Each	**VF-D51-A**
Thermo Scientific™ Vanquish™ Variable Wavelength Detector		Each	**VF-D40-A**

Workflow solution for AAV protein analysis and separation

The Thermo Scientific™ Hypersil GOLD™ Peptide Columns are an excellent choice for consistent retention of all peptides, including hydrophilic peptides and resolution of deamidated species. Designed to provide retention time stability and lot-to-lot consistency, the Hypersil GOLD Peptide column meets the needs for the development and manufacturing control of biopharmaceutical products.

Workflow solution for AAV protein analysis and host cell protein profiling

Description	Quantity	Cat. no
Thermo Scientific columns		
Thermo Scientific™ Hypersil GOLD Peptide Column, 1.9 µm, 50 × 2.1 mm	Each	**26002-052130**
Thermo Scientific™ Hypersil GOLD Peptide Column, 1.9 µm, 100 × 2.1 mm	Each	**26002-102130**
Thermo Scientific™ Hypersil GOLD Peptide Column, 1.9 µm, 150 × 2.1 mm	Each	**26002-152130**
Thermo Scientific™ Acclaim™ PepMap™ 100 C18 Column, 3 µm, 1.0 × 150 mm	Each	**164572**
Thermo Scientific vials, caps, and consumables		
Thermo Scientific™ SMART Digest™ Pepsin Kit	Each	**60109-110**
SureSTART 0.3 mL GOLD-grade clear glass insert	100/pack	**6PME02CG**
SureSTART 2 mL vial clear glass (for holding insert)	100/pack	**6ASV9-1P**
SureSTART 9 mm screw cap	100/pack	**6PSC9TST**
Thermo Scientific instruments		
Thermo Scientific™ Vanquish™ Horizon UHPLC System	Each	**IQLAAAGABHFAPUMZZZ**
Thermo Scientific™ Orbitrap™ Exploris™ 240 Mass Spectrometer	Each	**BRE725535**
Thermo Scientific™ Vanquish™ Neo UHPLC System	Each	**VN-S10-A-01**

Learn more at **thermofisher.com/biolc**

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