SARTORIUS

Method Guide

Full AAV Enrichment with CIMmultus PrimaS® HR

Introduction

CIM® General Instructions for Use (IFU) document can be accessed by scanning the QR code on the right or by following this hyperlink. This method guide is a separate document to guide the user on how to start the CIMmultus PrimaS® HR purification for full AAV enrichment.

CIMmultus PrimaS HR is a monolithic chromatographic column. PrimaS HR is a multimodal ligand that combines elements of hydrogen bonding with weak anion exchange chromatography. HR line is a new generation of pre-packed chromatographic monolithic products developed to meet the most demanding separation and purification needs. HR stands for High Reproducibility between batches and different scales.

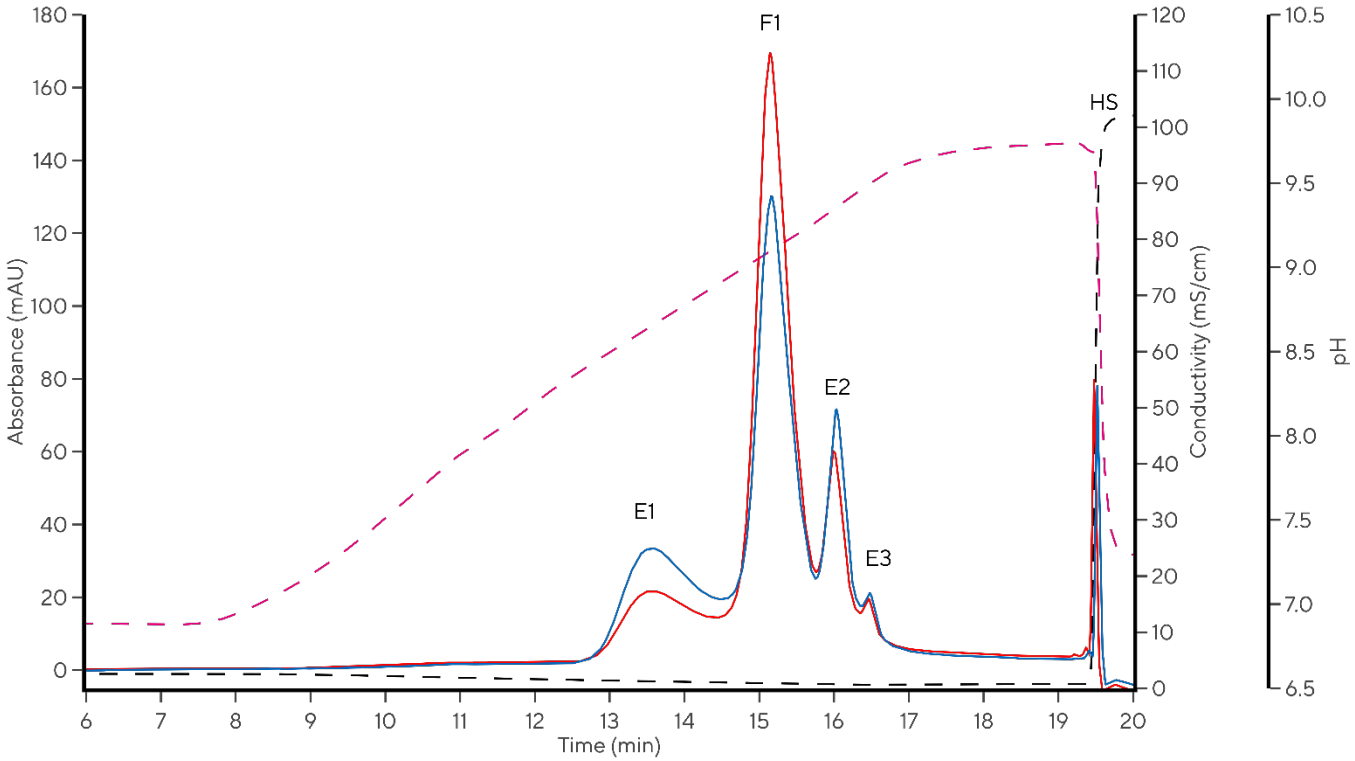
CIMmultus PrimaS HR allows binding of all AAV serotypes and provides advanced removal of product-related impurities, while maintaining high recovery rates. (Figure 1).

Main benefits of CIMmultus PrimaS HR:

- **High reproducibility**: Stringent quality control release test based on the results of AAV E/F separation, ensures consistent column performance across all column sizes or batches.
- **AAV binding at neutral or closer to neutral pH**: Multimodal properties of PrimaS HR allow binding of AAV in neutral or closer to neutral pH values compared to QA-based columns.
- **Endotoxin Removal:** Allows superior reduction of endotoxin compared to strong anion-exchangers (e.g., QA).

For more information, contact
help.bia@sartorius.com

Figure 1: *Separation of AAV8 post capture step on CIMmultus PrimaS HR 1 mL. Loading amount: 3.5E+13 vp/mL column. Legend: black dashed line represents conductivity, pink dashed line represents pH value, red solid line represents UV absorbance at 260 nm, blue solid line represents UV absorbance at 280 nm, enriched full AAV fraction (F1), empty subspecies (E1, E2, E3), and high salt wash (HS).*

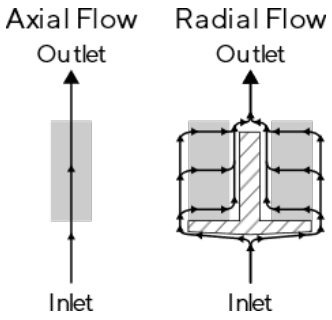


This method guide provides the recommended initial protocol for using the CIMmultus PrimaS HR column in the full AAV enrichment, along with general optimization tips. For specific questions that might not be covered in this method guide, please reach out to help.bia@sartorius.com.

Getting Started

In **CIMmultus® columns**, the flow is radial, moving from the outer side of the monolith through the bed height to the inner side of the monolith (Figure 2). It is essential to connect the column to the chromatography system with the **correct flow direction**. This is different from CIMac columns, which utilize axial flow through the monolith; therefore, when using CIMac, flow direction is not important. Note that some chromatography systems have default reverse-flow functions built into their software that can cause the flow direction to be reversed without warning. Make sure this function is disabled before conducting any experiments with CIMmultus columns.

Figure 2: *Comparison of axial flow and radial flow distribution within stationary phase of the chromatographic column.*



Purification Protocol

Column integrity test: It is highly advised to first perform an integrity test to check the monolith's integrity. Instructions are available by scanning the QR code on the right or by following this hyperlink.

Flow rate: Chromatography with monoliths ensures capacity and resolution independent of the flow rate. However, typical starting flowrate is about 5 CV/min for a CIMmultus 1 mL column. When scaling up, adjust the flow rate according to the IFU for specific CIMmultus column size.

Column preparation: Before each use, Storage solution should be washed out and column should be sufficiently equilibrated, as described in the Column equilibration section. In case of using cGMP column, it should be sanitized before use according to the IFU.

Recommended buffers for initial purification method are listed in Table 1. Recommended buffers for sample preparation and neutralization are listed in Table 2.

Table 1: *Buffers for initial purification methods and sample preparation.*

Buffer	Composition
Mobile phase A (MPA)	10 mM TRIS, 10 mM BTP, 2 mM MgCl2, 1 % sucrose, 0.1 % poloxamer 188, pH 7.0
Mobile phase B (MPB)	10 mM TRIS, 10 mM BTP, 2 mM MgCl2, 1 % sucrose, 0.1 % poloxamer 188, pH 10.0
Mobile phase C (MPC)	Use Column neutralization buffer
Cleaning solution	0.1 M NaOH + 1 M NaCl
Column neutralization buffer	0.1–0.5 M buffer (e.g., TRIS, Acetic acid, etc.) with high salt concentration (e.g., 1 M NaCl), pH 5.0 – 6.0 (refer to IFU)
Column storage solution	20 % ethanol (EtOH)
Sample dilution buffer	MPA

Column equilibration:

- **Initial wash**: If the column is stored in 20 % EtOH, begin by washing it with at least 10 CV of water. This step is crucial to prevent EtOH residues from mixing with the mobile phase buffers.
- **Equilibration (conditioning)**: Wash the column with 10 CV MPB, followed by 10 CV MPA or until conductivity and pH at column outlet match conductivity and pH at column inlet.

Sample preparation: After the capture step, samples should be buffer exchanged (BE) to MPA to minimize salt concentration and traces of residual capture step buffering system to enhance reproducibility of CIMmultus PrimaS HR purification results. Alternatively, instead of performing BE a dilution with MPA is needed to reach the conductivity value of sample below 2.5 mS/cm.

Sample loading: Observe the operating pressure and if necessary, reduce flow rate to maintain operating pressure within acceptable limits of the column (as noted in IFU) and within acceptable limits of the FPLC system.

Wash with MPA: Wash to return UV signal to baseline.

Elute with MPB gradient: For the initial purification run, perform a linear gradient elution by combining MPA and MPB, from 0 % to 100 % MPB over 50 CVs, hold at 100 % MPB for 15 CV. After the main gradient elution, a high salt wash is performed at 100 % MPC for 10 CVs. Once AAV elution conditions are defined, a step elution wash can be implemented.

Column cleaning: Column cleaning is recommended between purification runs. If needed, wash the column with 10 CV of water to prevent mixing of incompatible buffers. Wash the column with at least 10 CV of Cleaning solution. If needed wash the column with 10 CV of water. Wash the column with at least 20 CV of a Column neutralization buffer.

Note: *If needed, extend the contact time with cleaning solution or implement cleaning steps specific to the contaminants present in the sample. If sanitization is required, protocol is described in IFU.*

Column storage: After completed cleaning procedure (including neutralization step), wash the column with 10 CV of water and then with 10 CV of Column storage solution.

Optimization, Troubleshooting and Other Considerations

Optimization of the purification method requires tailoring purification process to specific AAV sample due to their high variability. When optimization is needed, consider screening at least the following variables:

- **pH value:** pH value impacts anion-exchange and hydrogen bond interactions of PrimaS HR with AAV. It is recommended to screen different pH values for loading and elution conditions. Note that the binding capacity of the weak exchanger should be greater at pH 7.0 and lower with increasing pH.

- **Buffering system:** When optimizing pH value, a different biological buffering system might be chosen to support buffer capacity at that value, but this can influence AAV elution performance.

- **Presence of salt:** Optimization of process where not only pH change elutes the AAV, but combination of pH-salt (simultaneous dual gradient). Samples can be loaded onto PrimaS HR at near-neutral pH values, and then exposed to relatively moderate pH to remove empty capsids before eluting full capsids in the presence of salt as described in this publication.

- **Specific additives to promote or inhibit hydrogen bonding effect interactions**: Effects of $MgCl_2$ in the load, Sorbitol, PEG, and Urea.

- **Column capacity:** Defining optimal loading range.

Ordering Information

Cat No.	Product Name
BIA-311.5119-2	CIMmultus PrimaS® HR 1 mL Monolithic Column (2 µm channels)
BIA-414.5119-2	CIMmultus PrimaS® HR 4 mL Monolithic Column (2 µm channels)
BIA-411.5119-2	CIMmultus PrimaS® HR 8 mL Monolithic Column (2 µm channels)
BIA-914.5119-2	CIMmultus PrimaS® HR 40 mL cGMP Compliant Monolithic Column (2 µm channels)
BIA-911.5119-2	CIMmultus PrimaS® HR 80 mL cGMP Compliant Monolithic Column (2 µm channels)
BIA-924.5119-2	CIMmultus PrimaS® HR 400 mL cGMP Compliant Monolithic Column (2 µm channels)
BIA-921.5119-2	CIMmultus PrimaS® HR 800 mL cGMP Compliant Monolithic Column (2 µm channels)
BIA-934.5119-2	CIMmultus PrimaS® HR 4000 mL cGMP Compliant Monolithic Column (2 µm channels)
BIA-931.5119-2	CIMmultus PrimaS® HR 8000 mL cGMP Compliant Monolithic Column (2 µm channels)

FAQ

What are the differences and similarities PrimaS and PrimaS HR?
CIMmultus PrimaS HR and CIMmultus PrimaS are both weak anion exchangers with hydrogen bond interactions, sharing the same chemical backbone. The differences between them arise from different release testing and criteria, leading to different reproducibility performance.

CIMmultus PrimaS is designed for purification of a wide range of biomolecules (such as mRNA, bacteriophage, AAV, etc.) and is suitable for robust separations and linear gradient methods, which allow for some variability in the elution of the target molecule. On the other hand, CIMmultus PrimaS HR product line is recommended for sensitive separations where reproducible chromatography is essential (e.g., AAV empty - full separation), as it ensures consistent column performance, regardless of batch or product scale.

Does the sample need to be purified prior polishing step?
Yes. Our recommendation is performing capture step on CIMmultus SO3 column (strong cation exchange column) prior to polishing step for AAV empty-full separation. Prior to capture step, pre-treatment is also important to further reduce impurities and to increase the binding capacity on CIMmultus SO3 column. Suggested methods for pre-treatment are (alone or combined): tangential flow filtration | DNAse treatment | CIMmultus OH chromatography | flocculation | solid phase extraction.

How much sample should we load in the initial screening run using the CIMmultus PrimaS HR 1 mL column?
For initial screening we recommend loading at least 1E+13 vp per 1 mL column.

Can linear gradient be switched to step elution?
Step elution method can be implemented. The steps are defined based on elution conductivities derived from an optimized linear gradient, aiming to minimize impurity binding and maximize the column's capacity for the virus.

What operating flow rate do you recommend for each column size?
The minimum and the maximum flow rate for each column is defined in IFU. Flow rate does not affect the resolution and capacity.

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