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SPOTLIGHT

AAV Analysis **Simplified**

Helping You Help Patients Faster

You are likely all too familiar with the challenges in gene therapy development, many of which relate to inadequate analytical techniques for viral vector characterization. Several techniques used currently are complex with long development times, and require enormous expertise and often generate poor sensitivity. Further, most are not QC-friendly. It is of no surprise then, that measuring CQAs of AAVs is extremely challenging (see Table 1).

You now need a solution! Something that can accelerate your gene therapy development journey to reach patients and save lives faster. How about an analytical technique that is fast, easy to use, and generates high quality data?

Meet Maurice, a fully integrated capillary electrophoresis (CE) platform for imaged capillary isoelectric focusing (icIEF) and capillary electrophoresis sodium dodecyl sulfate (CE-SDS). As a single automated instrument, Maurice lets you analyze multiple AAV CQAs like identity, purity, stability, and potency. Whether you're in analytical development or QC, Maurice will enable you to:

- **Move faster through the pipeline** – develop method conditions in a day or less. Save time and labor
- **Get results fast** – measure charge heterogeneity under 10 minutes, purity under 35 minutes
- **Be confident in your results** – get highly reproducible data, minimizing the need to rerun an experiment
- **Stay compliant** – maintain data integrity with Compass for iCE or Empower® CFR software

Identity

Without laborious gels or blots, quickly know if you have the right AAV serotype with clear, reproducible charge profiles of viral proteins. Just by the apparent pI values, you'll be able to differentiate serotypes that are otherwise highly similar in structure. In a day or less, you'll get reproducible data on your serotype identity so you can make decisions faster.

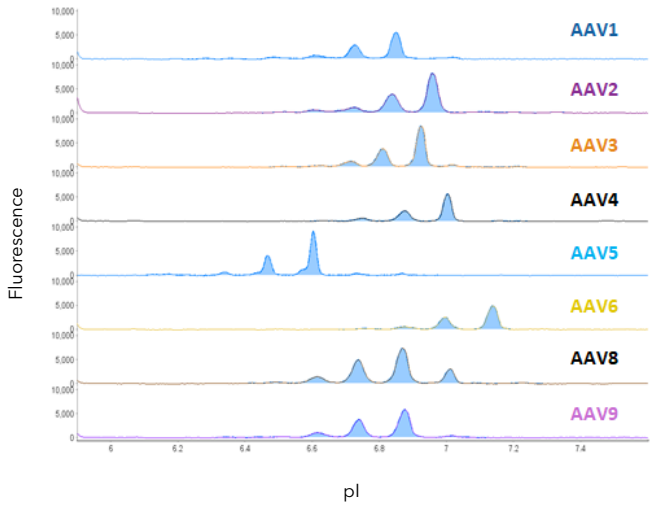
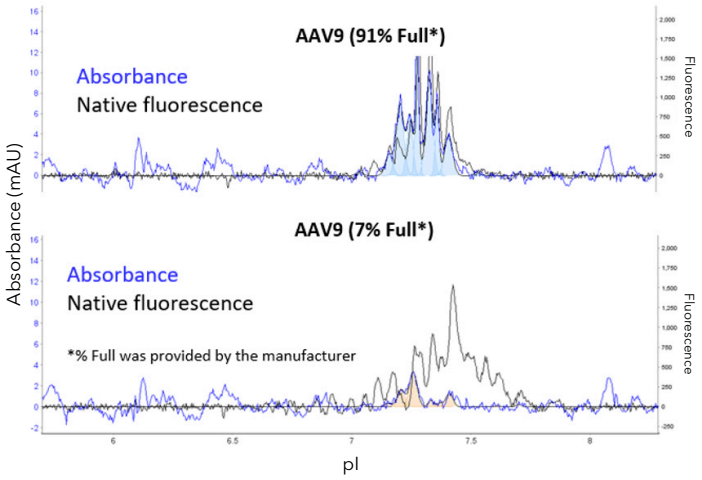


FIGURE 1. Apparent pI comparison of 8 different AAV serotypes with Maurice icIEF using native fluorescence detection. Distinct charge profiles are obtained for each serotype, demonstrating how Maurice can be used to determine AAV identity.

Purity

Gain a deeper understanding of what's contributing to your capsid content – DNA or protein – by leveraging absorbance and native fluorescence detection (NF) with icIEF. You can also use CE-SDS to confirm the purity of viral proteins. Instead of relying on a handful of highly specialized personnel, you can train your team on Maurice, develop robust methods, and transfer the same into QC without being hampered by experiment run times or reproducibility issues.

A



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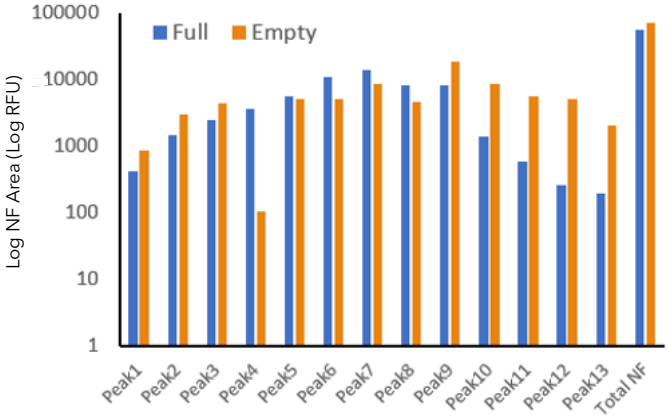

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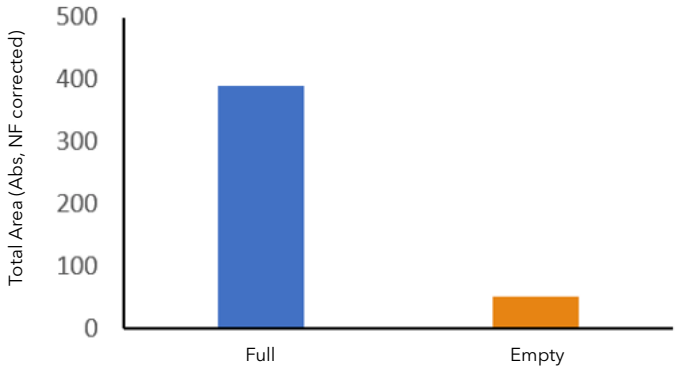


FIGURE 2. Evaluation of empty and full AAV capsids with icIEF using absorbance and native fluorescence detection modes. A) An overlay of absorbance and NF data for AAV9 at 91% full and 7% full. While both AAV samples show similar NF profiles, a stark difference is observed between the absorbance profiles, which is attributed to DNA's ability to absorb energy significantly at 280 nm. B) Peak area quantification of full and empty samples with NF detection, where the results are comparable. C) Quantification of the total area with absorbance normalized to NF, where the observed difference between full and empty samples is approximately 5-fold.

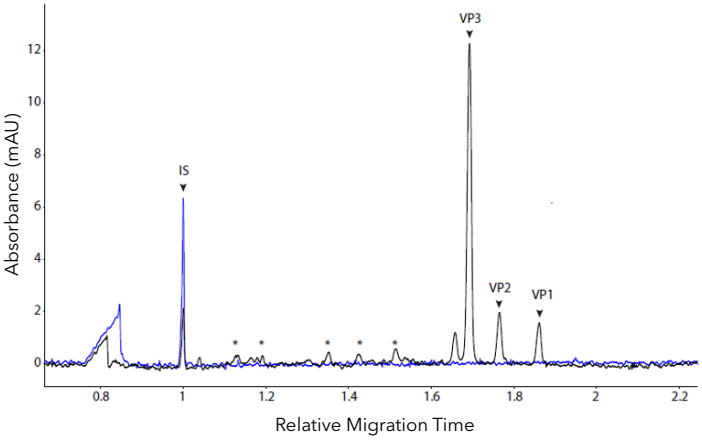
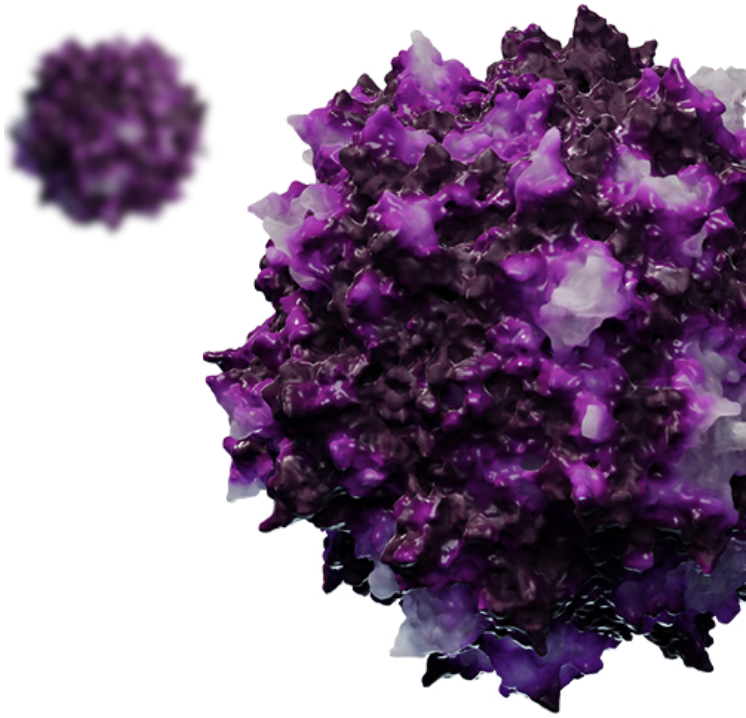


FIGURE 3. Determining AAV capsid purity with Maurice CE-SDS. This method detects and resolves AAV capsid proteins. The AAV2 sample is shown in the black trace and the blank sample is shown in the blue trace. The three viral proteins are clearly resolved, and the impurities are labeled with an asterisk. The internal standard is labeled as IS.



Stability

See changes in the charge profiles of AAVs, including the formation of aggregates, when they're subjected to different stress conditions. More importantly, you'll get an idea of how your product's potency has been impacted before you run any lengthy potency assays. Ultragenyx describes in their webinar how these altered charge profiles induced by stress correlated to the potency of their product.

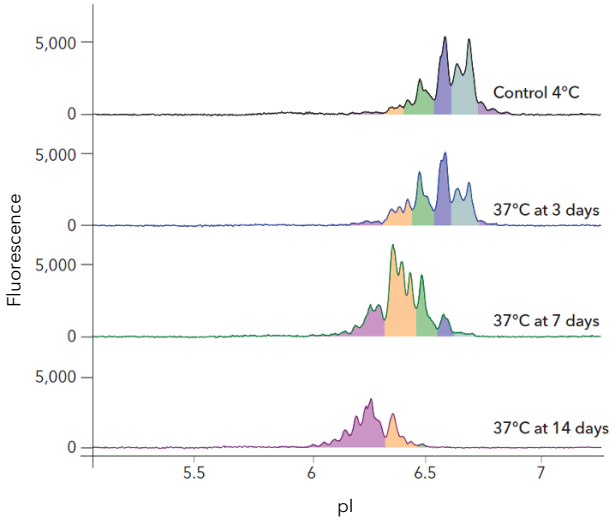


FIGURE 4. AAV stability evaluated with Maurice icIEF. AAV8 DS was incubated for 3, 7, or 14 days before analysis with Maurice. A control sample was used to compare the changes over time. Substantial profile changes are observed in as little as 7 days with the method. These changes correlate well with potency assays like mRNA expression, the data for which was presented by our collaborators at Ultragenyx, in the webinar Exploring the Charge Heterogeneity in Recombinant Adeno-Associated Virus with Imaged Isoelectric Focusing.

Summary

It is time to say goodbye to tedious techniques used for AAV analysis. By eliminating several steps in sample preparation and instrument setup, Maurice gives you the ease-of-use you need while quickly generating high-quality reproducible data. Instead of spending weeks on method development, training, and data analysis, simply insert an icIEF or CE-SDS cartridge into Maurice, develop method conditions within a day, and get the data you need for your AAVs.

The information presented in this spotlight is only a glimpse of how Maurice characterizes AAVs. For more data, refer to the resources below or visit us at proteinsimple.com/cell-and-gene-therapy.html

CQA	COMMON ANALYTICAL TECHNIQUES	KEY DRAWBACKS AFFECTING TIMELINE
Identity	SDS-PAGE, Western Blot, PCR	Poor quantitation and reproducibility
Purity	Transmission Electron Microscopy (TEM) and Analytical Ultracentrifugation (AUC)	Expensive, require highly trained personnel, prolonged method development, not QC-friendly
Stability	Light microscopy, TEM, AUC, size-exclusion chromatography/field flow fractionation with multi-angle light scattering (SEC-MALS/FFF-MALS)	Poor reproducibility, lack of assay robustness, time-consuming, long method development times

TABLE 1. Common analytical techniques for AAV analysis and their associated challenges.

Resources

Webinar

Exploring the Charge Heterogeneity in Recombinant Adeno-Associated Virus with Imaged Isoelectric Focusing

Application Note

Assessing Your AAV Product Quality? Get the Confidence You Need with Maurice

Application Note

icIEF Analysis of Adeno-Associated Virus (AAV) Proteins for Gene Therapy

Application Note

Characterization of Adeno-Associated Viral (AAV) Vector Proteins Using Maurice CE-SDS

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