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Application Note

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Octet® AAVX Biosensors for Rapid and Direct Quantitation of AAV Capsids

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Abstract

Together with the Octet® Bio-Layer Interferometry (BLI) platform, the Octet® AAVX Biosensor quantitation assay workflow allows the rapid, real time and high-throughput measurement of AAV concentration in samples across the AAV bioprocess workflow enabling quick process optimization, quality check and increased productivity. Adeno-associated virus (AAV) has been a vector of choice in gene therapy as a gene delivery tool. Multiple AAV serotypes including both native (wild type) and recombinant are used in part due to their tissue specificity (tropism). In the production and manufacturing bioprocess workflow of AAVs, the concentration of the virus capsid (viral particle) is an important quality attribute. Current methods used to quantitate virus capsid concentration, such as ELISA, are time consuming, laborious and suffer from high variability. In this application note we present the results of the evaluation of the Octet® AAVX Biosensor for AAV capsid titer measurement. The AAVX Biosensors offer a quantitation dynamic range of $8.5x10^{8}$ to $1.0x10^{13}$ vp/mL, high precision and broad AAV serotype binding specificity, allowing for the quantitation of 10 different serotypes. Further, the AAVX Biosensors were observed to be compatible in different samples matrices encountered in upstream and downstream process intermediates requiring minimal sample preparation. The AAVX Biosensors are amenable to be regenerated and re-used for up to 20 times, reducing the cost per measurement.

Introduction

Considerable advancement in technology used in gene manipulation, editing and gene delivery has led to a rapid development of viable therapeutics based on gene therapy strategies. As of 2022, the FDA has approved eight gene therapy products for clinical use.[1] Candidates for gene therapy include cancers, hematological conditions, ocular diseases, neuromuscular disease, immunodeficiencies and frequently, rare or inherited disorders.

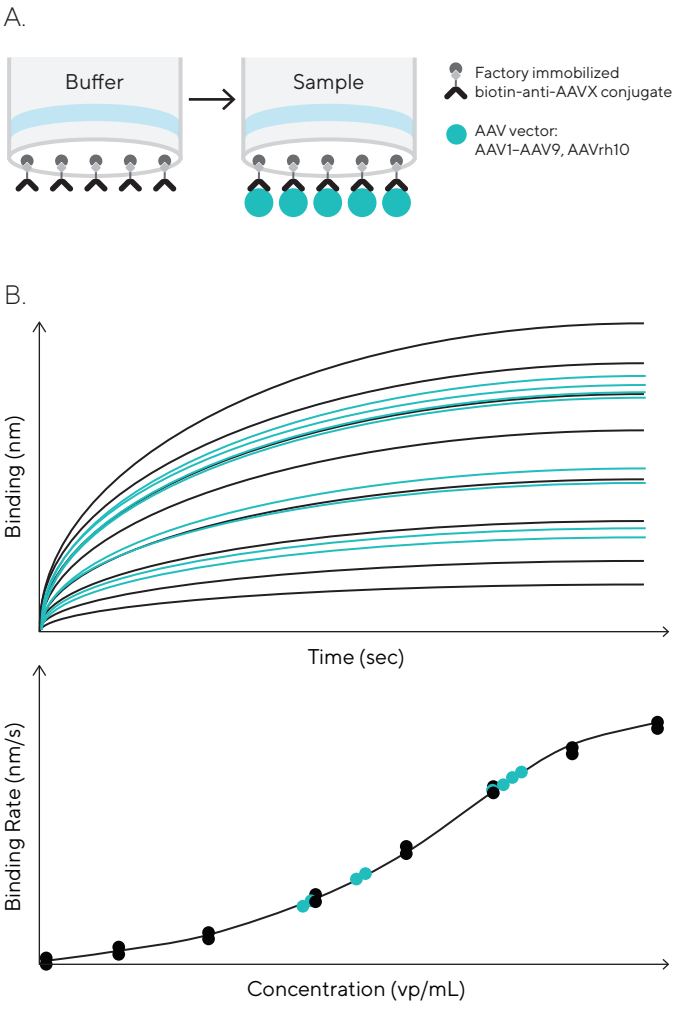
Key to the success of a gene therapy strategy is the development of an efficient process or method to deliver the gene product to the intended cell, tissue, or organ. Among the different viral vectors, the use of wild type and recombinant Adeno-associated viral (AAV and rAAV respectively) vectors has grown significantly. Production of AAV vectors at industrial scale requires the accurate measurement of the viral capsid concentration in both upstream and downstream AAV bioprocessing.

In this application note, we present the workflow and data around the use of the Octet® AAVX Biosensor for the quantitation of multiple AAV serotypes using a simple, direct quantitation assay design on the Octet® Bio-Layer Interferometry (BLI) label-free detection platform (Figure 1). The Octet® AAVX Biosensors use Sartorius' proprietary biosensor technology and are coated with the Thermo Scientific™ CaptureSelect™ Biotin Anti-AAVX Conjugate from Thermo Fisher Scientific Inc.

The AAVX Biosensors can be used on all models of the Octet® BLI platform except on the Octet® N1, where further assay optimizations would be required. This application note discusses key features and benefits of the AAVX Biosensor exemplified by its ability to bind to 10 different AAV serotypes (AAV 1–9 and AAVrh10), ability to quantitate both purified and crude viral samples, and good regenerability and re-use which helps reduce cost per assay.

Figure 2 presents a generalized workflow of AAV vector production and illustrates technology solutions offered by Sartorius. To learn more of the individual products, click on the image to access the product website.

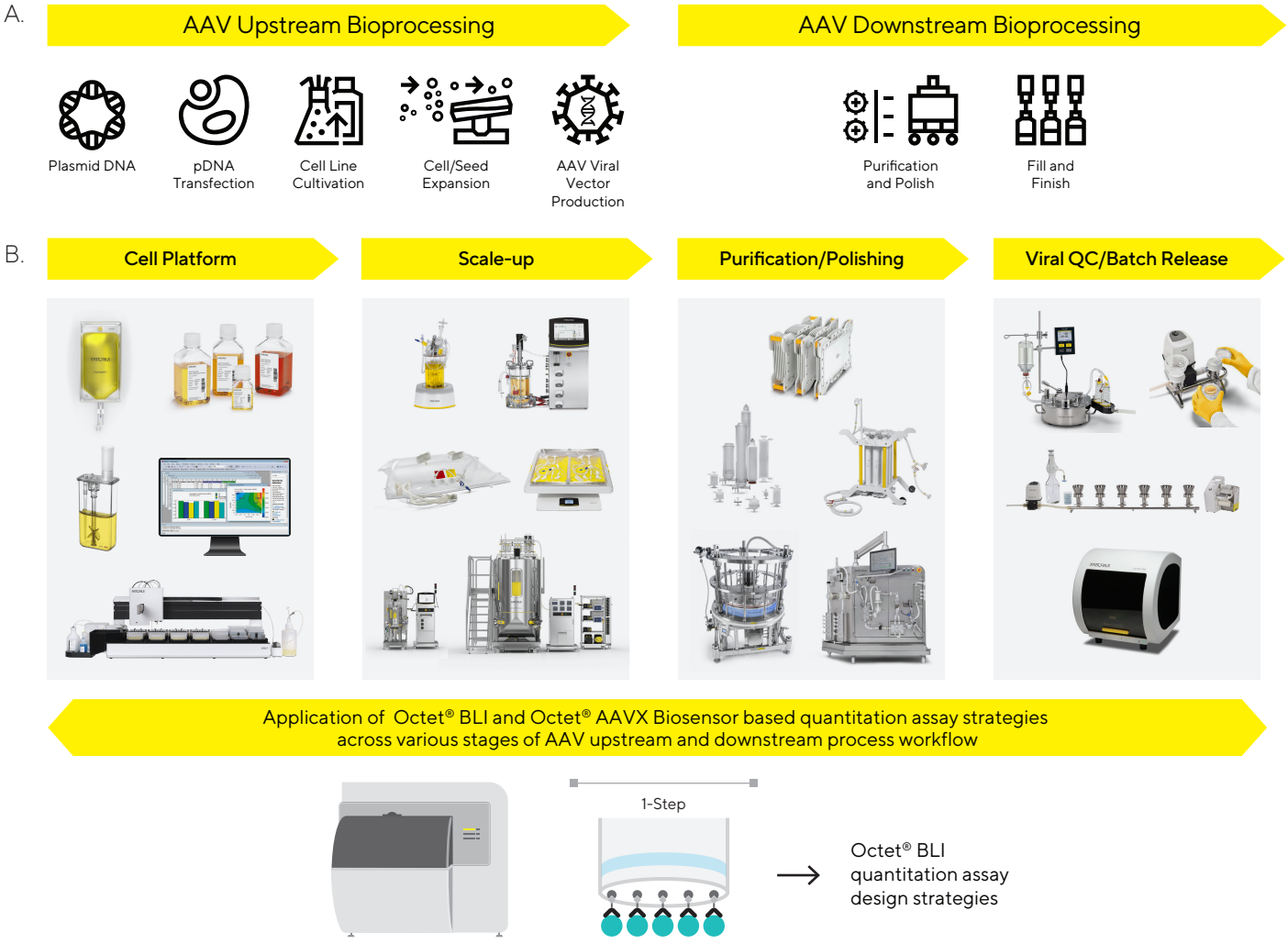
Figure 1
Quantitation Assay Workflow Using the Octet® AAVX Biosensors.



Note. (A) Assay illustration: Baseline equilibration in assay buffer (Buffer) followed by direct capture of AAV particles (Sample). (B) Schematic representation of quantitation analysis of AAV standards and unknown samples on the Octet® BLI platform.

Figure 2

Generalized Workflow for AAV Vector Production and Technology Solutions Offered by Sartorius to Support the AAV Production Workflow.



Note. (A) Generalized overview illustrating important steps/ processes in upstream and downstream AAV bioprocessing. (B) Sartorius products supporting applications across the different stages in the AAV production workflow. Click on individual product photos for more information.

Materials and Methods

Materials

- Octet® BLI system (R8, RH16, RH96) with Octet® BLI Discovery and Analysis Studio Software, version 13.0.1 and above.
- Octet® AAVX Biosensors (Sartorius Part No. 18-5160)
- AAV Standards: AAV 1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV8 and AAV9 were purchased from ViroVek, CA, USA. AAV 7 was purchased from Vigene Biosciences, MA, USA and AAVrh10 from Vector Biolabs, PA, USA.
- For all Octet® BLI systems: 96-well, black, flat bottom microplate, Greiner Bio-One Part No. 655209
  - Optional for Octet® RH16 and RH96 BLI systems: 384-well, black, flat bottom, polypropylene microplate, Greiner Bio-One Part No. 781209
- Buffers: The biosensors are compatible with a wide range of buffers. The quantitation assays were performed with the Octet® Sample Diluent buffer, pH 7.4 (Sartorius Part No. 18-1104).

Methods

For the studies discussed in the application note, in general, the quantitation assay workflow with the AAVX Biosensors took about 15–30 minutes to perform a measurement depending on the AAV serotype and concentration tested. The quantitation assay workflow included the following steps:

a. Biosensor hydration: AAVX Biosensors were dipped in the assay buffer for at least 10 minutes before use.
b. AAV direct quantitation assay design:
   i. Initial baseline equilibration: The hydrated AAVX Biosensors were dipped in assay buffer (Sample Diluent) for 180 seconds.
   ii. Direct quantitation: Following the baseline equilibration, the AAVX Biosensors were dipped into microplate wells containing the AAV serotype specific reference standards and test samples (unknown AAV concentration) for 900–1800 seconds to determine the binding rate of AAV vector to the AAVX antibody.
   iii. Biosensor regeneration for re-use: Biosensors were dipped into 10 mM glycine buffer, pH 1.7 for 5–10 seconds followed by a dip into assay buffer for 5–10 seconds. This process was repeated for 3 cycles.
   iv. All quantitation assays were performed at a sample stage with shake speed of 1000 rpm at 30°C.
   v. The binding rate for the standards was fitted using the 'initial slope' binding rate equation in the Octet® Analysis Studio Software and a standard curve was generated by applying a 4PL weighted Y fitting model.
   vi. The concentrations of the test samples were determined by interpolating their binding rates with the standard curve.

ELISA: Briefly, the ELISA assay protocol took about 4–6 hours to perform depending on the format and included the following generalized steps:

a. Capture of AAV standard and test samples: AAV serotype standard and test samples were incubated in anti-serotype-specific capture antibody coated microtiter plate. Followed by multiple wash steps in the assay buffer.
b. Detection step 1: The captured AAV capsid was detected using HRP (Horseradish peroxidase) tagged serotype-specific antibody followed by multiple wash steps in the assay buffer.
c. Detection step 2: Incubated with appropriate substrate such as TMB (tetramethylbenzidine) to develop a color reaction which is proportional to the concentration of AAV capsids in the sample. The color developed was measured at the recommended absorbance wavelength using an ELISA plate reader.

Results and Discussion

The serotype specificity and performance of the AAVX Biosensors in quantitation assays was evaluated internally at Sartorius as well as at customer sites.

Serotype Specificity and Dynamic Range of the Octet® AAVX Biosensors

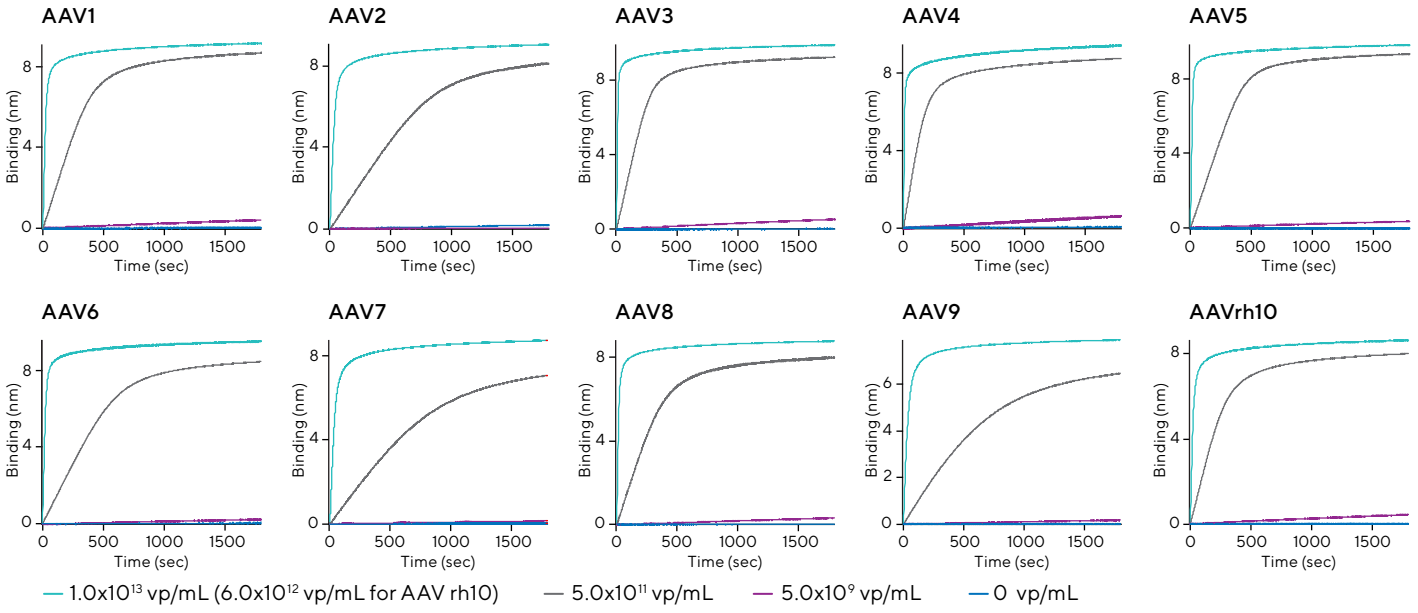
The serotype and binding specificity assays were performed using the Octet® BLI platform's established direct quantitation assay design as described in the methods section. Ten different AAV serotypes, namely AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9 and AAVrh10 were tested to evaluate the binding specificity of the Octet® AAVX Biosensors.

Figure 3A presents a 3-point dose-response binding data demonstrating the binding specificity of the AAVX Biosensors for individual serotypes measured over a broad concentration range. Further, the dynamic range of the AAVX Biosensor was evaluated and found to be between $8.5x10^{8}$ to $1.0x10^{13}$ vp/mL for most serotypes tested. This range spans approximately 5 orders of magnitude, which allows the accurate measurement of both high and low titer samples. Figure 3B–C illustrates with an example the dynamic range achieved using the AAVX Biosensors to quantitate AAV9, tested using concentrations from $7.62x10^{8}$ to $1.00x10^{13}$ vp/mL. A good % recovery (80–120%) and precision (CV <10%) was observed for all concentrations tested within this dynamic range (Table 1).

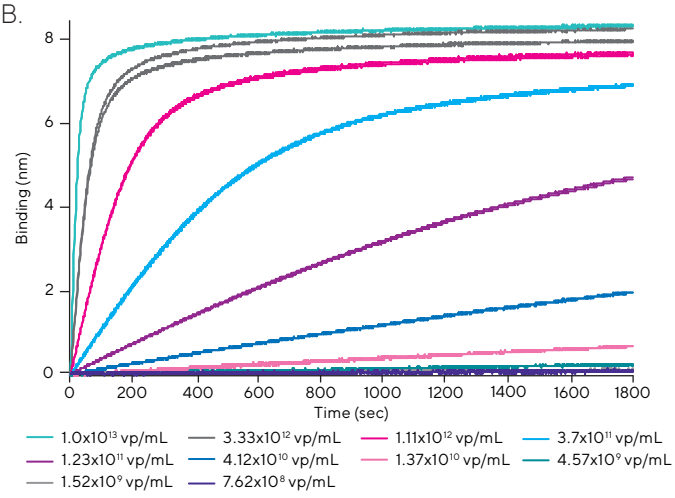
Figure 3

Quantitation Dynamic Range of Octet® AAVX Biosensors.

A.



B.



C.

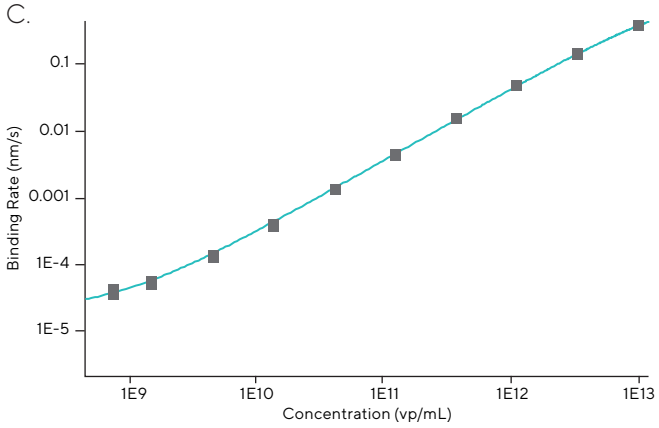


Table 1

AAV9 Capsid Quantitation Assay Results Generated Using Octet® AAVX Biosensors.

Known Titer, vp/mL	Average Calculated Titer (n=3), vp/mL	Titer %CV (n=3)	% Recovery
$1.00x10^{13}$	$1.00x10^{13}$	1.1%	100%
$3.33x10^{12}$	$3.30x10^{12}$	2.2%	99%
$1.11x10^{12}$	$1.13x10^{12}$	0.4%	101%
$3.70x10^{11}$	$3.85x10^{11}$	0.2%	104%
$1.23x10^{11}$	$1.17x10^{11}$	0.6%	95%
$4.12x10^{10}$	$3.90x10^{10}$	0.5%	95%
$1.37x10^{10}$	$1.16x10^{10}$	2.4%	85%
$4.57x10^{9}$	$3.95x10^{9}$	4.1%	86%
$1.52x10^{9}$	$1.32x10^{9}$	8.9%	87%
$7.62x10^{8}$	$7.62x10^{8}$	8.7%	100%

Note. (A) Binding specificity of the AAVX Biosensors tested for individual AAV serotype using a 3-point (high, medium and low concentration AAV titer) dose-response curve. (B) Dose-response binding curve of AAV9 capsids measured over a dynamic range of $7.62x10^{8}$–$1.0x10^{13}$ vp/mL. (C) AAV9 capsid titer standard calibration curve calculated using 4PL (weighted Y) fitting model. Log scale applied to x-axis and y-axis.

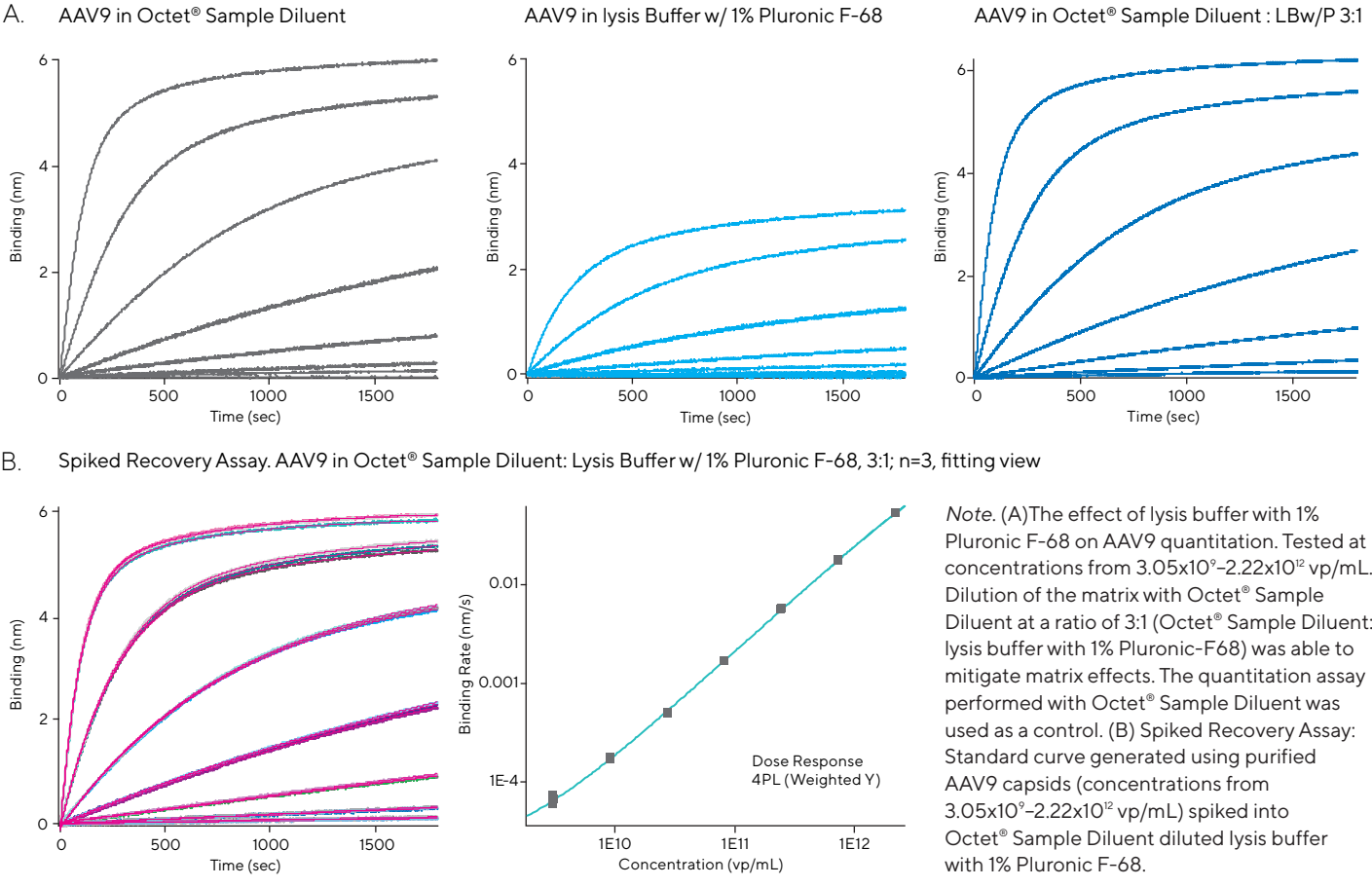
Matrix Interference Studies

A key advantage of the Octet® BLI platform is the ability to work with both purified and crude samples. Different buffer and media matrices are used across the AAV production workflow in both upstream and downstream bioprocessing. A common and simple approach to mitigating the effects of sample matrix is to dilute the crude sample in an appropriate assay buffer to reduce the concentration of matrix components affecting assay performance. We recommend a dilution factor of 4–10X in Octet® Sample Diluent to reduce matrix effect. As part of the assay optimization process, it is recommended to test a few dilutions of the sample (e.g. 4x, 8x, 16x, 32x or larger dilutions) along with the neat (undiluted) sample. Additionally, sample dilution helps to bring samples with high AAV titers fall into the dynamic range of the standard curve. To test the effect of matrix interference, a quantitation assay was performed as described in the methods section. Briefly, purified AAV samples were spiked into different matrices (undiluted and diluted) to generate a concentration series spanning from $3.05x10^{9}$–$2.22x10^{12}$ vp/mL. The change in the binding rate and response of the AAV to the AAVX Biosensor was evaluated under these conditions. The results of the spiked recovery assay performed using diluted lysis buffer (Octet® Sample Diluent:Lysis Buffer w/1% Pluronic F-68, diluted 3:1) is presented in Table 2.

Figure 4A exemplifies with an illustration the effect of lysis buffer with 1% Pluronic F-68 on AAV9 quantitation. A significant interference on the sensitivity and dynamic range of the assay was observed as indicated by reduced binding response. Further, the gain in assay robustness due to matrix dilution was evaluated by performing a spiked recovery assay with purified AAV9 capsids in diluted sample matrix (Figure 4B and Table 2). The %recovery for the samples were between 95–105% and a good precision (CV<10%) was observed for the concentrations tested indicative of very good assay robustness, mitigation of matrix effects by dilution and overall AAVX Biosensor compatibility when working with complex matrices. Additional studies performed at BridgeBio Pharma, Inc., to quantitate AAV5 in downstream bioprocess samples showed that a 1:10 to 1:20 dilution of the sample yielded accurate AAV5 titer measurement (Note: the samples

Figure 4

Effect of Sample Matrix and Assay Buffers on Robustness and Sensitivity of AAVX Quantitation Assays.



Note. (A)The effect of lysis buffer with 1% Pluronic F-68 on AAV9 quantitation. Tested at concentrations from $3.05x10^{9}$–$2.22x10^{12}$ vp/mL. Dilution of the matrix with Octet® Sample Diluent at a ratio of 3:1 (Octet® Sample Diluent: lysis buffer with 1% Pluronic-F68) was able to mitigate matrix effects. The quantitation assay performed with Octet® Sample Diluent was used as a control. (B) Spiked Recovery Assay: Standard curve generated using purified AAV9 capsids (concentrations from $3.05x10^{9}$–$2.22x10^{12}$ vp/mL) spiked into Octet® Sample Diluent diluted lysis buffer with 1% Pluronic F-68.

were diluted 1:20 to bring the AAV5 titer in the test samples within the dynamic range of the standard curve). The effect of the matrix on the quantitation assay was observed to be AAV serotype-dependent and a 4-fold to 10-fold dilution in sample diluent buffer was observed to mitigate the matrix effect significantly in most samples tested (Table 3).

Table 2
AAV9 Spiked Recovery Assay.

Known AAV9 Titer (vp/mL)	BR	BR CV	Calc. Conc	Conc. CV	Recovery
$2.22x10^{12}$	0.05313	0.4%	$2.22x10^{12}$	0.4%	100%
$7.41x10^{11}$	0.01777	1.1%	$7.38x10^{11}$	1.1%	100%
$2.47x10^{11}$	0.00568	0.3%	$2.51x10^{11}$	0.2%	102%
$8.23x10^{10}$	0.00171	0.7%	$8.22x10^{10}$	0.6%	100%
$2.74x10^{10}$	0.00051	2.2%	$2.61x10^{10}$	2.1%	95%
$9.14x10^{9}$	0.00017	2.6%	$9.11x10^{9}$	2.7%	100%
$3.05x10^{9}$	0.00007	8.8%	$3.05x10^{9}$	11.3%	100%

Note. Purified AAV9 capsids spiked into Octet® Sample Diluent diluted Pluronic-F68 matrix (3:1).

Table 3
Different Matrix Types Tested to Evaluate the Robustness of the AAVX Quantitation Assay.

Matrix Type	AAV Serotypes	Min. Dilution Factors in Octet® Sample Diluent
Octet® Sample Diluent Buffer	AAV2/5/8/9	Undiluted
Lysis buffer +1% Pluronic F68	AAV8/9	10- / 4-fold
Lysis buffer +0.5 mg/mL HEK293 cell lysate	AAV2/5	4-fold
Medium (DMEM +10% FBS)	AAV8/9	5-fold
Formulated product*	AAV5	10-fold
Concentrated lysate*	AAV5	10-fold
Clarified lysate*	AAV5	10-fold
Affinity neutralized eluate*	AAV5	20-fold#
Cell lysate†	AAV2/5/8	Undiluted
Clarified lysate using microfiltration†	AAV2/5/8	Undiluted
Affinity purified sample†	AAV2/5/8	Undiluted
Cell supernatant†	AAV8	Undiluted
Clear filtered supernatant†	AAV8	Undiluted
Undiluted Pel 15% PEG†	AAV8	10-fold
Purified AAV (pH 6.5, 1M NaCl)†	AAV8	Undiluted
Purified AAV (pH 8.5, 1M NaCl)†	AAV8	10-fold

* Data generated at BridgeBio Pharma, Inc.

† Data generated by the Lab Essentials Applications group (Sartorius).

Sample diluted 20-fold to bring the AAV5 titer within the dynamic range of the standard curve.

Correlation of Octet® AAVX Assays with ELISA

ELISA has traditionally been used to quantitate the AAV viral capsid titers and is often considered the method of choice for AAV titer measurement. Octet® AAVX assays provide a viable solution to some of the limitations associated with the ELISA technique, such as being an end-point assay often requiring considerable time and effort to perform. The Octet® BLI system design allows for rapid assay setup, analysis and displays data in real time which provides the ability to monitor and optimize every assay step. Figure 5A–B shows the scatter plot and corresponding data comparing the Octet® AAVX assay and ELISA for AAV8 and AAV9 quantitation, where a good correlation (R^2> 0.99) was observed. Table 4 presents the results of AAV2 titer in cell lysates samples taken from six Ambr15 (Sartorius) micro-bioreactor vessels 96h post transfection determined on the two platforms. The concentrations determined using the Octet® AAVX assay compared well with ELISA with a good correlation between the two.

Table 4
Quantification of AAV2 in Cell Lysate Samples.

Sample ID	Octet® AAVX Biosensor (vp/mL)	AAVX Biosensor Conc. CV (%)	ELISA (vp/mL)	ELISA Conc. CV(%)
NE	0	0	0	6.06
V1	$1.01x10^{12}$	2.13	$1.10x10^{12}$	6.06
V2	$1.26x10^{12}$	6.21	$1.11x10^{12}$	8.99
V3	$5.67x10^{11}$	0.68	$5.54x10^{11}$	1.68
V4	$6.01x10^{11}$	1.15	$5.58x10^{11}$	5.20
V5	$8.87x10^{11}$	1.29	$8.30x10^{11}$	4.69
V6	$5.80x10^{11}$	0.17	$5.35x10^{11}$	5.69

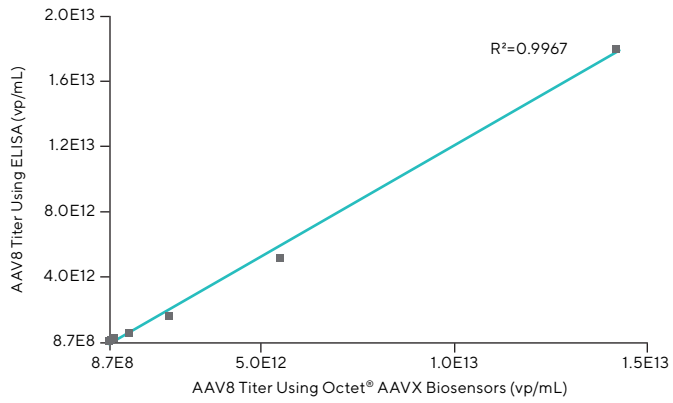
Note. Comparison between Octet® AAVX assay and ELISA. Samples (V1–V6) were collected from 6 different Sartorius Ambr® 15 automated bioreactor systems 96 h post-transfection. NE sample has been taken from non-transfected HEK293 cells. Data generated at Sartorius Stedim Biotech GmbH.

In another study to investigate the correlation between the Octet® AAVX assay and ELISA, scientists at BridgeBio Pharma Inc. and REGENXBIO, Inc. tested different AAV serotypes from samples at various steps in their AAV bioprocess workflow. In the study at REGENXBIO Inc., test samples (purified and process intermediate) of AAV8 and AAV9 were quantitated with the Octet® AAVX assay and ELISA, and a good correlation was observed (Table 5). At BridgeBio Pharma Inc., AAV5 titers in the range of $6.73x10^{9}$ to $9.59x10^{13}$ vp/mL from various downstream process intermediates were tested, and a good correlation between the Octet® AAVX assay and ELISA was observed (Figure 6).

Figure 5

Quantitation of AAV Serotypes Using Octet® AAVX Biosensors on the Octet® BLI Platform and ELISA.

A.

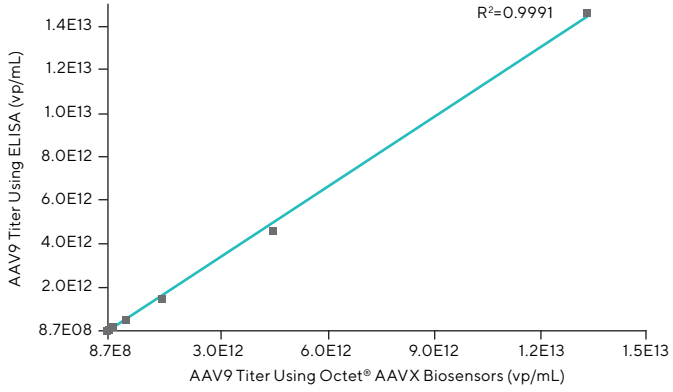


Note. Correlation of standard (reference) AAV8 at concentrations covering the Octet® AAVX Biosensor dynamic range measured on the Octet® BLI and ELISA.

AAV8	Octet® AAVX Biosensor (vp/mL)	ELISA (vp/mL)
Sample 1	$1.31x10^{13}$	$1.79x10^{13}$
Sample 2	$4.43x10^{12}$	$5.08x10^{12}$
Sample 3	$1.58x10^{12}$	$1.62x10^{12}$
Sample 4	$5.37x10^{11}$	$5.16x10^{11}$
Sample 5	$1.66x10^{11}$	$1.77x10^{11}$
Sample 6	$4.59x10^{10}$	$6.07x10^{10}$
Sample 7	$1.59x10^{10}$	$2.12x10^{10}$
Sample 8	$4.52x10^{9}$	$7.67x10^{9}$
Sample 9	$1.45x10^{9}$	$2.25x10^{9}$

Note. Comparison of AAV8 Quantitation Assay results generated using Octet® AAVX Biosensors vs. ELISA.

B.

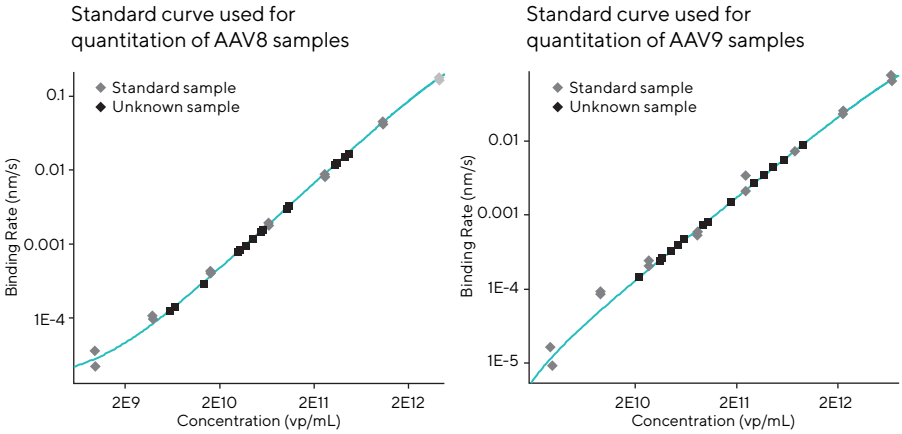


Note. Correlation of standard (reference) AAV9 at concentrations covering the Octet® AAVX Biosensor dynamic range measured on the Octet® BLI and ELISA.

AAV9	Octet® AAVX Biosensor (vp/mL)	ELISA (vp/mL)
Sample 1	$1.33x10^{13}$	$1.46x10^{13}$
Sample 2	$4.61x10^{12}$	$4.61x10^{12}$
Sample 3	$1.53x10^{12}$	$1.47x10^{12}$
Sample 4	$5.18x10^{11}$	$5.02x10^{11}$
Sample 5	$1.64x10^{11}$	$1.74x10^{11}$
Sample 6	$4.83x10^{10}$	$5.98x10^{10}$
Sample 7	$1.75x10^{10}$	$2.07x10^{10}$
Sample 8	$4.57x10^{9}$	$6.76x10^{9}$
Sample 9	$8.28x10^{8}$	$1.05x10^{9}$

Note. Comparison of AAV9 Quantitation Assay results generated using Octet® AAVX Biosensors vs. ELISA.

C.



Note. Quantitation of AAV8/9 Capsid Concentrations Determined in Crude and Purified Samples. (Left) AAV8: Standard curve used for quantitation of AAV8 samples. (Right) AAV9: Standard curve used for quantitation of AAV9 samples. Log scale applied to x-axis and y-axis to better illustrate the range of capsid concentrations tested. Data generated at REGENEXBIO Inc.

Table 5

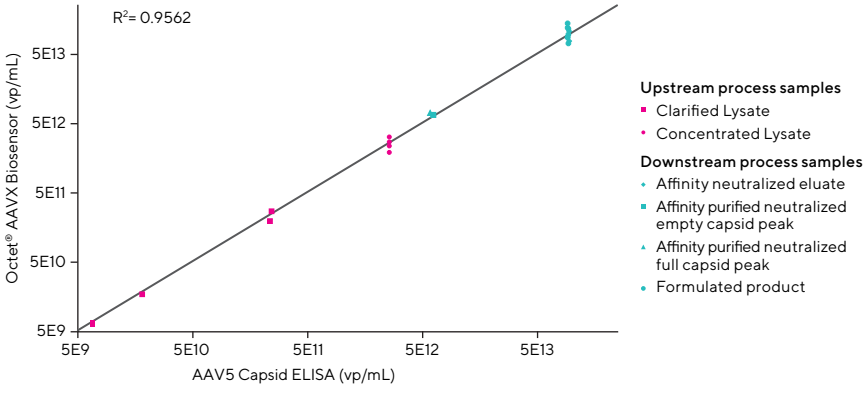
Quantitation of AAV8 and AAV9 in Purified and Crude Samples (Bioprocess Intermediates) by Octet® AAVX Biosensor and ELISA.

Serotype	Sample Number	Octet® AAVX Biosensor (vp/mL)	Commercial ELISA (vp/mL)
AAV8	1 (crude)	$5.86x10^{11}$	$5.10x10^{11}$
	2 (crude)	$1.15x10^{12}$	$1.13x10^{12}$
	3 (purified)	$3.20x10^{14}$	$2.91x10^{14}$
	4 (purified)	$4.11x10^{14}$	$4.42x10^{14}$
AAV9	5 (crude)	$3.63x10^{12}$	$2.36x10^{12}$
	6 (crude)	$6.95x10^{11}$	$5.49x10^{11}$
	7 (purified)	$7.39x10^{14}$	$6.58x10^{14}$
	8 (purified)	$4.19x10^{14}$	$4.02x10^{14}$

Note. AAV8/9 concentrations presented in the table were determined by extrapolation using the standard AAV8/9 curves shown. Log scale applied to x-axis and y-axis to better illustrate the range of capsid concentrations tested. Data generated at REGENXBIO Inc.

Figure 6

Quantitation of AAV5 Serotype Using Octet® AAVX Biosensors on the Octet® BLI Platform and ELISA.



Note. Quantitation of AAV5 Serotype Using Octet® AAVX Biosensors on the Octet® BLI Platform and ELISA. AAV5 capsid ELISA vs Octet® BLI comparison. Quantitation of AAV5 capsid titers from bioprocess intermediates. Log scale applied to x-axis and y-axis. Data generated at BridgeBio Pharma Inc.

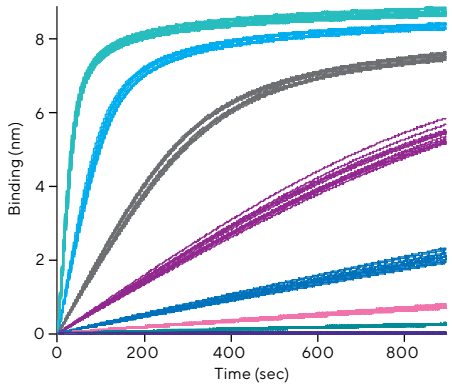
High-Throughput AAV Titer Analysis

High-throughput titer measurement capability offers customers the ability to optimize assays rapidly and test multiple samples simultaneously, increasing the ability to complete more projects per year. The Octet® BLI portfolio includes automated systems of varying throughput that includes the 8-channel, 16-channel and 96-channel systems that enable 8, 16 and 96 simultaneous titer measurements respectively, using either a 96- or 384-well sample plate. A high-throughput AAVX assay was setup to measure AAV titers; 96 samples of AAV8 serotype were tested over a concentration range from $3.05x10^{9}$–$2.22x10^{12}$ vp/mL on the three instrument models. The results of the study are presented in Figure 7A–C and the total assay time in Figure 7D. Compared to traditional ELISA which takes 4–6 hours to measure 96 samples, the time taken on all three Octet® models was significantly less, requiring just ~15 minutes on the Octet® RH96 BLI system. The Octet® RH96 BLI system offers the highest throughout both in terms of total number of samples that can be tested per assay as well as the ability to perform 96 simultaneous measurements.

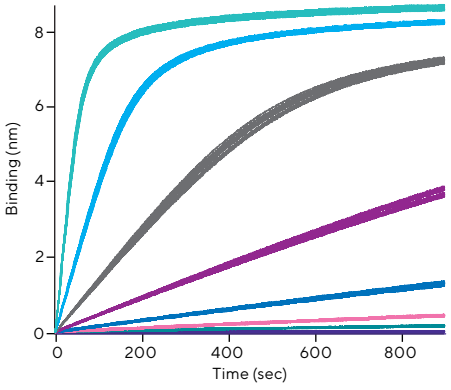
Figure 7

Titer Measurement of AAV8 Capsid on Different Octet® BLI Systems Using Octet® AAVX Biosensors.

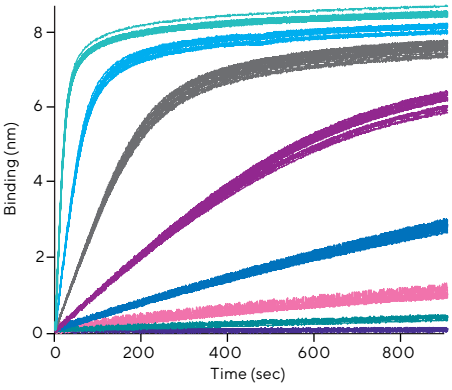
A.



B.



C.



D.

	Octet® R8 BLI System (8-channel)	Octet® RH16 BLI System (16 channel)	Octet® RH96 BLI System (96 channel)
Number of samples tested (AAV8)	96	96	96
Number of assays	1	1	1
Total assay time	180 min	90 min	15 min

Note. 96 samples of AAV8 serotype were tested over a concentration range from $3.05x10^{9}$–$2.22x10^{12}$ vp/mL (sensorgrams overlayed) on the Octet® R8 BLI system (A), Octet® RH16 BLI system (B) and Octet® RH96 BLI system (C). (D) Table listing the total assay time to perform a quantitation assay to measure 96 samples.

Summary

The Octet® AAVX Biosensor has been developed to enable the capsid quantitation of multiple AAV serotypes on the Octet® BLI platform. The Octet® AAVX assay follows an easy to setup assay format allowing the direct quantitation of AAV in bioprocess samples. A series of experiments were performed by the teams within Sartorius and at customer sites to evaluate the performance of the AAVX Biosensor. This application note presents the results of these studies and discusses the merits offered. The AAVX Biosensors showed broad serotype binding specificity to 10 different AAV serotypes as well as a broad dynamic range in quantitation experiments spanning about 5-orders of magnitude. The broad serotype specificity provides the advantage of requiring just one biosensor type to evaluate and test multiple AAV serotypes. Further, the AAVX Biosensors were used to quantitate AAV samples from different stages in the bioprocess workflow. The results presented illustrate the robustness of the AAVX Biosensor to accurately quantitate the AAVs with high precision in crude samples such as lysates, culture supernatants, and chromatography eluates.

Current AAV quantitation workflows commonly employ laborious and time-consuming ELISA as a method of choice. However, the Octet® AAVX assay offers an alternative, efficient quantitation workflow. It provides users with an easy to perform, direct, rapid assay with high accuracy and precision and broad serotype specificity, all of which are limited with ELISA technology. Feedback from the customers who presented studies in this application note echoed the above advantages. The results of the studies presented show good correlation (R^2 = 0.99), between the AAV concentrations measured on the Octet® BLI platform and ELISA for the different AAV serotypes tested, and across a broad AAV titer range (10^8–10^{13} vp/mL), matching the dynamic range of the AAVX Biosensors.

Further, the Octet® BLI platform offers customers the choice of multiple instrument models with varying throughput capabilities including 8-, 16- and 96-channel models that allows 8, 16 and 96 simultaneous AAV concentration measurements. Octet® AAVX Biosensors can be used on all Octet® BLI systems and data presented show good correlation and scalability in throughput across the three models tested. An important attribute tested was the regenerability of AAVX Biosensors, and it was shown that the AAVX Biosensor can be regenerated and re-used up to 10X–20X, providing significant reduction in cost per assay. For more information on regeneration of the AAVX Biosensors, see the Technical Note, Octet® AAVX Biosensors for AAV Capsid Titer Quantitation.

The features and advantages offered by combining Octet® AAVX Biosensors with the Octet® BLI platform discussed in this application note enables users working on AAV bioprocessing to quantitate their samples accurately and rapidly across the upstream and downstream workflow.

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