SARTORIUS

Application Note

March 23, 2021

Keywords or phrases
Adeno-associated virus (AAV), CIMmultus® QA, ion-exchange chromatography, empty full separation, single-step rapid method

Chromatographic Separation of Full and Empty AAV8 Capsids

Correspondence
E-Mail: monolith-purification@sartorius.com

Abstract

Adeno-associated virus (AAV) vectors of various serotypes are considered to have a high potential for gene therapy applications. Currently, the manufacturing of AAV vectors faces the challenge of co-production of incompletely formed particles lacking a recombinant viral genome.

Introduction

Empty capsids increase the dose of total AAV administered for efficient transduction and are thought to cause unwanted immunological reactions against the virus. Removal of empty capsids during manufacturing, as well as analysis of empty | full AAV particle content, is therefore a critical requirement for any AAV production process..

Materials

- CIMmultus® QA Monolithic column, bed volume 1 mL;
- Buffer A: 20 mM Bis-Tris propane (BTP), pH 9.0;
- Buffer B: 20 mM BTP, pH 9.0, 1 M NaCl;
- High salt buffer: 1 M NaCl in 20 mM BTP, pH 9.0;
- Sample loop 1 mL;
- All samples diluted in 1 mL buffer A

Methods

Gradient elution method	Wash after load: 10 columns volumes (CV) buffer A Linear gradient: 0 - 200 mM NaCl, 60 CV High salt wash: 1M NaCl in 20 mM BTP, pH 9.0 for 10 CV
Detection:	UV detection, 280 nm and 254 nm

Electron Microscopy: Ion-exchange purified AAV8 capsids were examined under TEM using the negative staining method. Twenty microliters of fractions were applied on formvar-coated and carbon-stabilized copper grids (400 mesh) at room temperature for 5 min and stained with 1% uranyl-acetate (SPI Supplies, West Chester, PA, USA). The samples were observed using a Philips CM 100 transmission electron microscope operating at 80 kV, and images were acquired with an ORIUS SC200 CCD camera using Digital Micrograph Software (Gatan Inc., Pleasanton, CA, USA).

Results

AAV8-containing cell lysate (HEK 293T; 3 freeze-thaw cycles) was benzonase-treated, filtered (0.45µm), and loaded onto AVB Sepharose (1 mL). AAV8 was eluted with 50mM Glycine pH 2.7 and neutralized by the addition of Tris pH 8.8 (30 mM final concentration). AAV8 was then buffer exchanged into PBS supplemented with 2.5mM KCl and 1mM $MgCl_2$ until further treatment.

When 1.29E+12 GC of AAV8 (affinity-purified) was loaded onto CIMmultus® QA (1 mL), 1.05E+12 GC was recovered in the second peak (80% recovery). EM images demonstrate the enrichment of empty capsids in peak 1 (middle panel) and full capsids in peak 2 (right panel). UV absorbance was monitored at 280 and 254 nm. Note the difference in 260/280 nm ratios, suggesting a difference in DNA content. GC: genome copies

Conclusion

A rapid method for separation of empty and full AAV8 particles by linear gradient elution on CIMmultus® QA monoliths with 80% recovery is demonstrated.

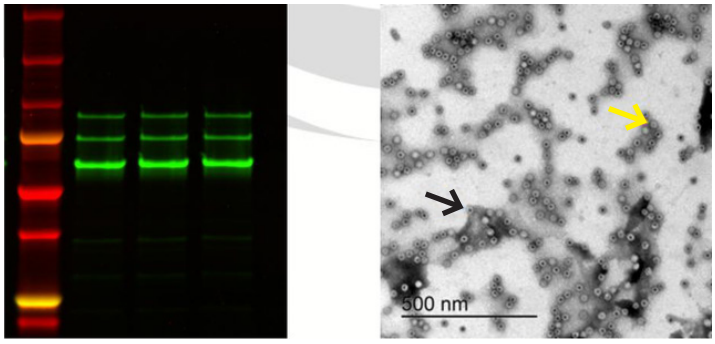
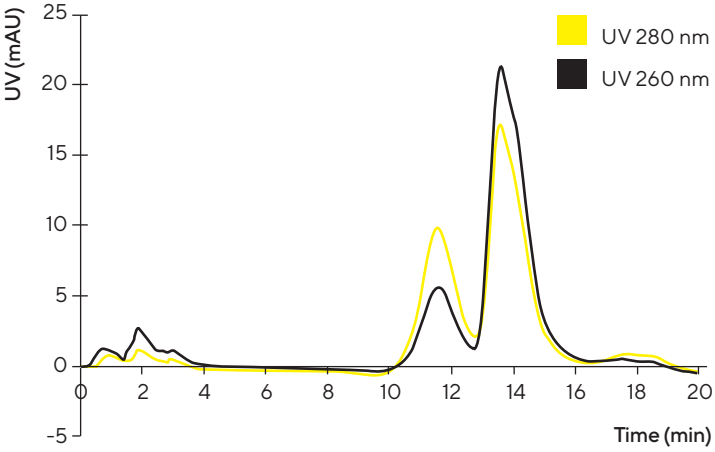


Figure 1: SDS-PAGE analysis of affinity-purified AAV8 shows the presence of VP1-3 (left). EM analysis reveals the presence of full (yellow arrow) and empty capsids (black arrow).



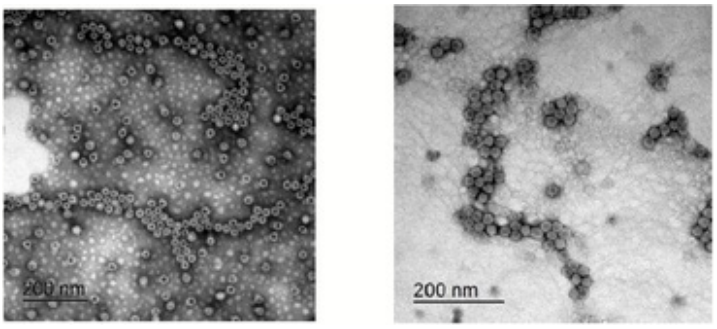


Figure 2: FPLC chromatogram and EM images of CIM® monolith ion-exchange chromatographic separation of full empty and full AAV8 capsids using a linear gradient of NaCl.

Germany
Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Stedim North America Inc.
565 Johnson Avenue
Bohemia, NY 11716
Toll-Free +1 800 368 7178

Slovenia
BIA Separations d.o.o.
A Sartorius company
Mirce 21
5270 Ajdovščina

For More Information, Visit
www.sartorius.com

Status 04 | 27 | 2021