SARTORIUS

# Octet® AAVX Biosensors for Quantitation of AAV Capsids

Technical Note

## Introduction

Adeno-associated virus (AAV), with low immunogenicity and toxicity, has become one of the most promising viral vectors in the field of gene therapy. A rapid and robust method for AAV capsid quantitation is essential for AAV bioprocess development and manufacturing. Conventional ELISA, HPLC and ddPCR methods for AAV titer measurement are time-consuming and labor intensive. This tech note introduces a rapid, label-free method for direct capture and quantitation of various AAV serotypes on the Octet® Bio-Layer Interferometry (BLI) Platform using the Octet® AAVX Biosensors.

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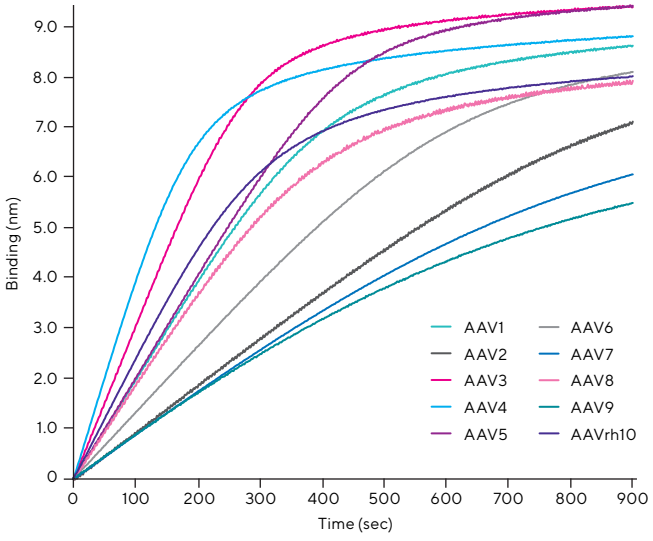
The Octet® AAVX Biosensors are pre-immobilized with Thermo Scientific™ CaptureSelect™ Biotin-Anti-AAVX Conjugate from Thermo Fisher Scientific Inc. and bind to a wide selection of AAV serotypes, including AAV1-AAV9 and AAVrh10 (Figure 1). The biosensor exhibits broad dynamic range from 8.5E8 to 1.0E13 vp/mL for the majority of the serotypes tested. It also shows high precision and accuracy for capsid quantitation of both purified and crude AAV samples. Furthermore, the biosensor can be regenerated and reused up to 20 times, providing a cost-effective and extremely useful solution for high-throughput AAV quantitation applications. This Technical Note describes in detail the AAV capsid quantitation assay workflow using the Octet® AAVX Biosensors.

**Figure 1**
*Binding of Various AAV Serotypes to the Octet® AAVX Biosensor.*

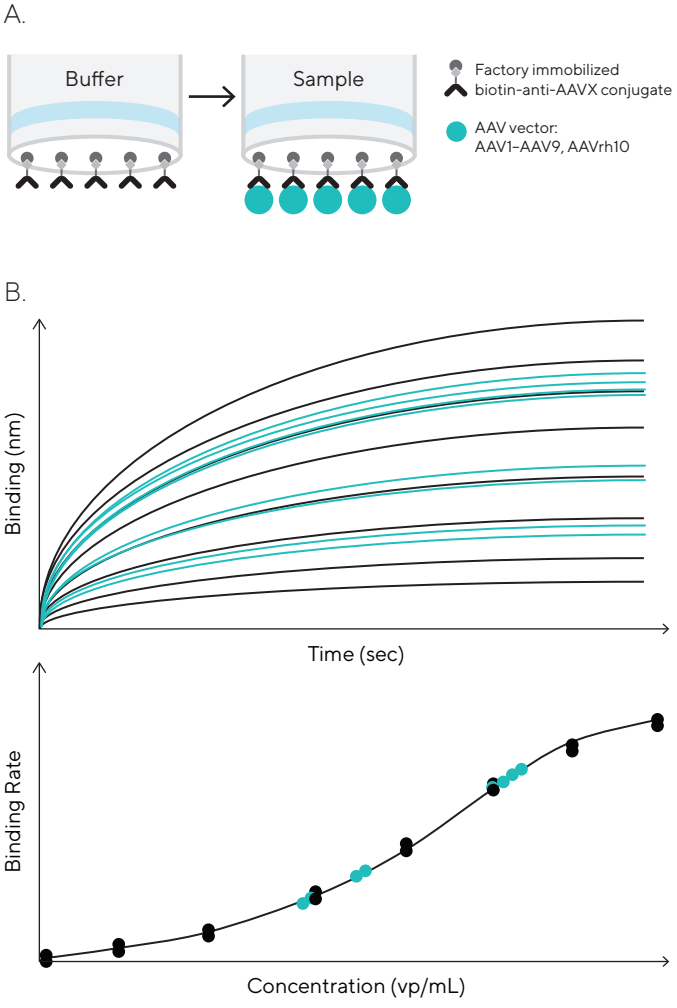



*Note.* All samples were tested with AAV titer at 5.0E11 vp/mL.

## Quantitation Assay Workflow

The Octet® AAVX Biosensors have high specificity towards AAV particles and enable direct quantitation of various AAV serotypes in both crude and purified samples. An example assay workflow utilizing the Octet® AAVX Biosensors to quantitate AAV capsids is shown in Figure 2.

**Figure 2**
*AAV Quantitation Assay Workflow Using the Octet® AAVX Biosensors.*




*Note.* (A) Quantitation assay workflow starts with equilibration of the Octet® AAVX Biosensors in the assay buffer (Buffer) followed by quantitation of AAV capsid (Sample). (B) Schematic representation of quantitation analysis of AAV standards and unknown samples on the Octet® BLI platform.

## Materials Required

- Octet® BLI system with Octet® BLI Discovery and Analysis Studio Software, version 13.0.1 or above
- Octet® AAVX Biosensors (Sartorius Part No. 18-5160)
- 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:
  - Octet® 384 Well Tilted bottom Plate, Sartorius Part No. 18-5080 (pack); 18-5076 (case)
  - 384-well, black, flat bottom, polypropylene microplate, Greiner Bio-One Part No. 781209
- Octet® Sample Diluent (Sartorius Part No. 18-1104)
- Regeneration buffer (10 mM Glycine, pH 1.7)
- Neutralization buffer: It should be the same as the assay buffer.
- AAV standards for calibration: Not included with the Octet® AAVX biosensors and should be provided by the user. The AAV standards should be the same serotype and ideally from the same cell line as the analyzed unknown samples.

## Assay Optimization Tips

- The dynamic range of the Octet® AAVX Biosensors is serotype dependent. In order to establish the actual dynamic range for user's serotype, it is recommended to prepare 5 – 8 standard sample solutions (using 2- or 3-fold serial dilutions) with concentrations covering the full dynamic range of the Octet® AAVX biosensors (i.e. 8.5E8 to 1.0E13 vp/mL).
- It is recommended to prepare standards in the assay buffer or matrix matching the one used for the unknown samples as closely as possible.
- Octet® Sample Diluent is recommended to be used as the assay buffer for dilution of all samples, purified or crude.
- When purified AAV samples are used as standards for quantitation analysis of crude unknown samples, it is recommended to dilute the crude samples with Octet® Sample Diluent to reduce matrix effects and achieve a better correlation between binding of the standards and the crude unknown samples to the Octet® AAVX Biosensors. For common matrices, typical minimum dilution factors in the Octet® Sample Diluent are shown in Table 1. It is recommended to prepare and analyze three to four consecutive 2-fold serial dilutions (e.g. 4x, 8x, 16x, 32x) for each unknown sample, to ensure that the titer in all or some of these diluted samples falls within the dynamic range previously defined for the user's specific AAV sample. The concentration average should then be calculated and used for accurate titer calculation.

**Table 1**

*Recommended Typical Minimum Dilution Factors for Common Matrices in AAV Manufacturing.*

| Matrix Type | AAV Serotype | Typical Minimum Dilution Factor in the Octet® Sample Diluent |
|---|---|---|
| Octet® Sample Diluent | AAV2/5/8/9 | Neat |
| Lysis buffer with 1% Pluronic F68 | AAV8/9 | 10-fold for AAV8<br>4-fold for AAV9 |
| Lysis buffer with 0.5 mg/mL HEK293 cell lysate | AAV2/5 | 4-fold |
| Medium (DMEM +10% FBS) | AAV8/9 | 5-fold |

*Note.* The minimum dilution factors vary based on different AAV serotypes and sample types.

- If crude samples are used as standards, the same matrix (free of AAV) should be used as the assay buffer throughout the assay.
- For each assay it is recommended to include and analyze a negative control sample along with the standards when generating calibration curve. A negative control is a sample that contains assay buffer/matrix only with no AAV particles present.
- Minimum volume needed for standards and samples is 200 µL/well for 96-well plates, 80 µL/well for flat bottom 384-well plates and 40 uL/well for the Octet® 384-Well Tilted bottom plates.

## Assay Settings

- In the Octet® BLI Discovery Software version 13.0.1 or higher, select the appropriate method template by clicking **Experiment -> New Experiment Wizard -> Advanced Quantitation -> AAV Quantitation**. Follow instructions to set up the sample plate to correspond with the location of standards, unknown samples, regeneration buffer (if applicable) in the sample plate. Enter titer values of the standards in the Sample Plate Table. Sample IDs and dilution factors can be entered in the Sample Plate Table as well.
- Two examples of the Plate Map and Assay Settings are shown in Figure 3 (A - without regeneration; B – with regeneration). The assay time in Assay Buffer should be set in the range of 60 – 180s to allow sufficient equilibration. The assay time for Sample capture should be set in the range of 600 – 1800s for accurate quantitation of AAV standards and unknown samples.

***Note:*** *The assay time for sample capture should be adjusted based on the titer range and binding signal resolution for your specific standards and samples. For*

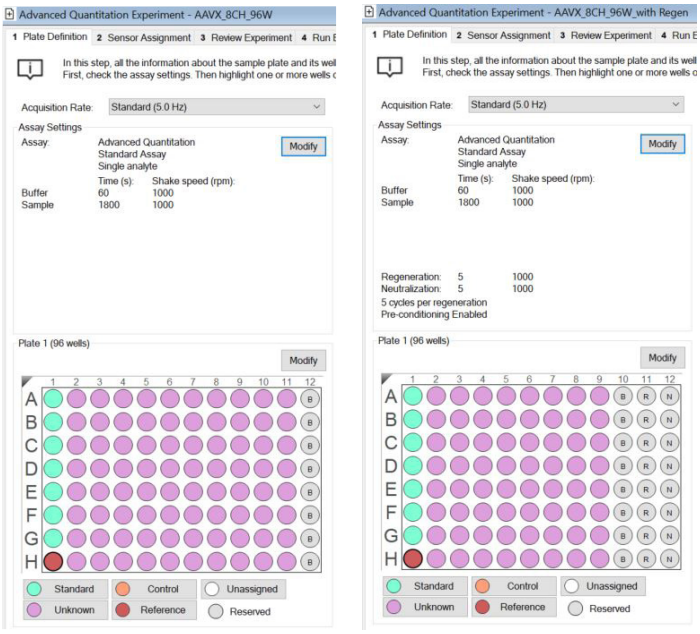
*example, when testing samples with linear binding at low titers (e.g. 10E8 – 10E9 vp/mL), the assay time for sample capture step could be increased to 1800s for increased binding curve resolution.*

- When setting up an assay with biosensor regeneration, it is recommended to include 5 initial pre-conditioning cycles (i.e. 5s in regeneration buffer and 5s in neutralization buffer for each cycle) prior to AAV Quantitation assay.

**Figure 3**

*Two Examples of Plate Maps and Assay Settings on the Octet® R8 BLI System.*

A. B.




*Note.* (A) Assay without Regeneration. (B) Assay with Regeneration.

## Performing the Assay

- Confirm that the assay temperature is set to 30°C under **Experiment -> Set Plate Temperature**.
- Prepare a 96-well hydration plate by dispensing 200 µL of the assay buffer in wells that match the locations of the biosensors to be used in this assay. Place the hydration plate in the instrument with or without the plate holder (depending on instrument model). Place the green biosensor tray on top of the hydration plate. ***Note:*** *The best results will be achieved when matrices for samples, standards and the assay buffer match as closely as possible.* For example, if the Octet® Sample Diluent was used for dilution of standards and unknown samples, it is recommended to use it as the assay buffer as well.
- Place the prepared sample plate in the instrument.
- Set the delay to 10 minutes to allow the biosensors to hydrate and the samples to warm up to the assay temperature (30°C).
- In the software, select the location where the data should be stored and create a new folder. Click the **GO** button to start the delay countdown. The assay will begin automatically after the delay timer reaches zero.

## Data Analysis

- Open the Octet® Analysis Studio Software version 13.0.1 or higher, browse and load the data to be analyzed.
- In the Preprocessed Data tab, designate the reference well. Next, select all standards, samples, and the reference well/s, right click and select **Subtract Reference for Selected Wells -> By Average**. In the Data Correction tab, binding curves will automatically align on the X-axis at 0.00 seconds. To change the X-axis alignment, select **Align X** and specify the time point for alignment of the binding curves. ***Note:*** *Reference subtraction is recommended to correct any artifacts arising from buffer mismatch, non-specific binding, or baseline drift etc.*
- In the Quantitation Analysis tab, click **Auto Fit -> Enable**, then select the appropriate Standard Curve Equation. It is recommended to use the **4PL fitting model** (unweighted, weighted Y or weighted Y2) for the AAV Quantitation Assay. If necessary, the standard curve equation can be changed to obtain the most suitable standard curve for user's specific serotype and titer range.
- Select the **Initial Slope for Binding Rate Equation** and verify that by default the data analysis read time matches the duration of the AAV sample loading step. Adjust the **Zero Conc. Threshold** to 0 and choose **Best Fit** in the Model Classifier in Advanced Settings to obtain calculated concentration and binding rate for individual binding curves. ***Note:*** *If using a software version prior to 13.0.1, adjust the Zero Conc. Threshold to 0 and tune the Low Conc. Threshold manually to obtain the best fit for individual binding curves. Refer to the Octet® Software User Guide for additional instructions on the Low Conc Threshold.*
- Once all the parameters are set, the standard curve and titer values for unknown samples will be calculated and tabulated automatically.
- ***Note:*** *It is recommended to check the binding rate for each individual binding curve and to exclude from the analysis those that are showing "too low" instead of a numerical value after reference subtraction. A "too low" binding rate indicates that the binding curve for this sample is either negative or not distinguishable from that of the negative control sample.*
- Click the **Export** button to generate and save a Microsoft® Excel® report from the data.

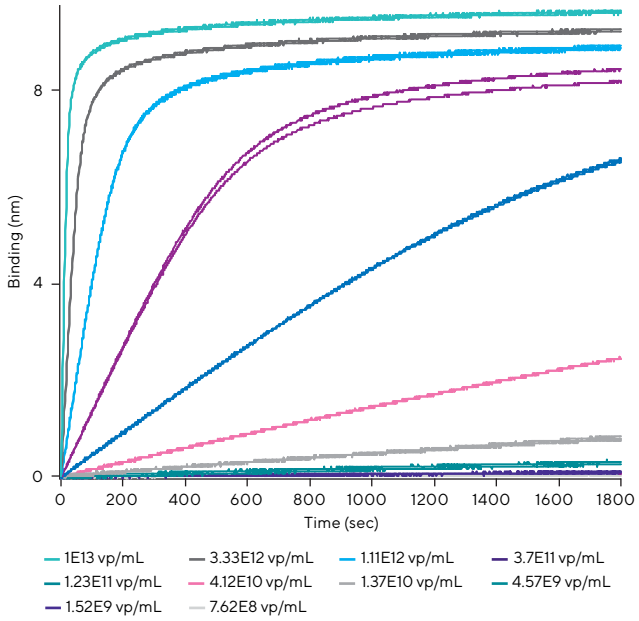
# Representative Data

Figures 4 and 5 show AAV8 and AAV9 quantitation assay using the Octet® AAVX Biosensors. Table 2 shows precision (CV ≤ 10%) and accuracy (recovery 100 ± 20%) for AAV9 capsid quantitation in Standards and Spiked samples.

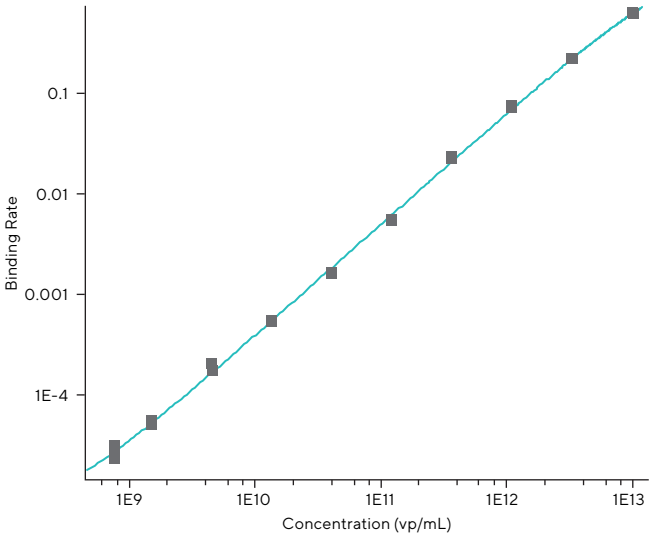
**Figure 4**

*Quantitation of AAV8 Using the Octet® AAVX Biosensors on the Octet® RH96 BLI System.*

A.



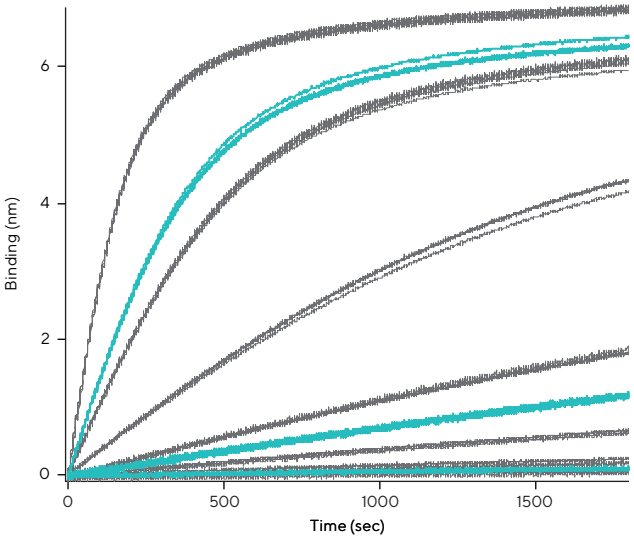

B.




*Note.* (A) AAV8 serotype dose response for capsid titer within the biosensor dynamic range of 7.62E8–1.0E13 vp/mL. (B) AAV8 capsid titer standard curve calculated using 4PL (weighted Y) fitting model (log scale applied to illustrate calibration curve accuracy at the low end). The Octet® Sample Diluent was used as the assay buffer.

**Figure 5**

*Quantitation of AAV9 in Standards and Spiked Samples Using the Octet® AAVX Biosensors.*




*Note.* The Octet® Sample Diluent was used as the matrix and all samples were analyzed in quadruplicate (binding curves corresponding to Standard Samples (gray) and Spiked Samples (teal)).

**Table 2**

*Results of AAV9 Quantitation in Standards and Spiked Samples.*

| | Known Titer, vp/mL | Average Calculated Titer, vp/mL | Calculated Titer %CV (n=4) | % Recovery |
|---|---|---|---|---|
| Standard Samples | 2.22E12 | 2.22E12 | 0.9 | 100% |
| | 7.41E11 | 7.43E11 | 0.5 | 100% |
| | 2.47E11 | 2.44E11 | 1.3 | 99% |
| | 8.23E10 | 8.37E10 | 2.0 | 102% |
| | 2.74E10 | 2.71E10 | 0.6 | 99% |
| | 9.14E9 | 9.65E9 | 7.3 | 106% |
| | 3.05E9 | 3.05E9 | 10.0 | 100% |
| Spiked Samples | 1.0E12 | 1.0E12 | 1.0 | 100% |
| | 5.0E10 | 4.67E10 | 0.9 | 93% |
| | 5.0E9 | 4.54E9 | 5.8 | 91% |

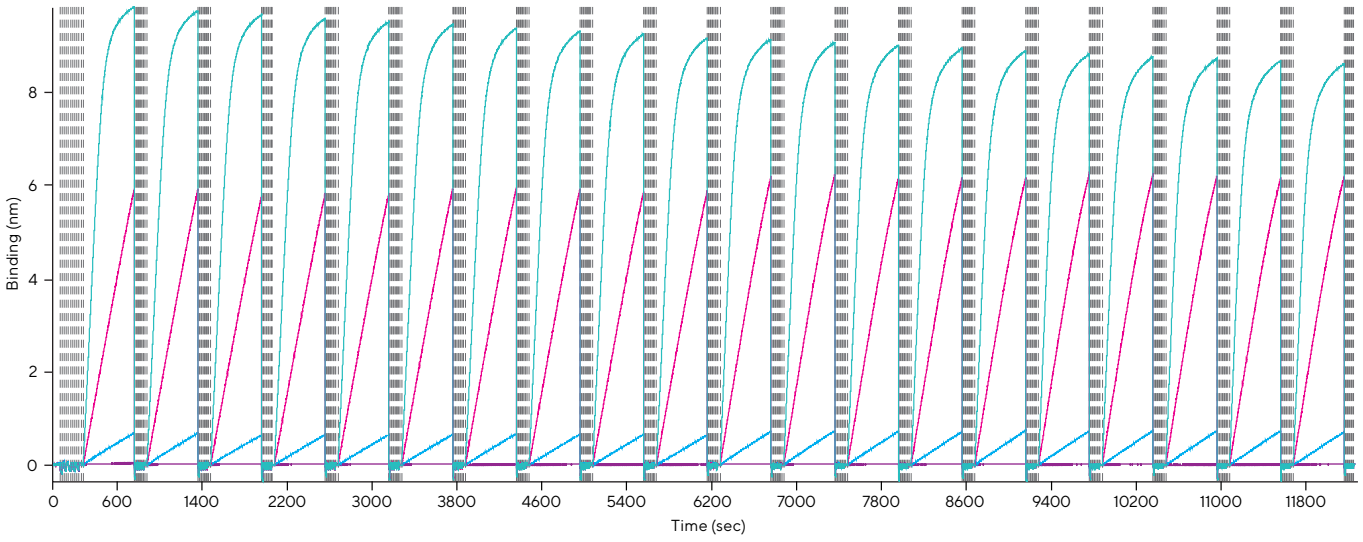
*Note.* The data was analyzed using the Initial Slope Binding Rate equation and 4PL (weighted Y) standard curve fitting.

# Regeneration of the Octet® AAVX Biosensors

The Octet® AAVX Biosensors can be regenerated and re-used up to 20 times providing an efficient and cost-effective solution for high-throughput AAV quantitation applications. As a regeneration cycle it is recommended to first dip the biosensors into 10 mM glycine, pH 1.7 for 5 seconds, followed by a dip in the assay buffer for 5 seconds. This regeneration cycle should be repeated 3 – 5 times in a sequence to fully remove bound AAV particles. For best results it is also recommended to pre-condition biosensors by running the regeneration protocol prior to capturing the AAV sample for the first time. Regeneration capability towards different AAV serotypes varies due to affinity differences. The exact number of possible regenerations in one assay should be determined by the user as it will depend on the required assay precision and accuracy. Figure 6 and Table 3 show an example of AAV5 quantitation at 3 concentrations after 20 consecutive regeneration cycles in one assay.

**Figure 6**

*Quantitation of AAV5 in the Octet® Sample Diluent over 20 Consecutive Octet® AAVX Biosensor Regeneration Cycles.*




*Note.* Regeneration buffer: 10 mM Glycine, pH1.7.

**Table 3**

*AAV5 Quantitation Assay Results After 10 and 20 Regeneration Cycles of the Octet® AAVX Biosensors.*

| Known Titer, vp/mL | Average Calculated Titer After 10 Regenerations, vp/mL | Calculated Titer %CV After 10 Regenerations | % Recovery After 10 Regenerations | Average Calculated Titer After 20 Regenerations, vp/mL | Calculated Titer %CV After 20 Regenerations | % Recovery After 20 Regenerations |
|---|---|---|---|---|---|---|
| 1.00E12 | 1.15E12 | 2.8% | 115% | 1.14E12 | 3.2% | 114% |
| 2.50E11 | 2.43E11 | 8.1% | 97% | 2.50E11 | 6.9% | 100% |
| 2.50E10 | 2.32E10 | 7.5% | 93% | 2.31E10 | 9.6% | 92% |

# High-Throughput AAV Capsid Quantitation

The AAV Quantitation assay can be performed on various Octet® BLI Systems, including the R8, RH16 and RH96 that offer a wide range of sample throughput capabilities and allow direct analysis of AAV capsids in up to 96 samples in as little as 15 minutes (Figure 7). Table 4 shows a comparison of the total assay time required to analyze 96 samples depending on the instrument capacity. As outlined in the Assay Settings section above, the total assay time might vary and should be adjusted based on the AAV titer range and binding signal resolution for user's specific standards and samples. Also, when using the Octet® R2, R4 or R8 BLI System for AAV titer analysis of a large number of samples in a single experiment, it is recommended to take into account potential reagent evaporation issues due to the overall length of the experiment. For example, if 96 samples need to be analyzed on the Octet® R8 BLI system, it is recommended to split the samples between two 96-well microplates (48 samples per microplate) and analyze these plates consecutively.

**Table 4**

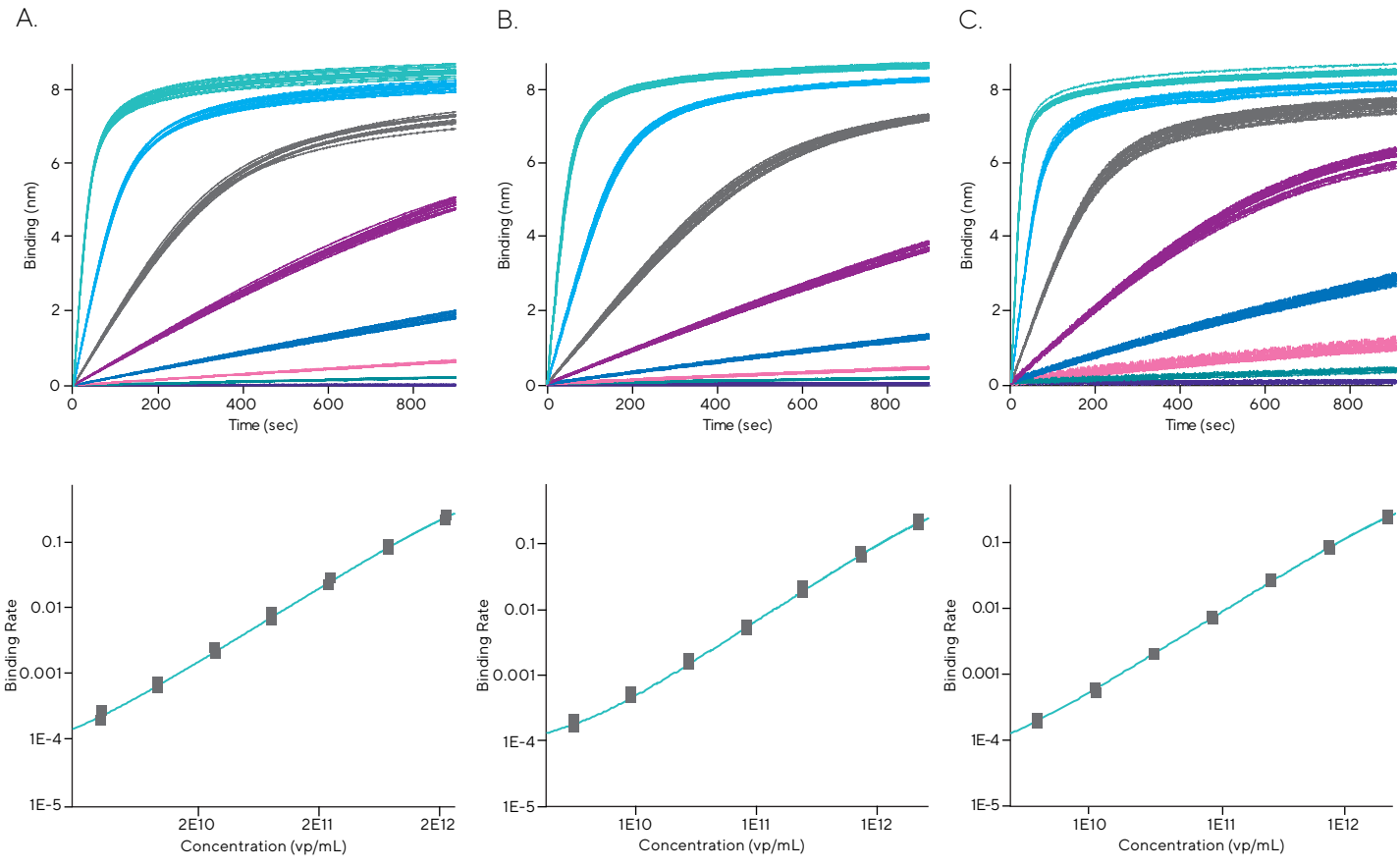
*Total Time Required for AAV Quantitation in 96 Samples on the Octet® BLI Platform Depending on Octet® BLI System Capacity.*

| Octet® BLI System (Acquisition Mode) | Octet® R8 BLI System (8-channel) | Octet® RH16 BLI System (16-channel) | Octet® RH96 BLI System (96-channel) |
|---|---|---|---|
| Number of Analyzed Samples Per Run | 96 | 96 | 96 |
| Total Assay Time | 180 min | 90 min | 15 min |

*Note.* Total assay time is based on the 15 minute acquisition time (capture step) for AAV8 capsid titer measurement.

**Figure 7**

*Dose Response Binding Curves (Top) and Standard Curves (Bottom) for AAV8 Quantitation.*




*Note.* AAV8 quantitation in the range of 3.05E9 – 2.22E12 vp/mL using the Octet® AAVX Biosensors on various Octet® BLI systems. Log scale applied in standard curve to illustrate accuracy at low end. (A) Octet® R8 BLI System (8-channel mode). Note: Two experiments were run with each experiment quantifying 48 AAV8 samples to minimize effect from reagents evaporation. The two runs were overlayed in the Octet® Analysis Studio Software for data analysis. (B) Octet® RH16 BLI System (16-channel mode). (C) Octet® RH96 BLI System (96-channel mode).

**Table 5**

*AAV8 Quantitation Results After Analysis of 96 Samples Using the Octet® AAVX Biosensors on Various Octet® BLI Systems.*

| Known Titer, vp/mL | Octet® R8 BLI System (8-channel Mode) | | | Octet® RH16 BLI System (16-channel Mode) | | | Octet® RH96 BLI System (96-channel Mode) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Average Calculated Titer, vp/mL | Calculated Titer %CV (n=12) | % Recovery | Average Calculated Titer, vp/mL | Calculated Titer %CV (n=12) | % Recovery | Average Calculated Titer, vp/mL | Calculated Titer %CV (n=12) | % Recovery |
| 2.22E12 | 2.22E12 | 2.0% | 100% | 2.22E12 | 4.2% | 100% | 2.22E12 | 3.0% | 100% |
| 7.41E11 | 7.33E11 | 2.1% | 99% | 7.4E11 | 3.2% | 100% | 7.34E11 | 2.2% | 99% |
| 2.47E11 | 2.59E11 | 2.5% | 105% | 2.53E11 | 4.0% | 102% | 2.56E11 | 2.9% | 104% |
| 8.23E10 | 7.76E10 | 3.1% | 94% | 7.65E10 | 2.7% | 93% | 8.01E10 | 3.0% | 97% |
| 2.74E10 | 2.69E10 | 3.4% | 98% | 2.98E10 | 3.3% | 109% | 2.64E10 | 2.1% | 96% |
| 9.14E9 | 9.4E9 | 2.5% | 103% | 1.03E10 | 2.1% | 112% | 8.44E9 | 4.2% | 92% |
| 3.05E9 | 3.05E9 | 3.0% | 100% | 3.05E9 | 5.1% | 100% | 3.05E9 | 5.7% | 100% |

*Note.* For analysis on the Octet® R8 BLI system, the 96 samples were split between 2 microplates (48 samples per plate) in order to minimize effects from sample evaporation due to the overall length of the assay. Then data from the two runs was overlayed in the Octet® Analysis Studio Software for data analysis.

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