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In this issue: DGUC is advantaged over affinity chromatography for viral vector purification

Issue: 011

When should I choose centrifugation instead of affinity chromatography for purifying viral vectors?

Affinity chromatography (AC) is commonly used for viral vector purification because it offers process scalability, throughput, and automatability.[1] Despite this adoption, several limitations exist, including costly resins, serotype-dependance, and co-purification of empty and full virus particles.[2] Density Gradient Ultracentrifugation (DGUC), however, is a cost-effective, serotype-agnostic method that efficiently separates empty and full particles. While chromatography still provides excellent throughput, you can address the scalability shortcomings of DGUC through simple concentration steps prior to centrifugation. Here, we present a brief summary of Yu *et al*, where they compare both methods for AAV purification.[2]

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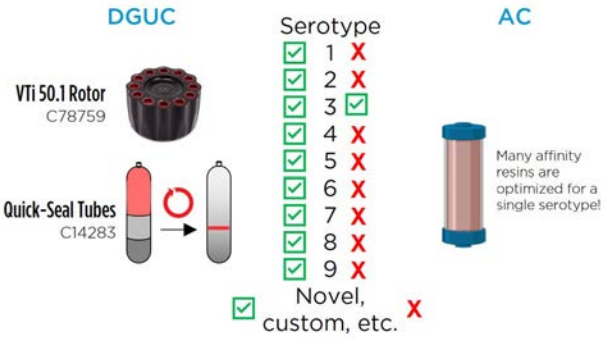
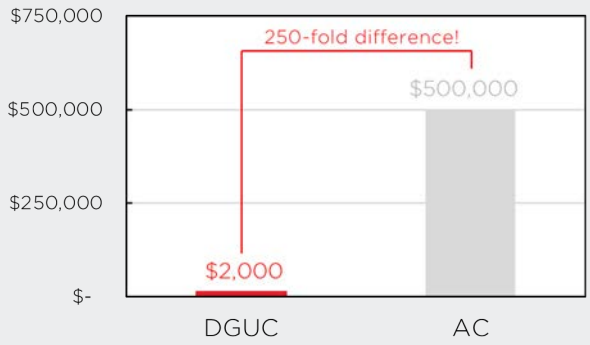
Advantages of UC for AAV purification

Serotype independence

Viral vectors have emerged as promising gene therapy delivery vehicles.[3] However, to continue the clinical advancement of viral vectors, purification schemes that are serotype-independent will be critical.[4] Many AC resins are only specific for a single serotype, and therefore require extensive method development efforts.[2] DGUC offers unparalleled flexibility since a single method can be applied to all serotypes. Moreover, many different serotypes can be purified in a single run.



COGs to purify 10^{16} vg of AAV

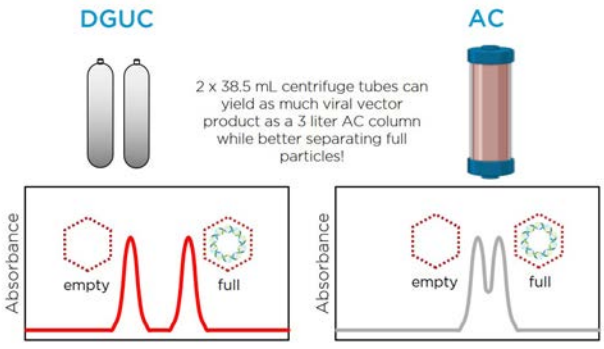


Cost

Yu *et al* estimate that 10^{16} viral genomes (vg) of AAV are required for a small-scale clinical trial. Generating this quantity using AC would require nearly 10 L of affinity resin and would cost approximately **$500,000 USD**.[2] In contrast, 10^{16} vg can be produced from a single run in a SW 28 rotor if a concentration step is utilized prior to DGUC. The cost of goods (COGs) for this DGUC purification is around **$2,000 USD**. Finally, although the SW 28 Ti rotor they used does work for AAV purification, shorter protocols and increased throughput can be achieved by using vertical and fixed-angle Beckman Coulter Life Sciences rotors, such as the VTi-50.1.

Process Quality: Yield and Purity

A major challenge in AAV manufacturing is generating the large quantities of virus sufficient for clinical studies. Yu *et al* purified over 3 x 10^{15} vg with DGUC using just two 38.5 mL Beckman Coulter centrifuge tubes after concentration of the input.[2] Since the binding capacity of AAV affinity media is in the range of 10^{12} vg/mL, it would take a 3 L column to purify an equivalent amount using AC. Furthermore, empty particles are usually co-purified using AC, which reduces the effective yield and presents significant patient safety risks.[5] DGUC, however, can efficiently separate empty from full virus particles.[2]



Summary

Density gradient ultracentrifugation (DGUC) offers several advantages for AAV purification including high purity and yield, serotype-independence, and the efficient removal of empty capsids (Yu). To learn how you can improve your viral vector purification, please visit beckman.com/centrifuges/ultracentrifuges

1 Smith, *et al.* (2009). *Mol Ther,* 17(11). DOI: 10.1038/mt.2009.128
2 Yu, *et al.* (2020). *Mol Ther Methods Clin Dev,* 17. DOI: 10.1016/j.omtm.2019.11.009
3 Büning, *et al.* (2019). *Mol Ther Methods Clin Dev,* 12. DOI: 10.1016/j.omtm.2019.01.008
4 Arden & Metzger (2016). *J Biol Methods,* 3(2). DOI: 10.14440/jbm.2016.102
5 Gao, *et al.* (2014). *Mol Ther Methods Clin Dev,* 1(9). DOI: 10.1038/mtm.2013.9

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