SARTORIUS

Method Guide

# AAV Capture Step with CIMmultus® SO3

## Introduction

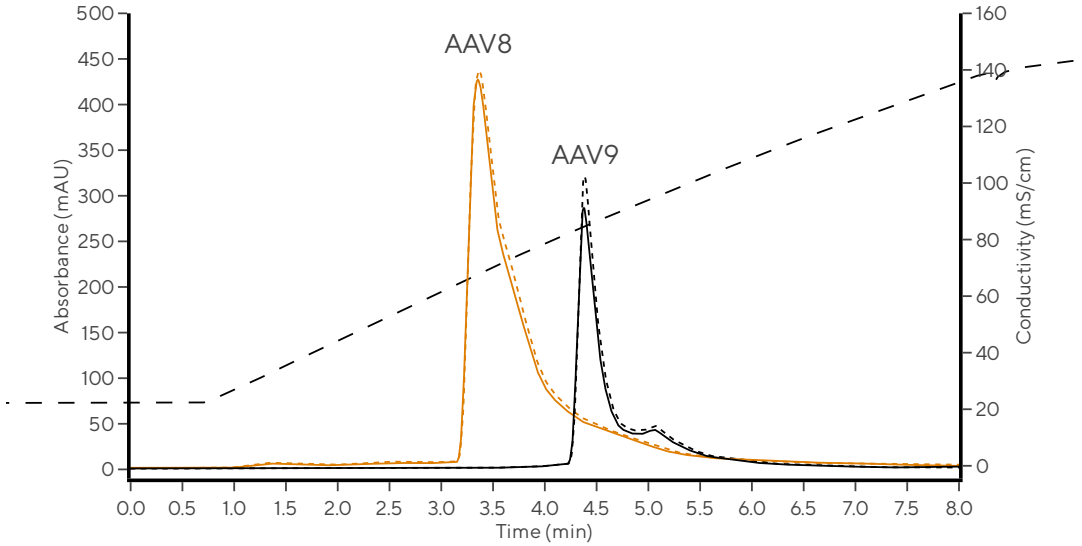
CIM® General Instructions for Use (IFU) document can be accessed by scanning the QR code on the right or by following this hyperlink. This method guide is a separate document to guide the user on how to start the CIMmultus® SO3 purification for AAV capture step.

CIMmultus SO3 is a monolithic chromatographic column. SO3 (Sulfonate) is a strong cation-exchanger that binds molecules with predominantly positive charge and repels molecules with predominantly negative charge across the entire pH range.

CIMmultus SO3 allows binding of all AAV serotypes and provides advanced removal of process-related impurities, while maintaining high recovery rates. To achieve binding of AAV to the negatively charged SO3, a sample acidification step is required prior to sample loading. Host cell DNA and remaining host cell proteins that were not removed in previous purification steps are depleted in the capture step. Full and empty AAV capsids are highly concentrated and co-eluted in a single peak (Figure 1).

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

Figure 1: *Overlay of AAV8 (orange) and AAV9 (black) elution profiles using CIMmultus SO3 column. Loading amount: AAV8 = 5.2E+13 vp/mL column, AAV9 = 4.0E+13 vp/mL column. Legend: black dashed line represents conductivity values, colored dashed lines represent UV absorbance at 260 nm, colored solid lines represent UV absorbance at 280 nm.*




Main benefits of CIMmultus SO3:

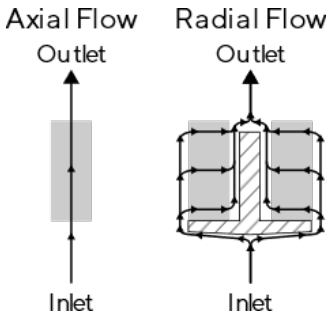
- Binds and elutes any AAV serotype (including engineered AAV capsids)
- Reusable column (cleanable with 1M NaOH)
- High recovery and purity
- Rapid processing time

This method guide provides the recommended initial protocol for using the CIMmultus SO3 column in the AAV capture step, 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 8 – 10 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.*

| | **Buffer composition** |
|---|---|
| Mobile phase A (MPA) | 50 mM Formic acid, 200 mM NaCl, 0.1 % Poloxamer 188, 1 % sucrose, pH 3.5 |
| Mobile phase B (MPB) | 50 mM Formic acid, 2 M NaCl, 0.1 % Poloxamer 188, 1 % sucrose, pH 3.5 |
| Cleaning solution | 1 M NaOH + 2 M NaCl |
| Column neutralization buffer | A concentrated buffer (e.g. >100 mM Tris, pH 7.0) with high salt concentration (e.g. > 1 M NaCl) is recommended to efficiently displace the counter ion. |
| Storage solution | 20 % ethanol (EtOH) |

Table 2: *Buffers for sample preparation and neutralization.*

| | **Buffer composition** |
|---|---|
| Sample acidification solution | 1 M Formic acid, pH 2.5 (titrated with NaOH) |
| Sample dilution buffer (optional) | MPA |
| Sample neutralization buffer | e.g. 1 M Tris pH 9.0 |

Note: *The capture step can be conducted at a higher pH, up to pH 5.0. If conducting the purification process at pH values other than pH 3.5, a different buffering system might be required (e.g., acetate, glycine, etc.). It is advisable to use the same buffering system for sample acidification as for MPA and MPB. If the process is run at a pH higher than 3.5, it may be necessary to lower the conductivity of sample and 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**: To reach target acidic conditions, gradually add Sample acidification solution until titration reaches the pH of MPA (at this stage expect an increase in turbidity). If needed, adjust conductivity to binding conditions by diluting with MPA. Incubate for ~0.5 h. After incubation, filter the acidified sample (e.g., Sartopore® 2 0.45 µm) to reduce sample turbidity and proceed with column loading.

Note: *Volume of added Sample acidification solution depends on the composition of the loading sample. Approx. 1 % v/v of 1 M acid is typically sufficient to acidify the sample to target pH value.*

**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 % MPB to 100 % MPB in 10 CVs (AAVs usually elute in 0.5-1 M salt).
Once AAV elution conditions are defined, a step elution gradient can be implemented.

**Neutralization of sample elution fractions**: Neutralize elution fractions by adding Sample neutralization buffer (approximately 3 % of total fraction volume) to achieve a pH of approximately 7.0-8.0.

**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 10 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 Storage solution. The column must be at neutral or near-neutral pH before introducing EtOH.

# 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:

**Column capacity**: Load the sample until UV breakthrough and determine dynamic binding capacity at 10 % breakthrough ($DBC_{10}$). It is recommended to load approximately 50-70 % of the column's $DBC_{10}$. Typical process loading ranges from 1E+13 to 2E+14 AAV particles per mL of monolith, depending on the sample purity (sample pretreatment).

**Sample pretreatment**: TFF is most commonly used, but it is advised to explore also: CIMmultus OH chromatography, flocculation, solid phase extraction, precipitation and/or nuclease treatment.

**Elution gradient**: It is recommended to screen different pH values for loading and elution conditions, as well as different salt types and concentrations.

# Ordering Information

| Cat No. | Product Name |
|---|---|
| 311.6157-2 | CIMmultus® SO3 1 mL Monolithic Column (Sulfonate) (2 µm channels) |
| 414.6157-2 | CIMmultus® SO3 4 mL Monolithic Column (Sulfonate) (2 µm channels) |
| 411.6157-2 | CIMmultus® SO3 8 mL Monolithic Column (Sulfonate) (2 µm channels) |
| 614.6157-2 | CIMmultus® SO3 40 mL Monolithic Column (Sulfonate) (2 µm channels) |
| 611.6157-2 | CIMmultus® SO3 80 mL Monolithic Column (Sulfonate) (2 µm channels) |
| 814.6157-2 | CIMmultus® SO3 400 mL Monolithic Column (Sulfonate) (2 µm channels) |
| 811.6157-2 | CIMmultus® SO3 800 mL Monolithic Column (Sulfonate) (2 µm channels) |
| 1014.6157-2 | CIMmultus® SO3 4000 mL Monolithic Column (Sulfonate) (2 µm channels) |
| 1011.6157-2 | CIMmultus® SO3 8000 mL Monolithic Column (Sulfonate) (2 µm channels) |
| 904.6157-2 | CIMmultus® SO3 4 mL cGMP Compliant Monolithic Column (Sulfonate) (2 µm channels) |
| 901.6157-2 | CIMmultus® SO3 8 mL cGMP Compliant Monolithic Column (Sulfonate) (2 µm channels) |
| 914.6157-2 | CIMmultus® SO3 40 mL cGMP Compliant Monolithic Column (Sulfonate) (2 µm channels) |
| 911.6157-2 | CIMmultus® SO3 80 mL cGMP Compliant Monolithic Column (Sulfonate) (2 µm channels) |
| 924.6157-2 | CIMmultus® SO3 400 mL cGMP Compliant Monolithic Column (Sulfonate) (2 µm channels) |
| 921.6157-2 | CIMmultus® SO3 800 mL cGMP Compliant Monolithic Column (Sulfonate) (2 µm channels) |
| 934.6157-2 | CIMmultus® SO3 4000 mL cGMP Compliant Monolithic Column (Sulfonate) (2 µm channels) |
| 931.6157-2 | CIMmultus® SO3 8000 mL cGMP Compliant Monolithic Column (Sulfonate) (2 µm channels) |
| BIA-944.6157-2 | CIMmultus® SO3 40000 mL cGMP Compliant Monolithic Column (Sulfonate) (2 µm channels) |

# FAQ

**How much sample should we load in the initial screening run using the CIMmultus SO3 1 mL column?**
When no pretreatment is used (loading only acidified clarified lysate), we recommend loading 1E+13 AAV particles per mL monolith.

**Why sample pretreatment is important?**
Pretreatment of clarified AAV lysate before entering the capture step is of a great importance, because it contributes to higher capacity of the capturing columns and higher purity of the final product.

**What is the importance of adding additives?**
Surfactants (e.g., Poloxamer 188) are added to mobile phase buffers to suppress non-specific interactions with tubing and containers. Sugars (e.g., sucrose) are added to maintain structural integrity of AAV (preserving infectivity) and to prevent aggregation of AAV particles.

**What are the expected recoveries?**
Step recovery using SO3 is typically above 80 %.

**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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