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# CIM® SO3 Monolithic 96-Well Plate for Fast and Efficient rAAV Capture Step Screening After Kryptonase-TFF Pre-Treatment

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## Introduction

Standard 96-well design offers a great advantage for screening many samples or conditions and supports process automatization. Our approach was multi-parallel screening of different mobile phases for rAAV capture step using CIM® SO3 0.05 mL Monolithic 96-well plates. Buffers of different pH, sodium chloride concentrations and use of Poloxamer 188 were screened to purify AAV2/9 clarified lysate obtained from Sf9 cells. Sample was pretreated by tangential flow filtration (TFF) coupled with nuclease treatment – Kryptonase™*[1]. It was shown that the optimal conditions were buffers of pH 3.5, 500 mM NaCl, with addition of Poloxamer 188. Verification of results with selected buffer resulted in high capacity (1.44 E14 capsids/mL SO3), great recovery (87.7%) and excellent protein and DNA reduction (99.98 and 99.25%).

*Kryptonase™ was discontinued in January 2023

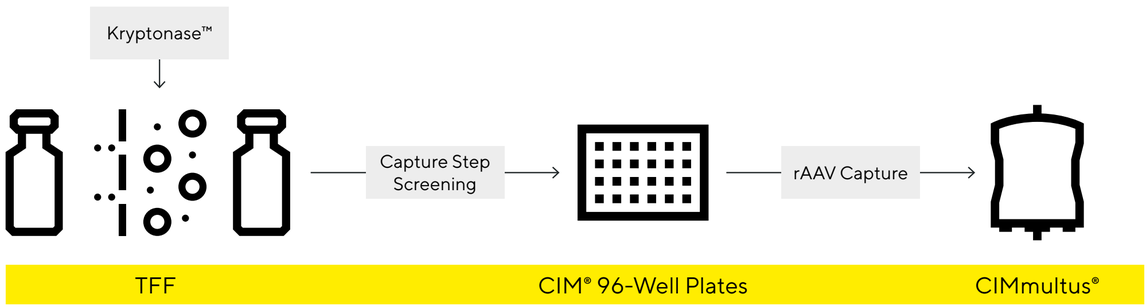


**Figure 1:** *Experimental Design to Optimize rAAV Capture Step Using CIM® SO3 0.05 mL Monolithic 96-Well Plates*

## Methods and Automating Analytics From CIM® SO3 0.05 mL Monolithic 96-Well Plate

Kryptonase-treated rAAV2/9 TFF retentate was acidified to selected pH (pH 3.5, 4.0, 4.5 or 5.0) and filtered using Sartorius Minisart PES 0.45 µm and loaded to the pre-conditioned CIM® SO3 0.05 mL Monolithic 96-well Plate with 2 µm channels. Plate was washed with different mobile phases A (MPA; different pH, sodium chloride and poloxamer conc.) for 10 column volumes (CV). Elution was performed using 4 CV of mobile phase B (MPB; buffers with corresponding pH and poloxamer conc. with 2 M NaCl). Cleaning in place (CIP) and regeneration was performed as recommended in Instruction manual. To determine optimal combination of parameters for SO3 capture step wash and elution fractions were analyzed using fluorescence microplate reader (Tryptophan native fluorescence; ex. 280 nm, em. 348 nm), rAAV9 capsid-specific ELISA and SDS-PAGE.

| Wash Fraction | | NaCl | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 mM | | | 250 mM | | | 500 mM | | | 750 mM | | | |
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | |
| pH 3.5 | A | 65,233 | 65,297 | 66,259 | 65,798 | 67,156 | 68,122 | 67,159 | 68,513 | 68,884 | 67,105 | 68,105 | 66,690 | - |
| | B | 64,787 | 66,494 | 66,406 | 66,662 | 67,134 | 67,265 | 66,832 | 67,821 | 67,988 | 67,914 | 66,925 | 67,037 | + |
| pH 4.0 | C | 66,772 | 68,385 | 68,177 | 66,125 | 68,879 | 68,415 | 69,240 | 69,276 | 70,025 | 81,885 | 81,469 | 81,405 | - |
| | D | 66,560 | 68,089 | 67,259 | 67,294 | 66,827 | 67,787 | 67,254 | 68,115 | 69,321 | 81,638 | 81,007 | 80,275 | + |
| pH 4.5 | E | 67,265 | 67,333 | 69,174 | 69,389 | 68,808 | 69,671 | 83,254 | 83,583 | 84,566 | 85,424 | 85,168 | 84,563 | - |
| | F | 65,721 | 66,717 | 67,707 | 66,986 | 67,780 | 67,879 | 81,455 | 82,679 | 82,103 | 83,709 | 83,805 | 84,200 | + |
| pH 5.0 | G | 66,588 | 66,672 | 67,798 | 81,413 | 81,855 | 82,225 | 88,428 | 87,930 | 88,305 | 89,215 | 90,079 | 88,251 | - |
| | H | 65,627 | 66,379 | 66,821 | 79,630 | 79,784 | 80,494 | 87,216 | 87,809 | 88,554 | 88,715 | 87,967 | 87,645 | + |

Poloxamer 188

**Figure 2A:** *Fluorescence Read for Wash Fractions*

| Elution Fraction | | NaCl | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 mM | | | 250 mM | | | 500 mM | | | 750 mM | | | |
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | |
| pH 3.5 | A | 57,049 | 58,073 | 58,338 | 62,732 | 63,517 | 63,877 | 54,687 | 64,699 | 63,230 | 64,552 | 62,345 | 64,528 | - |
| | B | 60,374 | 60,884 | 60,969 | 60,953 | 61,033 | 60,056 | 61,057 | 62,966 | 61,622 | 62,839 | 61,278 | 61,121 | + |
| pH 4.0 | C | 59,292 | 59,371 | 60,820 | 62,027 | 61,732 | 60,927 | 63,985 | 62,535 | 64,211 | 52,750 | 52,993 | 53,262 | - |
| | D | 64,016 | 64,642 | 64,748 | 66,076 | 64,309 | 65,874 | 65,003 | 65,700 | 64,938 | 52,560 | 52,321 | 53,178 | + |
| pH 4.5 | E | 61,617 | 63,219 | 63,786 | 66,022 | 62,957 | 61,910 | 50,397 | 50,285 | 49,660 | 54,142 | 54,885 | 55,507 | - |
| | F | 66,733 | 68,392 | 69,320 | 68,254 | 66,904 | 67,831 | 53,875 | 53,322 | 53,203 | 52,794 | 53,693 | 53,870 | + |
| pH 5.0 | G | 59,611 | 62,250 | 62,281 | 53,926 | 52,547 | 51,991 | 49,957 | 51,924 | 51,158 | 54,221 | 55,022 | 55,586 | - |
| | H | 71,391 | 69,522 | 69,936 | 60,368 | 59,550 | 60,353 | 54,160 | 53,145 | 54,353 | 53,883 | 53,364 | 53,815 | + |

Poloxamer 188

**Figure 2B:** *Fluorescence Read for Elution Fractions*

Fluorescence readings (FLD) of elution and wash showed stronger signal in wells where rAAV was detected. For wash fractions, wells with higher pH and/or sodium chloride concentration in mobile phase A showed inefficient binding observed as high flourescence signal (Figure 2A). On the other hand, higher FLD values in elution fractions correlate with successful rAAV binding. For pH 3.5 all tested NaCl concentrations up to 750 mM showed binding of rAAV to the SO3 monolith, whereas binding at higher pH resulted in tolerating lower NaCl conditions (Figure 2B). Impurity profile and efficiency of rAAV2/9 capture were verified with capsid specific ELISA analytics (Figure 3A) and SDS-PAGE (Figure 3B).

| ELISA Analysis \| Elution Fraction | | NaCl | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 mM | | | 250 mM | | | 500 mM | | | 750 mM | | | |
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | |
| pH 3.5 | A | | | | | | | | 1.49E+12 | | | 1.48E+12 | | - |
| | B | | | | | | | 2.32E+12 | | | 2.10E+12 | | | + |
| pH 4.0 | C | | | | | 1.74E+12 | | | 1.81E+12 | | | 4.54E+10 | | - |
| | D | | | | 2.24E+12 | | | | 1.83E+12 | | | 9.70E+10 | | + |
| pH 4.5 | E | | 2.04E+12 | | | 2.73E+12 | | 4.77E+10 | | | | | | - |
| | F | | 2.11E+12 | | | 2.27E+12 | | 9.73E+10 | | | | | | + |
| pH 5.0 | G | | 2.17E+12 | | | 3.14E+11 | | | | | | | | - |
| | H | | 2.11E+12 | | | 4.18E+11 | | | | | | | | + |

Poloxamer 188

**Figure 3A:** *Additional Analytics of Elution Fractions for rAAV Titer*

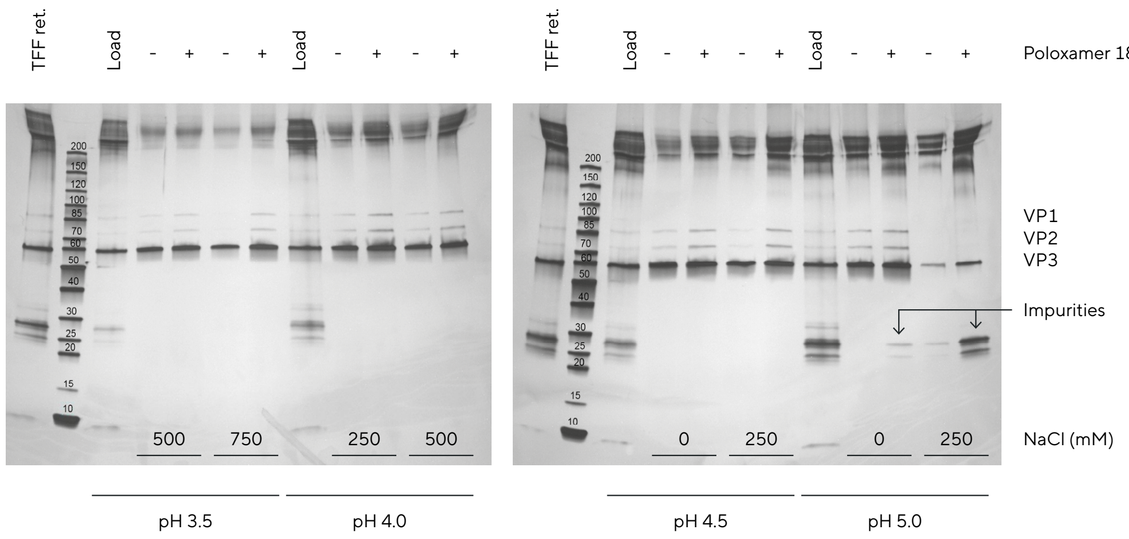


**Figure 3B:** *Additional Analytics of Elution Fractions for Protein Distribution Using SDS-PAGE*

The combination of parameters (different pH, sodium chloride and poloxamer concentration) that gave us the highest virus titer (vp/mL) value and lowest impurity content in elution fractions was selected as the most optimal mobile phase A (pH 3.5 and 500 mM NaCl in presence of Poloxamer 188).

## Verification of Results on CIMmic® and CIMmultus® Line With Additional Analytics

Optimal mobile phase A selected in CIM® 96-well plate screening, was utilized for dynamic binding capacity (DBC) testing on CIMmic® 0.1 mL SO3 disc (Figure 4B). Experimentally defined capacity was 1.44 E14 vector capsids/mL of SO3 monolith. Verification of results was performed also on CIMmultus® preparative line using sodium chloride linear gradient (Figure 4A). Elution fraction was analyzed for total protein (BCA) and DNA (Picogreen) content. High vector recovery and strong reduction of impurities were determined for selected capture step parameters, confirming successful optimization of downstream process (Figure 4B).

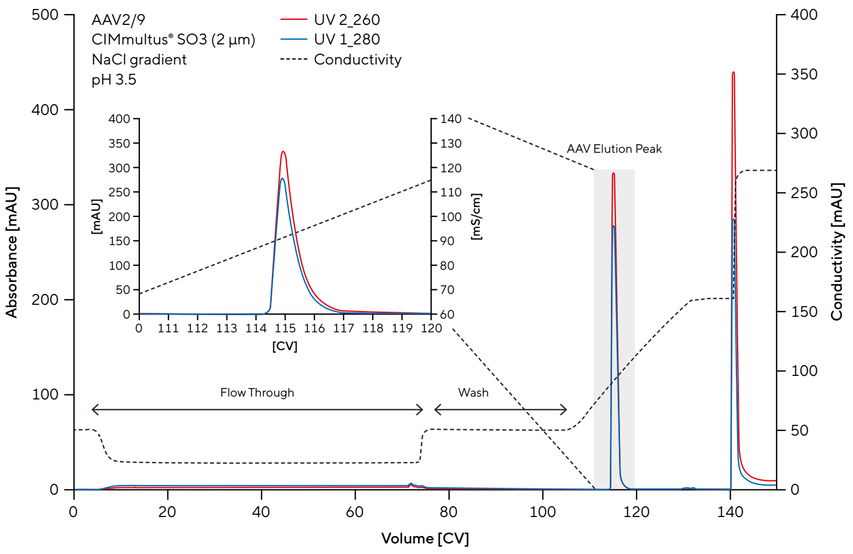


**Figure 4A:** *Cation Exchange Chromatography of AAV2/9 Sample on CIMmultus® SO3 1 mL. Sample: TFF Retentate, Acidified to pH 3.5. MPA: 500 mM NaCl, pH 3.5 and Poloxamer 188, MPB: 2 M NaCl, pH 3.5 and Poloxamer 188, MPC: 1 M NaOH + 2 M NaCl. Method: Sample Load, 30 CV MPA Wash, 25 CV Linear Gradient 0 to 100% MPB, 10 CV Step MPB, 3 CV MPC. Detection: Absorbance (260 nm, 280 nm)*

| Pre-treatment | |
|---|---|
| TFF - Kry | rAAV Elution |
| SO3 step recovery | |
| 87.70 % | |
| Protein reduction | |
| 99.8 % | |
| DNA reduction | |
| 99.25 % | |
| Lysate capacity/mL SO3 | |
| 1,652 mL | |
| Dynamic binding capacity | |
| 1.44E+14 capsids/mL SO3 | |

**Figure 4B:** *Under Selected Conditions Capture Step Was Robust and High Purity Was Observed in the Elution AAV Fraction*

## Conclusion

- CIM® SO3 0.05 mL Monolithic 96-well plates are efficient and fast tool for rAAV capture step screening, including automating of procedure and analytics steps
- Obtained results can be applied to CIMmultus® preparative line
- Sample obtained by TFF coupled with Kryptonase™ treatment gave high recovery and high protein | DNA reduction after optimized capture step
- Other applications and optimizations are possible with usage of CIM® 96-well plates

## References

1. Gagnon P., Leskovec M., Goričar B., Štrancar A. Streamlining Industrial Purification of Adeno-Associated Virus. Bioprocess International, December 2020.