SARTORIUS

Method Guide

Purification of rAAV With CIM® SO3 Monolithic 96-Well Plate

Introduction

CIM® Monolithic Well Plates ensure robust and reliable results for screening multiple chromatographic conditions simultaneously or high-throughput purification of large biomolecules such as viruses, nucleic acids, exosomes, bacteriophages, etc. Each well in our monolithic plates is prefilled with a defined amount of monolith to enable uniform high flow rates across the plate. The monoliths in the wells of the plate have the same chromatographic properties as our preparative line of chromatographic monolithic columns. CIM plates can be disposable or multi-use and are made from medical grade polypropylene (PP), a material that prevents target molecules from binding to the plastic. They are manufactured according to ANSI standards and are automation compatible. They can also be operated manually with a vacuum, centrifuge or positive pressure.

Presented method describes quick and simple high-throughput purification of crude rAAV lysates using CIM® SO3 0.2 mL Monolithic 96-well Plate (2 µm channels). Its main advantage is purification of low volume rAAV upstream samples (such as 1 mL of lysate) that can be then used for various analytics that require certain purity and concentration: mass photometry for capsid species, PATfix E/F or SEC-MALS or other. It is of particular use when many crude lysates should be purified simultaneously, such as rAAV capsid optimizations, library screening, transfection optimization or simply when small volume of purified lysate is needed for downstream analytics.

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How to Purify rAAV With CIM® SO3 0.2 mL Monolithic 96-Well Plate (2 µm Channels)

Plate design: Prior to starting the experiment design 96-well plate experiment, marking number or samples, replicates, and options of purification gradient. For preliminary testing, we recommend testing both Option 1 (pH-NaCl gradient) and Option 2 (pH gradient).

Figure 1: *Example of 96-well plate design for rAAV purification.*

Gradient		1	2	3	4	5	6	7	8	9	10	11	12
pH-NaCl	A	Sample 1, replicate 1	Sample 2, replicate 1	Sample 3, replicate 1	…								
pH-NaCl	B	Sample 1, replicate 2	Sample 2, replicate 2	Sample 3, replicate 2	…								
pH-NaCl	C	Sample 1, replicate 3	Sample 2, replicate 3	Sample 3, replicate 3	…								
pH	D	Sample 1, replicate 1	Sample 2, replicate 1	Sample 3, replicate 1	…								
pH	E	Sample 1, replicate 2	Sample 2, replicate 2	Sample 3, replicate 2	…								
pH	F	Sample 1, replicate 3	Sample 2, replicate 3	Sample 3, replicate 3	…								
	G												
	H												

Preparation of Solutions and buffers (not included with a product): Prior to starting the experiment prepare buffers and solutions as described in Table 1. Calculate accordingly the number of buffers used based on number of experiments and gradient options used.

Table 1: *List of buffers and solutions needed for each purification option.*

Solution	**Option 1 (pH-NaCl gradient)**	**Option 2 (pH gradient)**
Column conditioning buffer	/	50 mM formic acid 2 M NaCl 1 % saccharose 0.1% Poloxamer 188 pH 3.5
Acidification buffer	1 M citric acid pH 2.5	1 M citric acid pH 2.5
Binding buffer	50 mM acetate 0.2 M NaCl 1 % saccharose 0.1% Poloxamer 188 pH 3.6	50 mM formic acid 0.2 M NaCl 1 % saccharose 0.1% Poloxamer 188 pH 3.5
Elution buffer	50 mM acetate 1.5 M NaCl 1 % saccharose 0.1% Poloxamer 188 pH 5.5	20 mM TRIS, 150 mM NaCl, 2 mM MgCl2, 0.01 % Poloxamer 188, pH 7.5
Neutralization buffer	1 M TRIS pH 9.0	/
Dilution/Buffer Exchange buffer	20 mM TRIS, 150 mM NaCl, 2 mM MgCl2, 0.01 % Poloxamer 188, pH 7.5	20 mM TRIS, 150 mM NaCl, 2 mM MgCl2, 0.01 % Poloxamer 188, pH 7.5
CIP Solution	1 M NaOH, 2 M NaCl	1 M NaOH, 2 M NaCl
pH-Restoration Buffer	0.5 M TRIS, 1M NaCl pH 7.5	0.5 M TRIS, 1M NaCl pH 7.5
Storage Solution	20 % EtOH	20 % EtOH

If the same protocol is used for entire 96-well plate, prepare amount of buffer as in Table 2:

Table 2: *The number of buffers and solutions needed for each purification option for entire 96-well plate.*

Solution	**Option 1 (Volume in L)**	**Option 2 (Volume in L)**
ddH_2O	1	1
Column conditioning buffer	/	0.6
Acidification buffer	0.01	0.01
Binding buffer	0.8	0.8
Elution buffer	0.6	0.2
Neutralization buffer	/	0.002
Dilution/Buffer Exchange buffer	various	various
CIP Solution	0.4	0.4
pH-Restoration Buffer	0.4	0.4
Storage Solution	0.4	0.4

Well volume (WV): 1.6 mL
Monolith volume (MV): 0.2 mL

Preparation of 96-well plate prior to sample load (removal of storage solution and conditioning):
Follow guidelines for plate operations as in Instructions for use. The plate does not require a fully automated robot system and can be operated using a vacuum manifold, positive pressure manifold or a centrifuge. Operating parameters can be found under Technical Data. Before use, remove the top and bottom cover seals and remove storage solution by vacuum or centrifugation. It is possible to use limited number of well in the plate, if less than 96 wells are planned to be used. In this case, remove the top cover lid only for the wells in use to ensure unused wells are still in storage solution. Bottom cover can be removed for entire plate.

For robust and consistent operation of the plate, equilibration should be performed before starting with sample analysis when the plate was stored, regenerated or cleaned in place.

- Flush any storage or cleaning solution out of each well by washing with 2 WV of deionized water.
- Wash each well with at least 3 WV of either Elution Buffer (Option 1) or Column Conditioning Buffer (Option 2).
- Wash each well with at least 2 WV of Binding Buffer.

Samples and acidification: Prepare samples of crude rAAV lysates that will be used for purification. We recommend starting with 1.0 mL of sample per well. Samples after acidification should have pH 3.5.

- Acidify samples by adding 8 % (v/v) of Acidification buffer. Vortex for 10 seconds and incubate for 10 min.

Clarification:

- Centrifuge samples at 9500 g for 10 min. Carefully remove supernatant to proceed to next step and discard pellet.

Sample loading:

- Load clarified acidified lysate to pre-conditioned 96-well plate.

Wash plate:

- Wash plate with 2 WV of corresponding Binding buffer.

Elution:

- Option 1:
  - Elute rAAV with 1.5 MV of Elution buffer.
  - Neutralize elution by adding 4 % (v/v) of Neutralization buffer (12 µL per 1.5 MV elution).
- Option 2:
  - 6-times of 1.5 MV each of Elution buffer to raise pH and elute AAV. Collect each fraction in a separate collection plate and determine in which fraction AAV elutes.

Preparing sample for analytics:

- For option 1, lower NaCl concentration in the sample by dilution with Dilution/Buffer Exchange buffer. When purifying samples with lower rAAV titer, exchange buffer composition to Dilution/Buffer Exchange buffer using Vivaspin® 2 Centrifugal Concentrator Polyethersulfone, 100 kDa membrane (Sartorius, Cat. no. VS0241 or VS0242).
- For option 2, proceed straight to analytics, or in case of high rAAV titer dilute samples using Dilution/Buffer Exchange buffer.

Cleaning in Place (CIP) and Storage:

- Wash plate with 1-2 WV of of ddH_2O.
- Wash plate with 2 WV of CIP Solution.
- Wash plate with 2 WV of ddH_2O.
- Wash plate with 2 WV of pH-Restoration Buffer.
- Wash plate with 2 WV of ddH_2O.
- Wash plate with 2 WV of Storage Solution.
- Add storage solution to each well.
- Seal the plate and store it at the temperature specified in the table Technical Data.

Figure 2: *Results of analytics from rAAV purification protocol: a) Mass Photometry, b) PATfix empty/full analytics, c) PATfix total AAV.*

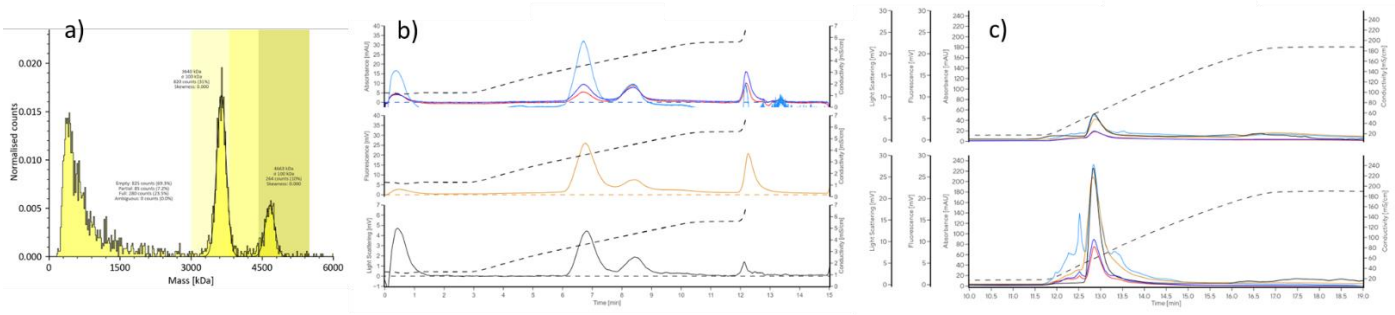
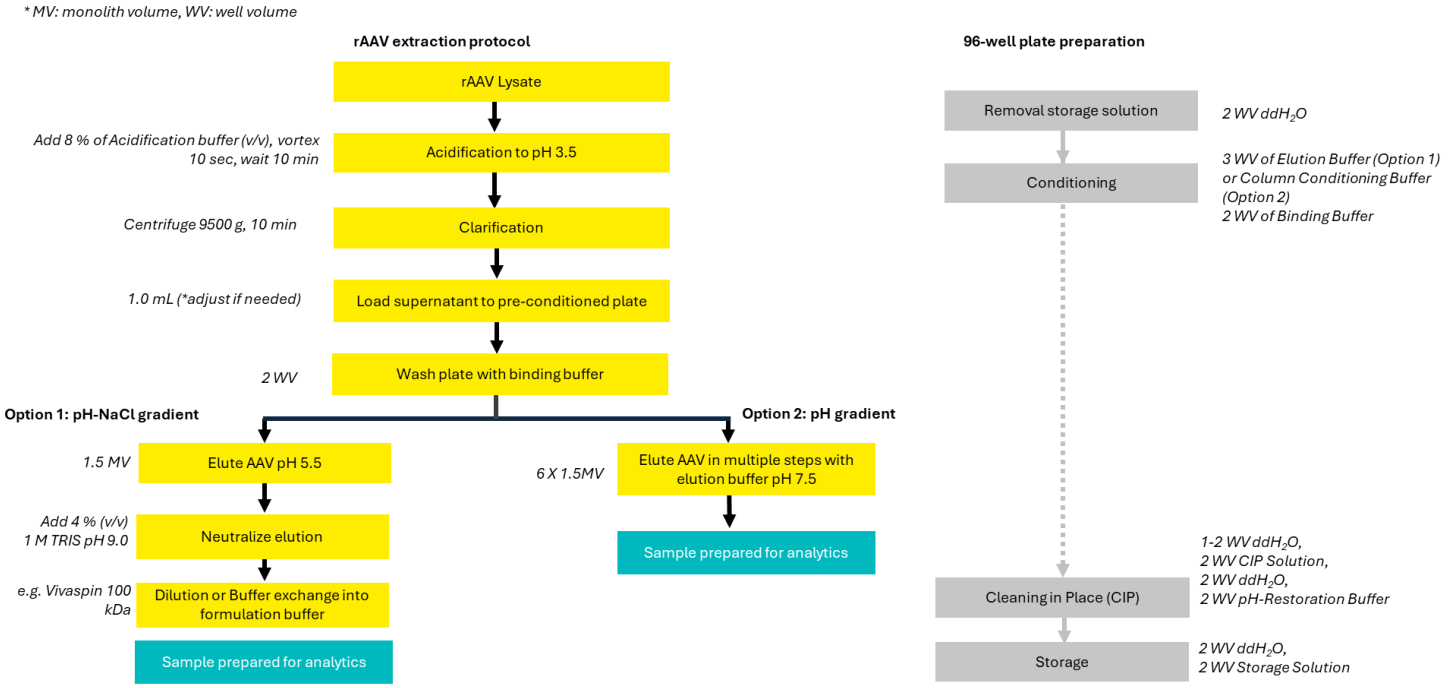


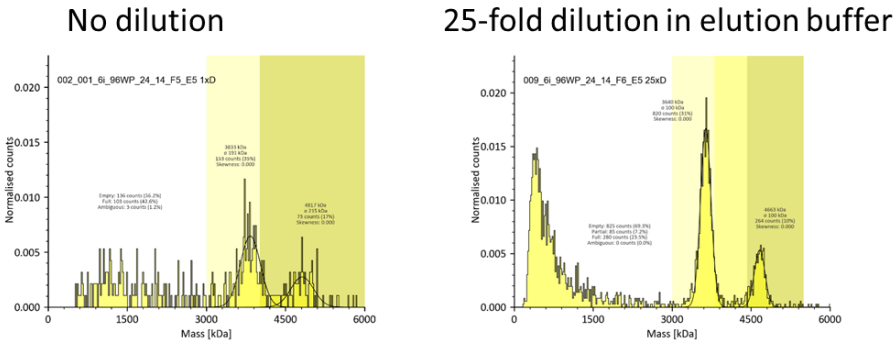
Figure 3: *Scheme of rAAV extraction protocol.*



Variations, Optimization, and Troubleshooting

No virus is detected in following analytics: it is possible that due to matrix/buffer/sample characteristics, eluted sample do not give desired analytical result. For example, for mass photometry (MP) analytics, in limited cases, there was observed improvement of results when dilution of Option 2 elution fraction was performed, as described in Figure 4.

Figure 4: *Improvement of MP result after dilution.*



Amount of lysate material per well: it is possible to adjust amount of rAAV lysate material used per each well. In this case, we recommend loading increasing amount of lysate per each well and test maximum loading amount for each sample. rAAV titer and especially protein amount in the samples are main drivers for determination of maximum loading amount.

rAAV recovery during purification: it is possible to calculate recovery during 96-well plate rAAV purification by determining rAAV titer prior and post purification, taking in account volume of the sample loaded per well and volume of elution fraction. Use the chromatogram as a guide for optimizing the duration of the individual steps described above.

Ordering Information

Cat No.	Product Name
120.6157-2	CIM® SO3 0.2 mL Monolithic 96-well Plate (2 µm channels)

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