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Case Study

Quick and Easy Separation of Empty | Full AAV Particles Using Sartobind® Q Lab

Sartobind® Q Lab anion exchange membrane chromatography units can be used to separate empty from genome-filled adeno-associated virus (AAV) particles. Compared to classical resin columns, these membrane adsorber units allow for significantly higher flow rates to be used. In this case study, we summarize an empty | full separation process for AAV8 particles, although it should be noted that this can also be applied to other serotypes. Our step gradient elution method showed a 5.3-fold enrichment of full particles, making Sartobind® Q Lab an ideal choice for empty | full separation in AAV purification workflows.

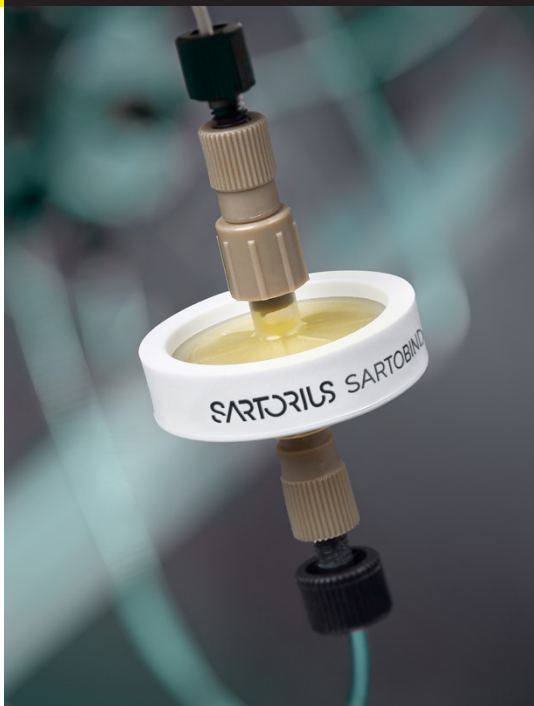
Materials

- Affinity-purified AAV sample
- Sartobind® Q Lab (0.41 mL membrane volume)
- Purification buffers (50 mM Tris-HCl pH 8.5 with 2 – 50 mM $MgCl_2$)
- Liquid chromatography system or syringes
- AAV reference standards
- Octet® R8 BLI system with AAVX Biosensors
- dPCR system

Case Profile

Objective:
This case study shows how Sartobind® Q Lab can be used to improve the efficiency of empty | full separation during the research and development of new AAV-based gene therapies. High flow rates and low shear forces speed up the polishing step used to enhance AAV purity.

Keywords:
AAV, adeno associated virus, empty | full separation, full capsid enrichment, gene therapy, membrane chromatography, Octet®, Sartobind® Lab , anion exchange chromatography

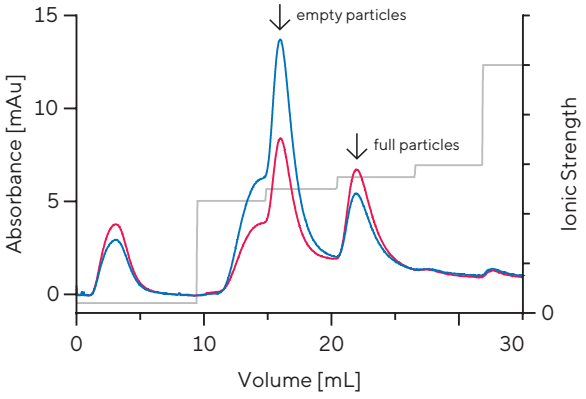


Results

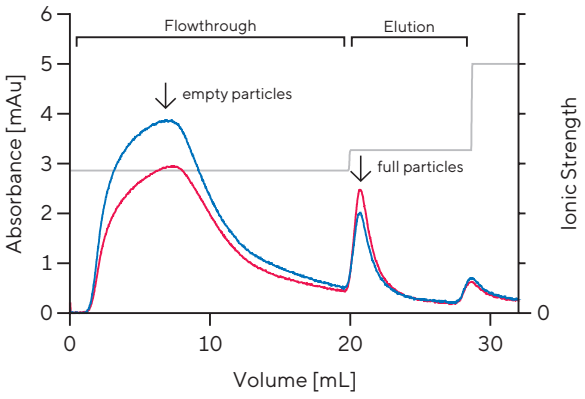
Ion exchange chromatography is commonly used to enrich genome-filled AAVs by exploiting the difference in isoelectric point between empty and full particles. **Figure 1** shows a 5.3-fold enrichment of full AAV8 particles when Sartobind® Q Lab was used in combination with a step elution gradient, where the loaded material and second elution peak contained 12.1% and 63.9% full particles, respectively.

To simplify the empty | full separation process, a flowthrough approach was developed. Sartobind® Q Lab was equilibrated at an ionic strength at which only full AAV8 particles would be captured during loading. **Figure 2** shows that loading and elution at 25 mM and 32.5 mM $MgCl_2$, respectively, resulted in a single elution peak containing 44.9% full particles—a 3.7-fold enrichment factor.

1. Step Elution E | F Separation



2. Flowthrough E | F Separation



Conclusion

Our results show that Sartobind® Q Lab can be effectively used for empty | full separation of AAV particles. Step elution and flowthrough approaches resulted in up to 5.3-fold enrichment of full AAV8 particles, relative to the affinity-purified starting material. Further data for AAV8 and other serotypes are available in our Application Note: Establishing a Small Scale AAV Empty | Full Separation Process using Sartobind® Q Lab.

These methods can increase empty | full separation efficiency because the convective Sartobind® Q membrane supports faster flow rates than diffusive, resin-based columns. In addition, the flowthrough approach is easy to perform using syringes, eliminating the additional time and effort required to set up a liquid chromatography system. Therefore, Sartobind® Q Lab offers distinct advantages to accelerate and simplify empty | full separation of AAVs in research and development applications.

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