BioReliance®
Contract Testing Services

Technical Note

Empty/Full Capsid Ratio of AAV by Sedimentation Velocity – Analytical Ultracentrifugation

Analytical Development for AAV

Challenge

'Empty' capsids (i.e. vectors that do not contain the gene of interest) are a common product-related impurity of recombinant adeno-associated virus (AAV) products. Empty AAV capsids have the potential to impact safety and efficacy, since they may stimulate an unwanted immune response without delivering the intended therapeutic benefit. As such, it is important to characterize the relative amounts of empty and filled capsids within an AAV product.

Sedimentation velocity (SV) is an analytical ultracentrifugation (AUC) method widely recognized as the gold standard for the characterization of AAV empty/full capsid ratio due to its ability to resolve empty, partially filled and filled capsids.

Methodology

As the sample is centrifuged, boundaries of viral particle concentration are formed, which are monitored over time by measuring the absorbance at 230 nm. Analysis of the boundary data using direct modelling methods results in a sedimentation distribution plot. Empty, partially filled and filled capsids are resolved since they exhibit different sedimentation rates due to their different masses. The relative abundance of each species is determined from the percentage area of each integrated peak in the distribution plot.

Assay Details

Up to four samples can be analyzed in a single experiment. A minimum of 500 µL sample at 4.0 x 10^{12} vp/mL is required for the analysis.

A reference buffer solution must be provided with each sample set and this solution must be identical to the bulk solution in which the AAVs are dissolved. Since the assay is based on UV absorbance, there should be no interferents at the detection wavelength (230 nm) – see Table 1 for details.

Table 1: Formulation buffer requirements

Component	Concentration
Glycerol, sucrose, trehalose, pluronic acid	<0.05% w/v
Buffer salts (NaCl, KCl, NH4Cl, other)	10 mM < [buffer] < 150 mM

Case Study Results

The results detailed below were generated from an internal study using AAV8 capsids. Figure 1 shows the boundary data for a mixture of empty and filled AAV8 capsids and displays the evolution of two visibly distinct boundaries during the experiment. Analysis of this data results in sedimentation distribution profiles of the dissolved species such as that shown in Figure 2. Within this distribution, empty, partially filled, filled and higher molecular weight AAV species are resolved.

To challenge the performance of the assay, a series of solutions were prepared with different levels of empty capsids spiked into filled AAV8. A plot of observed empty capsids versus expected empty capsids is shown in Figure 3. These results demonstrate that assay can accurately quantify empty capsids at or above 5% of the total capsid content and the response of the assay is linear (R2 ≥ 0.99) over the range tested. The limit of detection is 2% empty capsids (data not shown).

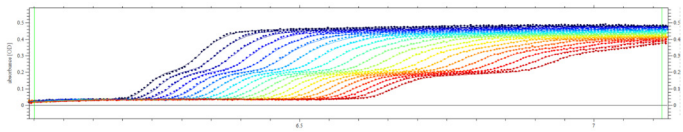

Figure 1: Boundary data generated from the sedimentation of a mixture of empty and filled AAV8 capsids

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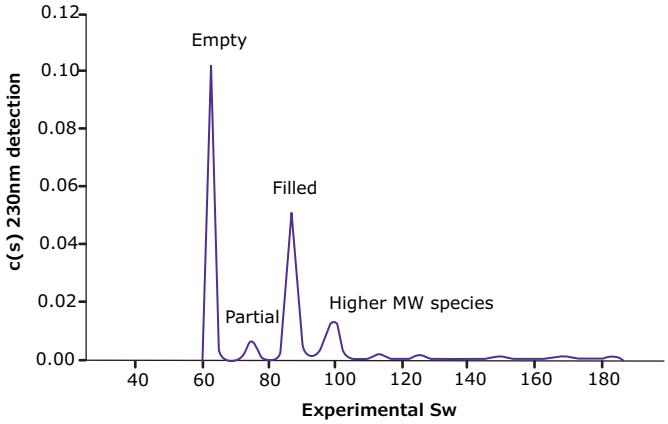

Figure 2: Sedimentation distribution plot for a mixture of empty and filled AAV8 capsids. Partially filled capsids and higher molecular weight species are observed also.

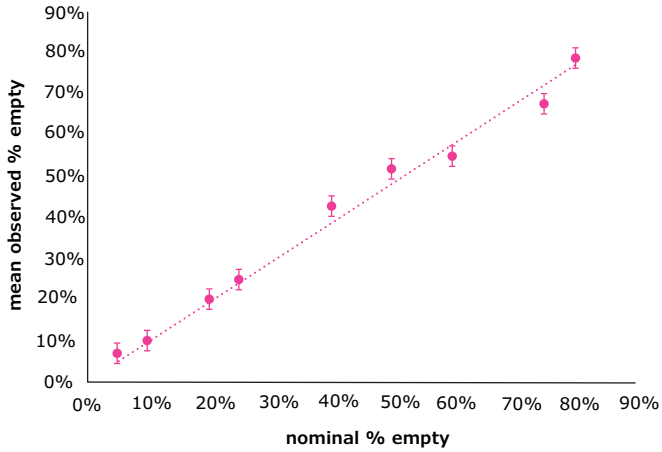

Figure 3: Quantitation of empty AAV capsids by SV-AUC

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