High yield & full-empty ratios achieved with a scalable GMP-ready AEX chromatography

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Abstract

A minimum of 70% full particles is typically recommended for the majority of clinical AAV applications since an excess of non-functional or empty capsids can impact both, therapeutic efficacy and safety. The design of plasmids and overall AAV upstream manufacturing platform significantly impacts the level of full-length AAV packaging. Technologies such as our EpyQ® can make further enrichment of full particles dispensable, resulting in higher AAV vector titers and overall process recoveries. When a full capsid enrichment step is required in downstream processing, it is very challenging to develop a robust and scalable step to significantly reduce empty capsids. Here we describe a systematic process development strategy to optimize full/empty separation using anion exchange chromatography (AEX), with a focus on methodical screening and stepwise refinement.

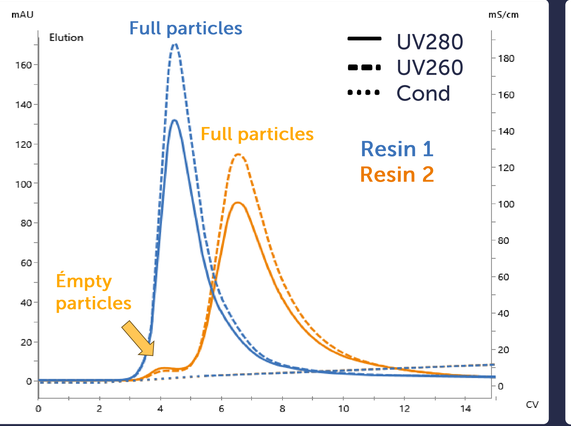
Development began with a screening phase to identify a suitable AEX resin to separate full and empty AAV9 capsids. Three commercially available AEX resins were evaluated under linear gradient elution conditions. Following resin selection, the buffer system was optimized by testing various salt compositions and dual salt combinations to ensure operational simplicity and compatibility with previous and following downstream processing steps. Linear gradient experiments with different salt types provided insights into the optimal elution profiles for full capsid enrichment.

Based on these findings, the chosen resin and buffer system were further evaluated to develop a step gradient protocol, replacing the linear gradient for enhanced process reproducibility and scalability. The step gradient resulted in an enrichment to ≥70% full of the already high % full starting material (~50% full for AAV9 & 3kb GOI) with a step recovery of more than 65 %, sufficient to include it in the purification process. Mass photometry was employed as a reliable analytical tool to determine the enrichment factor for full capsids. Concurrently, droplet digital PCR (ddPCR) was applied to quantify vector genome (vg) titers, enabling accurate calculation of step recovery. These two analytical approaches ensured a comprehensive evaluation of process performance.

This study highlights the critical role of robust and qualified analytical tools and a stepwise, well-structured process development for establishing an efficient method to separate full and empty capsids. A scalable, GMP-ready AEX step was developed first for AAV9, and is now available for the serotypes AAV2 and 8 as well. These studies offer a good basis for future projects with different serotypes and genes of interest.

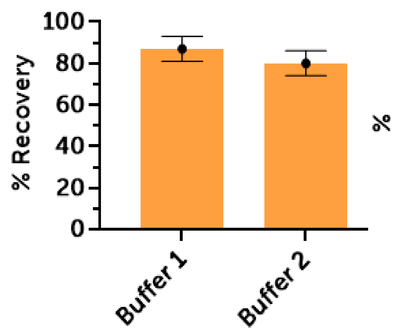
1. AEX Resin Screening

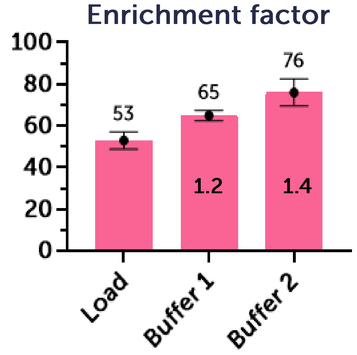
- AAV9 material purified by affinity chromatography was diluted and applied to different AEX resins
- A linear gradient elution with successive increasing salt concentration was used
- Different results were obtained as shown in the graph
  a. Resin 1: separation of full & empty particles by binding only full particles to the column
  b. Resin 2: separation of full & empty particle by elution at different conductivities
- Both, resin 1 & 2, allowed %full enrichment and vg step recoveries >70%
- **Resin 1 was selected for further development, since %recovery was higher at a comparable %full reached.**



2. Buffer System Selection

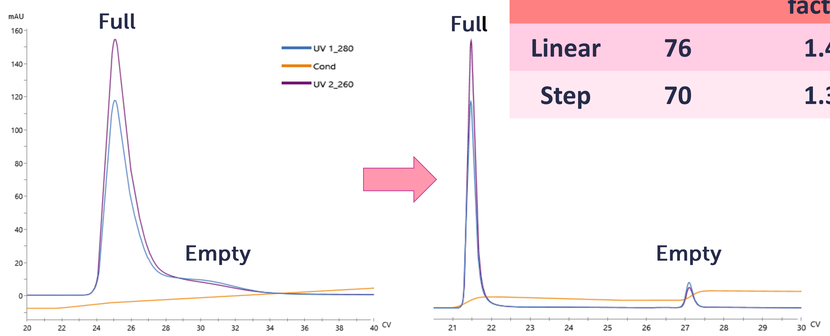
- Various salts and salt combinations were tested on Resin 1 using a linear gradient elution protocol.
- The here shown buffer systems 1 and 2 resulted in ~80% vg recovery and allow F/E enrichment.
- However, % full & enrichment factor for buffer system 2 was higher. Therefore, buffer system 2 was selected for step gradient development.





*Mass photometry **GOI specific ddPCR

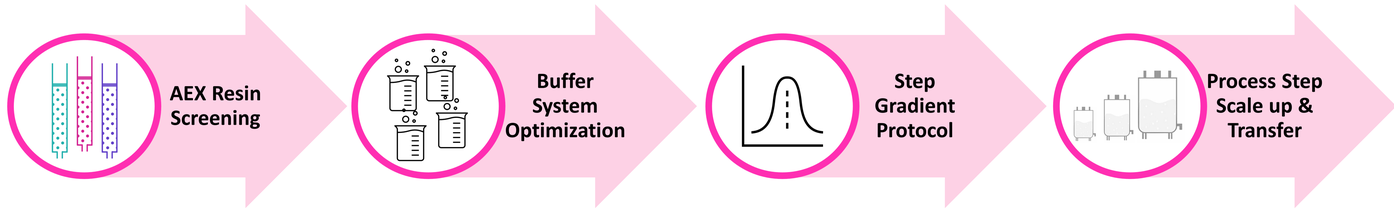
3. Step Gradient development



Elution	% Full*	Enrichment factor	Vg Step Recovery**
Linear	76	1.4	80
Step	70	1.3	65

*Mass photometry **GOI specific ddPCR

AEX Process Development



Process development is comprehensively supported by Ascend's analytical department, utilizing advanced analytical tools from early-stage development to commercial stage manufacturing. Robust and precise analytics are mandatory for generating reliable data, on which important decisions are made throughout the entire development cycle.

Summary

AAV9 serotype presents unique challenges in achieving efficient full-to-empty particle separation, since it is binding only at a low loading conductivity to an AEX resin. Leveraging our scientific expertise in process and analytical development, we successfully developed a scalable, GMP-ready anion exchange chromatography (AEX) step specifically optimized for AAV9. This robust platform has been further extended to include AAV2 and AAV8 serotypes, demonstrating its versatility and adaptability. These advancements provide a strong foundation for future gene therapy programs involving a wide range of serotypes and genes of interest.

Aim higher

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