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Innovative Octet® Biolayer Interferometry (BLI) Approach for Non-Disruptive and Fast AAV Capsid Ratio Determination in Complex Biological Matrices

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- **Rapid Method Development:** Utilizes Biolayer Interferometry (BLI) to quickly determine AAV capsid empty/full ratios in complex biological matrices.
- **Serotype Compatibility:** The method is compatible with different AAV serotypes and offers a straightforward, one-step assay, significantly saving time.
- **Error Reduction:** Eliminates compounded error by not requiring separate DNA titer determination.
- **High Throughput and Cost Effective Detection:** Employs Octet® AAVX Biosensors for high throughput detection, which can be regenerated and reused.

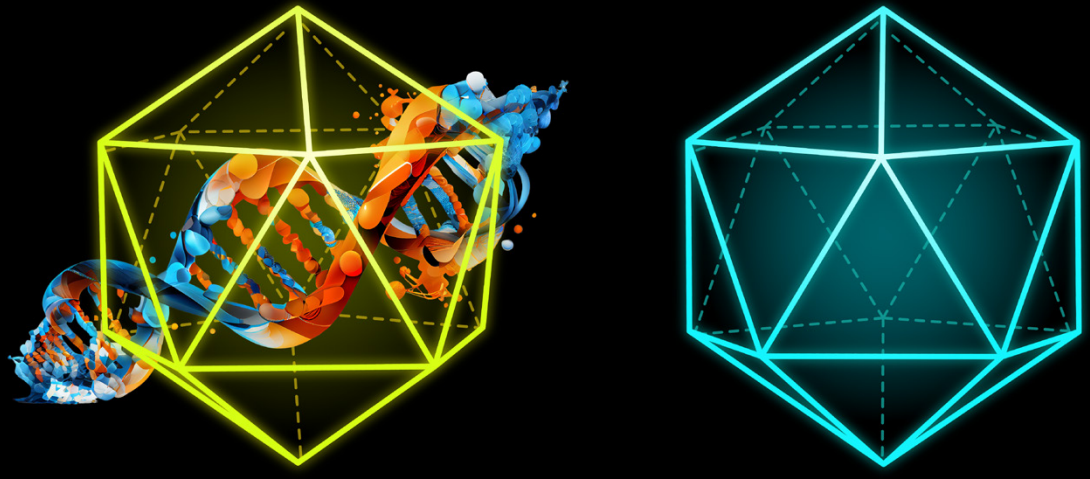
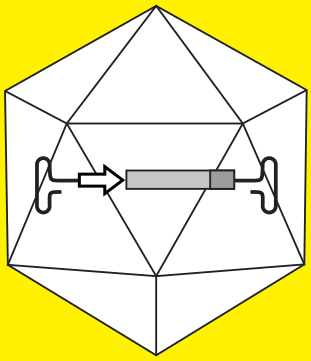
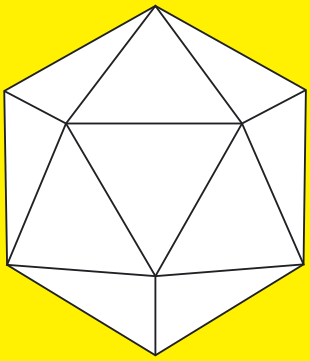


Figure 1: *Schematic of Full AAV Capsid with Genomic Insert (A) and Empty Capsid with No Insert (B)*

A



B



Note. At an equivalent capsid titer, the two exhibit different binding saturation signals on the Octet® BLI Platform that can be used for the determination of E/F ratio in an AAV bioprocess sample.

Introduction

Adeno-Associated virus (AAV) is composed of an icosahedral protein capsid ~26 nm in diameter and a single-stranded DNA genome of ~4.7 kb that can either be the plus (sense) or minus (anti-sense) strand. The AAV genome consists mainly of two viral genes: rep (replication) and cap (capsid), flanked by inverted terminal repeats (ITRs). The ITRs have a palindromic nucleotide sequence and create characteristic T-shaped hairpin structures, providing essential structural elements for viral genome replication and packaging. The open reading frame (ORF) of rep encodes several nonstructural proteins that are required for gene regulation, replication, transcription, and encapsidation. The ORF of cap encodes three structural proteins including virion protein 1 (VP1), VP2, and VP3. Distinct tissue tropism of different AAV serotypes results from variations in the processing of this cap ORF, leading to variable immune and transduction profiles. Different serotypes therefore have tropism for specific organs and tissues of the body and can be selectively deployed in gene therapy to target different organs.[1,2,3]

In gene therapy, gene replacement is considered a key strategy; it aims to deliver a gene product to compensate for loss-of-function mutations in various disease states.[4] AAV vectors are the leading platform for gene delivery for the treatment of a variety of human diseases. During development and production, AAV in-process samples often yield mixed populations of particles that include full capsids, partially full capsids, and empty capsids.

This heterogeneity during production necessitates that product characterization be efficiently done to establish the empty to full capsid ratios (E/F ratios). While there are many techniques that have been deployed to detect these ratios, they are often either expensive or time-consuming in addition to many other challenges hence making it difficult for developers to identify an ideal cost-effective technique.

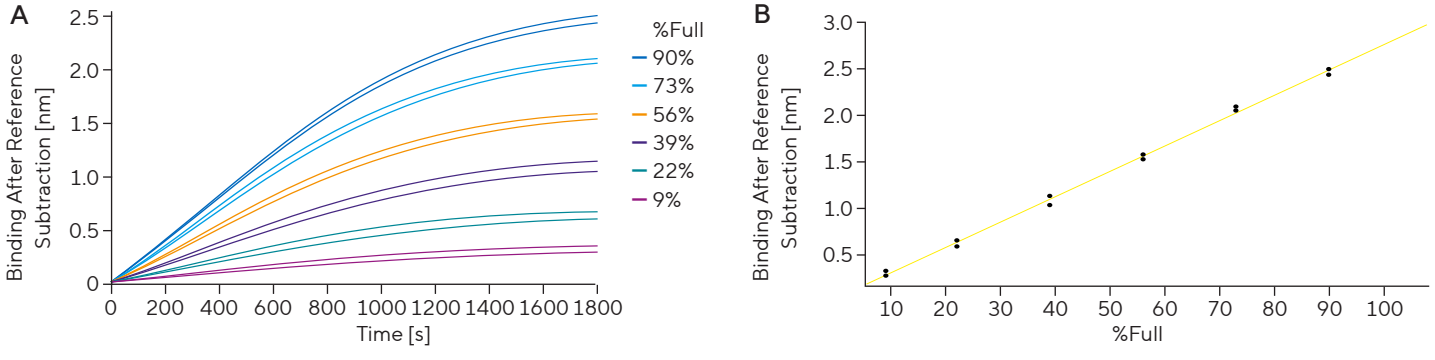
Octet® AAVX Biosensor was developed to enable rapid, real-time, and high-throughput measurement of AAV capsid titer in samples across the AAV workflow enabling quick process optimization, quality checks, and increased productivity. The AAVX Biosensors have broad serotype specificity allowing for the quantitation of 10 different serotypes, have high precision, and offer a capsid quantitation dynamic range of 8.5E8 to 1.0E13 vp/mL. Further, the Octet® AAVX Biosensors are compatible with different sample matrices encountered in upstream and downstream process intermediates thus allowing the analysis of crude samples without purification or treatment, requiring only sample dilution. With minimal assay optimization and with the appropriate reference standards, Octet® AAVX Biosensor users can now extend the same biosensors to detect AAV E/F ratios in an assay that takes < 60 min.

Principles for the Detection of AAV E/F Capsid Ratio Using the Octet® AAVX Biosensor

The principles of the BLI technology are such that binding of the analyte on the tip of the biosensor results in a molecular layer that increases in thickness as more analyte molecules bind to the surface. The spectral pattern changes as a function of the optical thickness of the molecular layer is monitored at the detector. While the AAVX biosensor utilizes an anti-AAV antibody to capture capsid proteins, the principles of detection of empty capsids relative to full capsids on BLI capitalize on the fact that although both bind to the antibody via the capsid proteins on the surface of the virion, the genomic insert in the full capsid imparts a density difference between the two (Figure 1) that leads to a difference in the binding signal at saturation.

As a fact, at a nominally equivalent capsid titer, full genomic capsids will exhibit a binding response higher than an equivalent amount of empty capsids at any given point in time, in which the difference in BLI signal is solely due to the density difference between equal numbers of AAV particles, with samples of higher % full generating higher signals (Figure 2). Even though both initial binding rate and saturation level report on %full differences, saturation analysis provides added benefits in that minor differences in sample titer can be tolerated, which eliminates compounded error — a limitation intrinsic to other methods that measure viral genome and capsid titers separately. This method is rapid, easy to perform, and accommodates a wide variety of matrices including untreated crude cell lysate, providing an attractive solution to upstream development efforts in AAV manufacturing. By analyzing intact viral capsids, the assay eliminates the need to release the packaged DNA, which would require extensive optimization and validation because of the complexities introduced by sample treatment and DNA detection. The main requirement however is that the capsid titer be (≥ 2E11 vp/mL).[5]

Figure 2: *Binding Response of AAV8 in the Octet® Sample Diluent Buffer (A) at Different % Full Ratios (90, 73, 56, 39, 22, 9) with the Resulting Standard Curve by Endpoint Analysis (B)*



Note. Samples were generated by mixing full and empty capsids to achieve the desired % full. Both full and empty capsids were titered at 2.5E11 vp/mL.

Matrix and Serotype Compatibility of the Assay

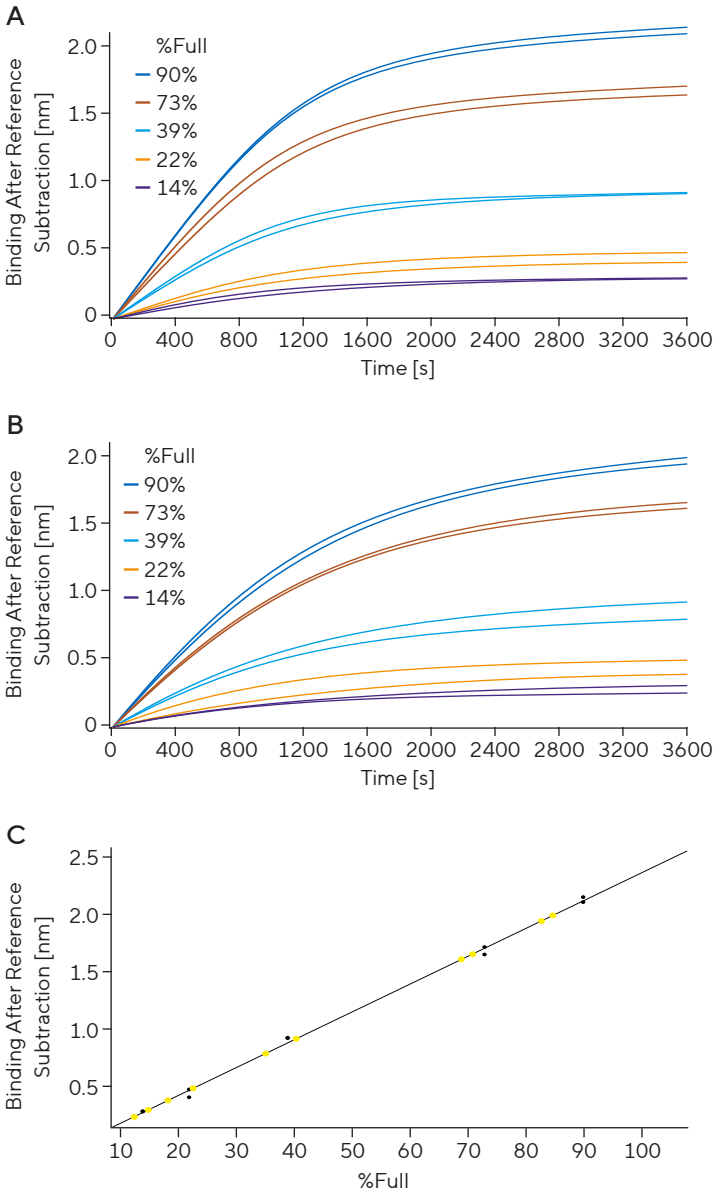
The Octet® AAVX Biosensor for AAV capsid titer measurement can be used with multiple AAV serotypes including AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9 and AAVrh10. However, for the analysis of the empty vs. full capsid ratio, the biosensor has been currently tested for AAV2, AAV5, and AAV8 serotypes. Other serotypes may require user optimization, keeping in mind assay condition requirements as stipulated in the related Octet® AAVX Biosensor E/F technical note.[5] Since this assay takes advantage of the density differences between full and empty capsids, the genomic insert's size and the normalized capsid titer are the key determinants to achieving a robust assay.

Table 1: *Method Compatibility with Common AAV Sample Matrices*

Matrix Category	Matrix Type	AAV Serotype	Minimum Dilution Required
Buffer	Octet® Sample Diluent	AAV2/5/8	Neat
Culture Media	Chemically defined, protein-free medium (Viral Production Media*, FreeStyle 293 Expression Medium*)	AAV5/8	Neat
	DMEM+10%FBS	AAV5/8	5-fold in Octet® sample diluent
Cell Lysis Solutions (in protein-free culture media)	1x Lysis Buffer with 1% Tween-20	AAV5/8	Neat
	0.5 M NaCl	AAV5/8	Neat
Cell Lysate	2 mg/ml HEK293 Cell Lysate with 1x Lysis Buffer and 0.5 M NaCl	AAV5/8	Neat

Note. The observed sample matrix compatibility of the Octet® AAV E/F method makes it highly suitable for a wide range of AAV purification workflows.

Figure 3: *Assay Matrix Compatibility Using AAV8 Samples Spiked in Octet® Sample Diluent Buffer (A) and in Crude Cell Lysate (B) with the Resulting Standard Curve by Endpoint Analysis (C)*



Note. Samples in B (yellow dots) and standards in A (black dots) generated comparable signals on the standard curve (C).

Table 2: *Results of the Octet® AAV E/F Assay Analyzing AAV8 Samples Spiked the Octet® Sample Diluent and in Crude Cell Lysate*

	Known %Full	90%	73%	39%	22%	14%
Samples spiked in the Octet® Sample Diluent	Average Calculated %Full (N=2)	90%	72%	41%	21%	14%
	%CV of Calculated %Full (N=2)	1.4%	2.6%	0.3%	9.6%	0.7%
	%Recovery*	100%	99%	104%	94%	102%
Samples spiked in Crude Cell Lysate	Average Calculated %Full (N=2)	84%	70%	38%	20%	14%
	%CV of Calculated %Full (N=2)	1.6%	1.9%	9.9%	15.0%	12.7%
	%Recovery*	93%	96%	97%	93%	98%

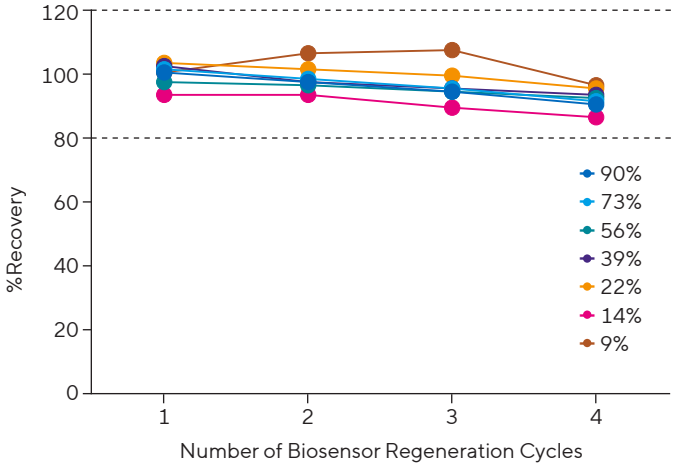
* Recovery refers to the percentage ratio of determined %Full (mean of two replicates) and theoretical %Full values.

Note. All standards and samples have a titer of 2.5E11 vp/mL.

Octet® AAVX Biosensor Regeneration for AAV E/F Ratio Analysis

The Octet® AAVX Biosensors can be regenerated for reuse 20 times when used for capsid titering. They can also be reused, although for a limited number of cycles, for AAV E/F ratio analysis, providing an efficient and cost-effective solution for high-throughput needs. The endpoint measurement used in the E/F ratio assay requires more stringent removal of analytes captured in the previous cycle; therefore a modified regeneration scheme from the one outlined in titer quantitation is recommended (see Octet® AAVX Biosensor E/F technical note[5]). The number of possible regenerations should be determined by the user as it depends on the sample, buffer and assay conditions used, especially for AAV serotypes that are prone to aggregation. Figure 4 shows an example of the AAV5 E/F detection assay in which the biosensor is regenerated four times.

Figure 4: *Octet® AAVX Biosensor % Recovery of Different %Full AAV5 Samples in the Octet® Sample Diluent Buffer Monitored Over Four Regeneration Cycles*



Conclusion

While some optimization may be needed for certain AAV serotypes and some matrix conditions, the Octet® AAV Empty vs. Full capsid ratio assessment approach enables Octet® BLI platform users to now extend the usage of the AAVX biosensors beyond AAV capsid titer quantitation. The proposed method does not require the lengthy and often inefficient capsid lysis to expose the ssDNA for its subsequent detection by comparative methods. The only critical requirements are that 1) the genomic insert is large enough to yield sufficient signal differences between empty and full genomic capsids, 2) the capsid titers of the empty reference and unknown samples are matched, and 3) reference materials against which sample E/F ratios are determined need to be well characterized in terms of their titer and % full capsid content to allow for accuracy. When combined with the high-throughput capabilities of the Octet® RH16 and RH96, the method accords users throughput advantages and time savings, often a challenge for some of the more established and industry-accepted methods such as analytical ultracentrifugation (AUC) and cryo-electron microscopy (cryo-EM).

Table 3: *Octet® AAV E/F Ratio Assessment Assay Time Required to Analyze One Fully Filled 96-Well Plate*[5]

	Octet® R8 (8-channel system)	Octet® RH16 (16-channel system)	Octet® RH96 (96-channel system)
Number of Analyzed Samples in one 96-well plate	77 samples + 11 Empty Ref + 8 Buffer	90 samples + 6 Empty Ref	95 samples + 1 Empty Ref
Total Run Time	6 hours	3 hours	30 min

References

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2. Carter, P.J.; Samulski, R.J. Adeno-associated viral vectors as gene delivery vehicles. Int. J. Mol. Med. 2000, 6, 17–27.
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4. Wang D, Tai PWL, Gao G. Adeno-associated virus vector as a platform for gene therapy delivery. Nat Rev Drug Discov. 2019 May;18(5):358-378. doi: 10.1038/s41573-019-0012-9. PMID: 30710128; PMCID: PMC6927556.
5. Technical Note: AAV Empty/Full Ratio Assessment Using the Octet® AAVX Biosensors.

Patent pending on methods disclosed in this document.

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