BECKMAN COULTER
Life Sciences

Get Insights Further Upstream

Quantifying crude AAV Samples using DGE-AUC

Objective

Perform minimal sample cleanup to get insights into adeno-associated virus (AAV) population distribution early in the production process.

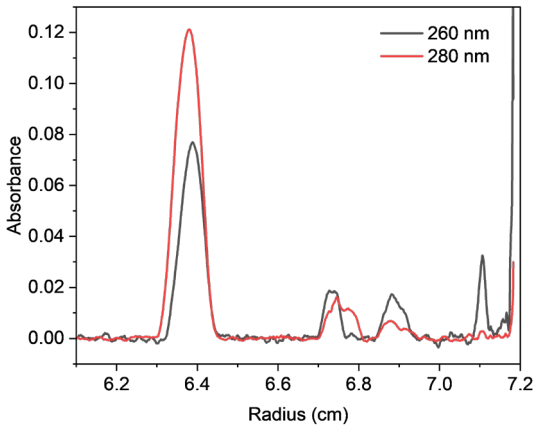
Method

Clean up crude AAV lysate using your method of choice. Here, we used simple centrifugal spin filters, but other viable and potentially more efficient options include affinity purification and precipitation. Once your sample is minimally cleaned up, determine appropriate density gradient equilibrium analytical ultracentrifugation (DGE-AUC) run conditions by screening for starting sample amount, density, and run speed. Since AUC is serotype agnostic, you can utilize an established protocol for all AAV samples. Typically, higher speeds provide better sensitivity (e.g., peak height).

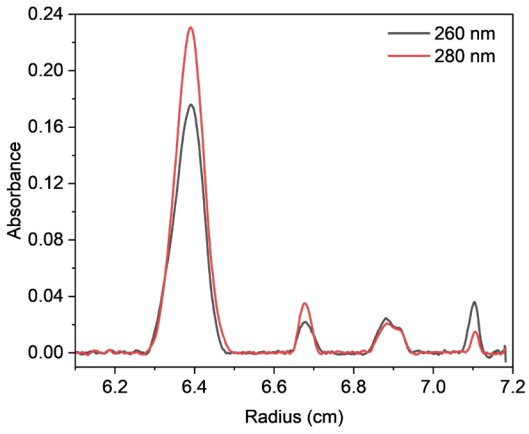
Results

DGE-AUC analysis of semi-pure AAV9 samples generated using two separate transfection conditions reveals multiple species.

Transfection Condition 1



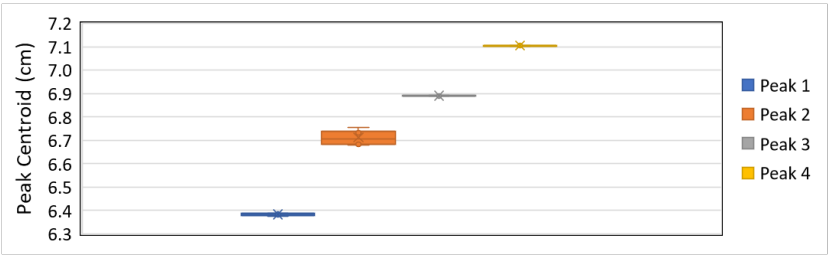
Transfection Condition 2



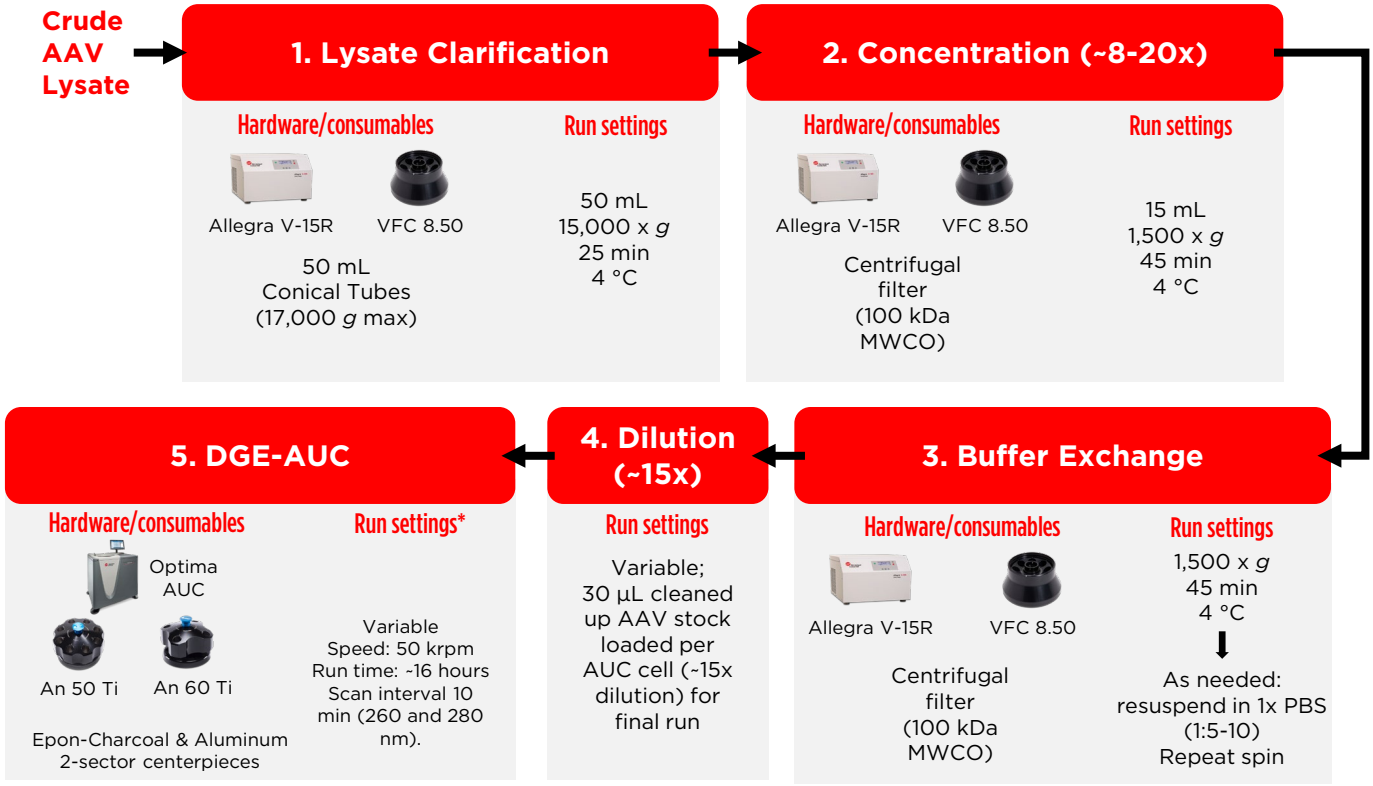
260/280 ratios of each peak suggest that the major peak corresponds to empty capsids, while lower abundance peaks are postulated to be partially-loaded, full, and overloaded capsids.

Sample	260/280 Ratio			
	Peak 1	Peak 2	Peak 3	Peak 4
Transfection Condition 1	0.56	1.35	2.31	16.51
Transfection Condition 2	0.78	0.72	1.12	2.92

Peak centroids at both wavelengths (260 and 280 nm) are highly consistent, indicating reproducibility across this limited sample size.



Methods



*Scan averaging and radial dilution correction were implemented prior to analysis.

Conclusions

- A simple workflow for cleaning up crude AAV9 lysate was developed which requires only 30-60 min of hands-on time. This workflow is a simple option for labs with limited instrumentation, but potential improvements include implementation of small-scale affinity chromatography, desalting, precipitation, or other crude purification kits.
- DGE-AUC identified multiple species (3-4) in both AAV9 samples. Peaks are expected to be empty, partial, full, and overloaded particles based on their position in the density gradient, as well as consistency across peak centroids. 260/280 ratios seemingly support this hypothesis, but low intensity of some peaks precluded complete peak identification.

For more information about DGE-AUC, refer to the Getting Started with DGE-AUC guide, the DGE-AUC Analysis in Origin guide and companion video, and the webinar with OriginLabs.
Please contact your local sales rep or visit beckman.com/request-quote for additional information or a customized quote.

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