

Strengthening USP AAV Reference Standards with cryoTEM characterization



Introduction

Accurate assessment of empty and full capsids is essential for the successful development of AAV-based gene therapy products. Although empty/full analysis is a well-established critical quality attribute, laboratories rely on a variety of analytical approaches, as there is no universally employed method. This variability creates challenges in method development, validation, and bridging studies, especially without an adequate reference standard. USP has developed two AAV Reference Standards, AAV8 (Empty Capsids, [catalog # 1000301](#)) and AAV8 (Full Capsids, [catalog # 1000302](#)), designed to ease the burdens associated with empty/full capsid analysis. These standards were designed to be suitable for a wide range of analytical techniques and to overcome limitations commonly encountered with commercially available materials, such as insufficient volume, low concentration, insufficient characterization, and poor capsid quality.

Expanded Characterization with cryoTEM

USP has expanded the analytical testing panel for the AAV8 reference standards to include cryogenic transmission electron microscopy (cryoTEM). Initial characterization included assessment by analytical ultra centrifugation (AUC), size-exclusion chromatography with multi-angle light scattering (SEC-MALS), charged detection mass spectroscopy (CD-MS), mass photometry, and UV-spectroscopy ([Figure 1](#) ([see application note](#))). The addition of cryoTEM strengthens the robustness and relevance of the standards by incorporating a widely used orthogonal technique that offers unique advantages over direct visualization of capsid populations.

CryoTEM is a powerful, high-resolution imaging technique that is used to directly analyze AAV particles. Before being imaged under an electron beam, samples are flash-frozen, which preserves the native structure and minimizes artifacts.

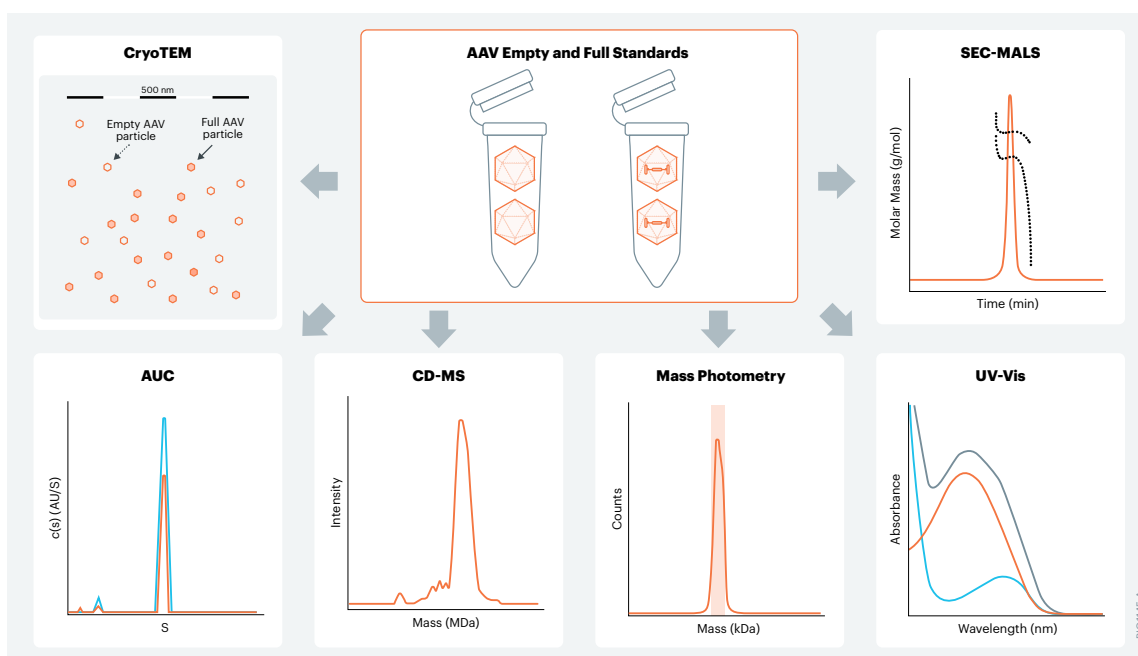


Figure 1. Overview of analytical methods used to analyze USP AAV Reference Standards.

Unlike many other techniques, cryoTEM directly visualizes individual capsid particles, allowing quantification of empty, full, and partially-filled AAV capsids.

Test Results

Both USP AAV8 (Full Capsids) and AAV8 (Empty Capsids) were measured undiluted on a Glacios cryoTEM instrument at 200 kV. Analysis of the electron density allowed for complete capsid characterization of these standards. Data from a multi-laboratory study of USP's AAV8 (Full Capsid) by cryoTEM revealed 92% full, 3% partially filled, and 5% empty capsids (**Figure 2**). AAV8 (Empty Capsid) had 1% full and 98% empty capsids, as measured by cryoTEM. These values align well with other techniques used to measure the USP AAV standards, as can be seen in **Table 1**. These results demonstrate strong agreement with orthogonal methods, highlighting the accuracy and reliability of cryoTEM, as well as reinforcing the value of the USP AAV standards.

Conclusion

The addition of cryoTEM data significantly enhances the value of the USP AAV reference standards by expanding their reach in both development and release testing. With six unique methods used to quantify the percentage of empty and full capsids, the USP standards offer a comprehensive and versatile solution for laboratories throughout the analytical lifecycle, including use in bridging studies or standards for release testing.

USP remains committed to continuously improving the characterization of its standards. As new methods emerge, USP will continue to update the testing panel to ensure that USP standards remain aligned with evolving industry practices. USP is committed to offering extremely high quality and well-characterized materials that can be used to assist testing laboratories. By integrating cryoTEM into the characterization panel, USP further strengthens confidence in the accuracy, reliability, and applicability of its AAV reference standards.

Ordering information:

AAV8 (Empty Capsids): store.usp.org/product/1000301

AAV8 (Full Capsids): store.usp.org/product/1000302

AAV analytical guide: genetherapyanalyticalguide.usp.org
and **additional application note**



More information: www.usp.org/biologics/aaav

Questions: uspbiologics@usp.org

USP store: store.usp.org

Table 1. Compiled data for AAV8 (Empty Capsids) and AAV8 (Full Capsids) using six methods.

Testing Method	AAV8 (Full Capsids)		AAV8 (Empty Capsids)	
	% Full	% Empty	% Full	% Empty
CryoTEM	92	8	2	98
SEC-MALS	98	2	2	98
Mass Photometry	91	6	5	95
CDMS	80	4	1	99
AUC	91	3	1	91
UV (A260/A280)	95	5	0	100

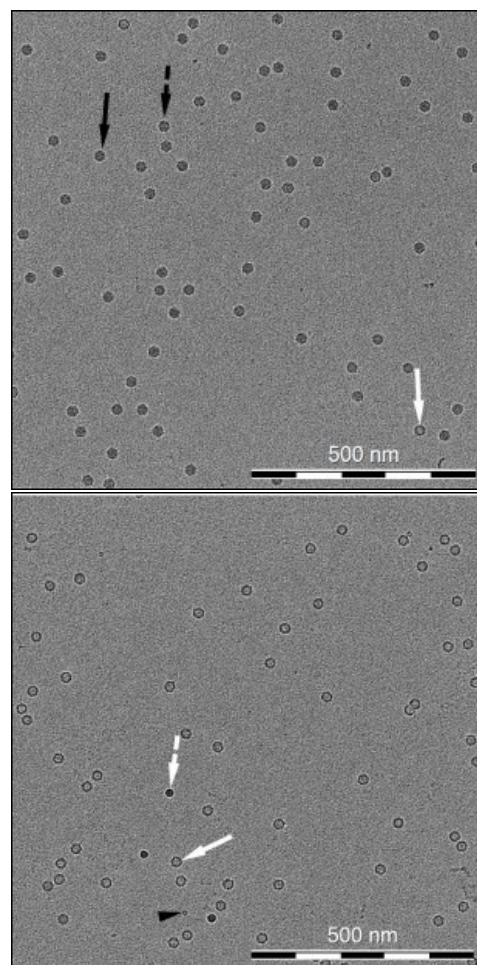


Figure 2. CryoTEM image for AAV8 (Full Capsids), below, and AAV8 (Empty Capsids), above. For AAV8 (Full Capsids), the solid black arrow represents full or overfull capsids, solid white represents empty capsids, and dashed black represents partially-filled capsids. For AAV8 (Empty Capsids), the solid white represents empty capsids, while the solid black is proteasome and dashed white is cryogenic contamination.