Simplified rAAV lysate purification for complex analytics of upstream samples

Bioprocess experts from Sartorius BIA Separations and BridgeBio Gene Therapy illustrate an approach with potential to expedite AAV upstream analytics and lower overall process development costs.

OPTIMISING UPSTREAM production of recombinant adeno-associated viruses (rAAV) is essential for reducing costs, improving quality, and enhancing efficiency. Producing full capsids—vectors containing complete therapeutic genes—is crucial for rAAV therapeutics. This process occurs during the complex upstream phase, necessitating precise analytical methods and relatively pure samples to determine capsid ratios.

A rapid and efficient high-throughput protocol

The production of rAAV vectors requires a streamlined approach to ensure the generation of full capsids. This paper introduces a rapid and efficient high-throughput protocol for purifying crude rAAV lysates using the CIM® SO3 0.2 mL Monolithic 96-well Plate (2 µm channels). This method is designed to purify low-volume rAAV upstream samples, suitable for various analytics requiring specific purity and concentration levels.

The purification protocol involves preparing the sample and the 96-well plate. The plate is flushed with deionised water, equilibrated with elution buffer, and washed with binding buffer. rAAV samples are acidified, vortexed, incubated, centrifuged, and loaded onto the plate. Elution is performed using either high salt and acidic-neutral pH or neutral pH without a salt gradient. The protocol was tested in-house with AAV8, AAV2, and AAV9, and externally by BridgeBio with AAV5 and AAV9 samples, both with single-step AAV elution (**Figure 1**) and with two different GOI.

Objective

This study aims to present the workflow and data surrounding the use of CIM® SO3 0.2 mL Monolithic 96-well Plate (2 µm channels) for rapid and parallelised purification of small-scale rAAV harvests. The primary objective is to enable high-throughput purification of multiple small-scale bioreactor harvests (~1 mL) to support plasmid ratio screening, transfection optimisation, and media evaluation. The method is further intended to streamline sample preparation for analytical workflows including vector genome (vg) and capsid particle (cp) titer measurement, cell-based potency assays, post-translational modification profiling, and CE-SDS.

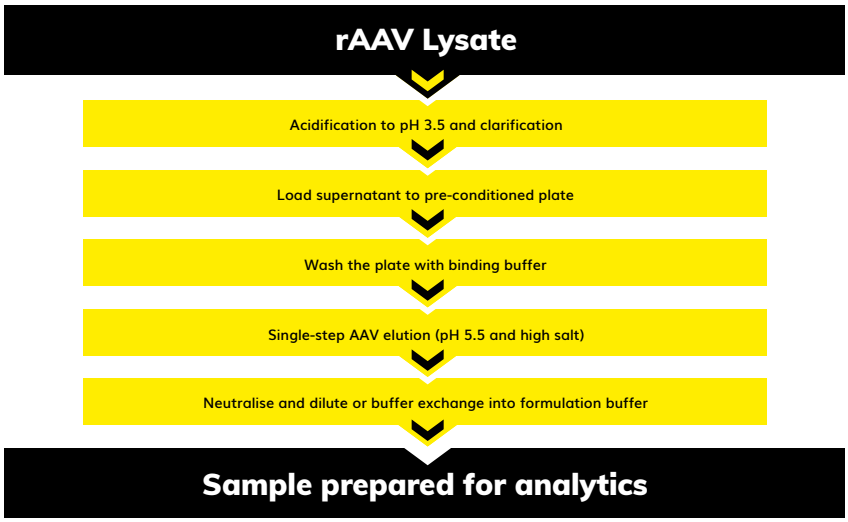

Figure 1: Scheme of BridgeBio's performed single-step AAV elution

Results and discussion

The performance of the CIM SO3 96-well plate was evaluated by BridgeBio Gene Therapy. When coupled with a vacuum manifold, the workflow enabled an easy, low volume requirement means for purifying bioreactor harvests across several serotypes containing different genes of interest. A total of 96 samples were processed in less than an hour with minimal hands-on time.

Crude harvest material across the serotypes and genomes of interest was produced in-house using transient transfection of HEK293 suspension cells cultured in its respective media. The cells were triple-transfected at pre-defined plasmid ratios and harvested 96 hours post-transfection. Depending on the preparation, the crude harvest was either treated with a (i) commercial endonuclease followed by chemical lysis, or (ii) salt and detergent combination for lysis.

These bioreactor lysates spanned various serotypes (AAV5 and AAV9), encompassing three different genomes of interest were purified using the above-mentioned workflow. Owing to varying capsid particle concentrations of the bioreactor harvests and fixed 1 mL volume of loading per well plate, these preparations were loaded across varying load factors of capsid particles normalised to packed monolith volume (cp/mL MV).

Figure 2 presents the capsid recovery percentage of (left) AAV9, and (right) AAV5 capsid particles across various load factors and containing different genomes of interest. The workflow enabled ~90 percent and ~60 percent capsid recovery for AAV9 and AAV5, respectively demonstrating favorable binding profiles for AAV9 over AAV5 with cation exchange-based monolith. These results are noteworthy when compared to standard industry practices. Affinity-based purification, often considered the gold standard for early-phase AAV vector recovery, typically yields 70–90 percent recovery for AAV9, depending on resin type and harvest quality.

The current method—using cation exchange with a pH + salt elution—matches or exceeds these expectations for AAV9, while offering a more cost-effective and platform-flexible alternative. For AAV5, the observed ~60 percent recovery is somewhat lower than that of affinity capture methods (which can reach 70–80 percent). Taken together, this performance positions the workflow as a robust

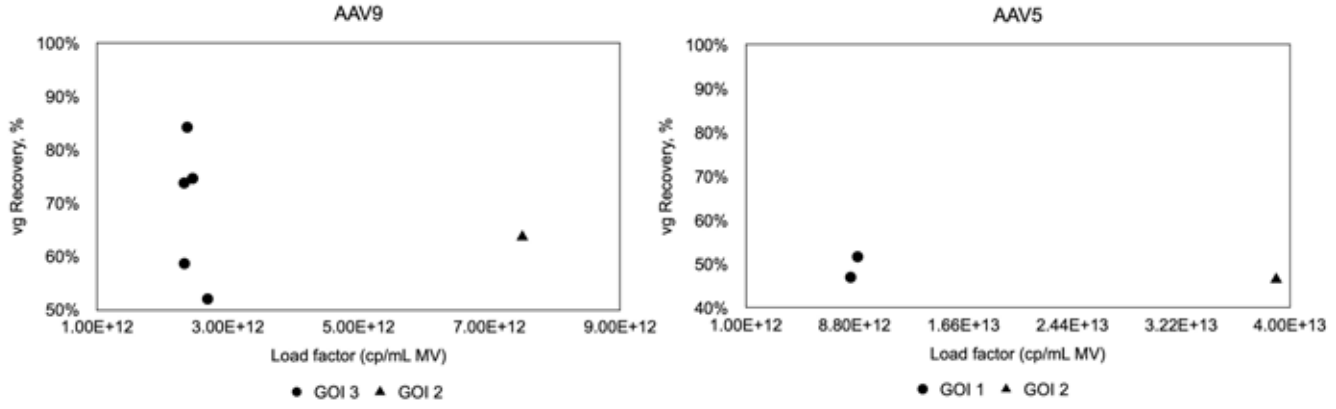

Figure 2: AAV capsid recovery of (left) AAV9 and (right) AAV5 vectors encompassing different genes of interest (GOI).

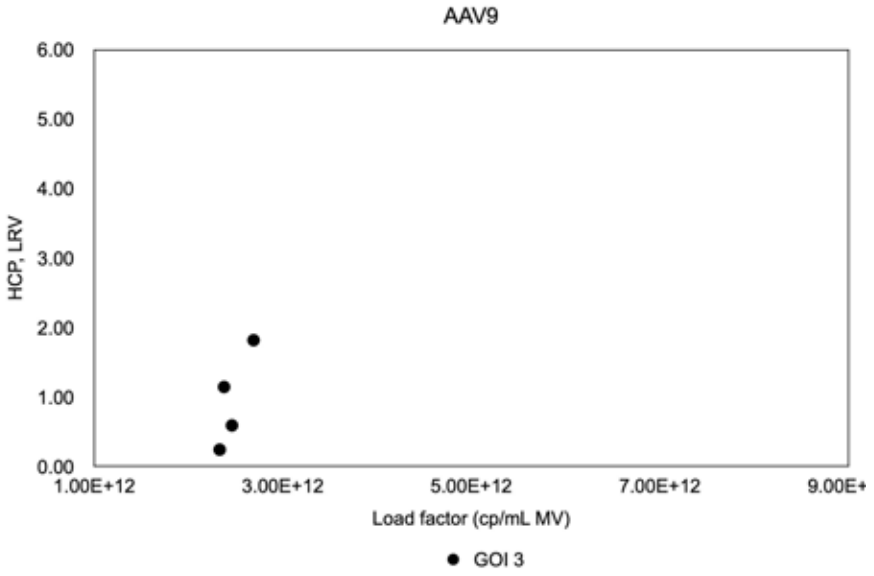

Figure 3: Clearance of host cell proteins (HCP) expressed in log reduction values (LRV) at various capsid particle loading factor.

alternative to affinity purification, particularly for early development scenarios where high-throughput screening, cost control, and downstream assay compatibility are prioritised. The single-step AAV elution method—utilising pH 5.5 and salt—enabled direct use of purified material for downstream quantification assays, including capsid titer, vector genome content, host cell protein (HCP), and host cell DNA (hcDNA), without the need for additional sample preparation.

To assess the process-related impurity clearance capability of the developed workflow, host cell protein (HCP) log reduction values (LRVs) were measured across varying load factors. As shown in **Figure 3**, ~98 percent HCP reduction is observed with pH + salt elution conditions. While the clearance is moderately relative to typical industry targets, the observed performance is notable given the simplicity, speed, and small-scale format of the method. The results support the platform's suitability for early-stage process development, enabling high-throughput evaluation of upstream parameters while delivering purified material suitable for downstream analytical assays.

BridgeBio's outlook

The CIM SO3 96-well monolith platform represents a powerful chromatography-based alternative to affinity purification for early-stage rAAV purification. It enabled rapid, small-scale processing with high recovery, ease of use, and broad assay compatibility. These attributes position it as a valuable tool in the gene therapy process development toolbox—supporting faster iteration, deeper analytical insight, and a more flexible purification strategy.

A tool for your process development

We have successfully developed a serotype-agnostic tool for screening and analysing AAV upstream samples, applicable to any AAV. It aids scientists in expediting AAV upstream analytics, ensuring required purity and concentration. Combined with other analytics, this approach can refine AAV production. The ability to purify multiple small samples in one run is beneficial for library screening or transfection optimisation, reducing time and lowering overall process development costs.

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