

Octet® AAVX Biosensors

For Quantitation of
AAV Capsids



Key Features

- Rapid, direct quantitation of AAV capsids in crude and purified samples
- Specificity to various AAV serotypes, including AAV1 – AAV9 and AAVrh10
- Good correlation with traditional time-consuming analytical methods, such as ELISA

Quick Facts

- Dynamic range: $8.5E8$ – $1.0E13$ vp/mL for most AAV serotypes
- Throughput: analyze AAV capsid titer in up to 96 samples in parallel in as little as 15 min using the Octet® RH96 BLI system
- Efficient and cost-effective regeneration for biosensor re-use up to 10 – 20 times

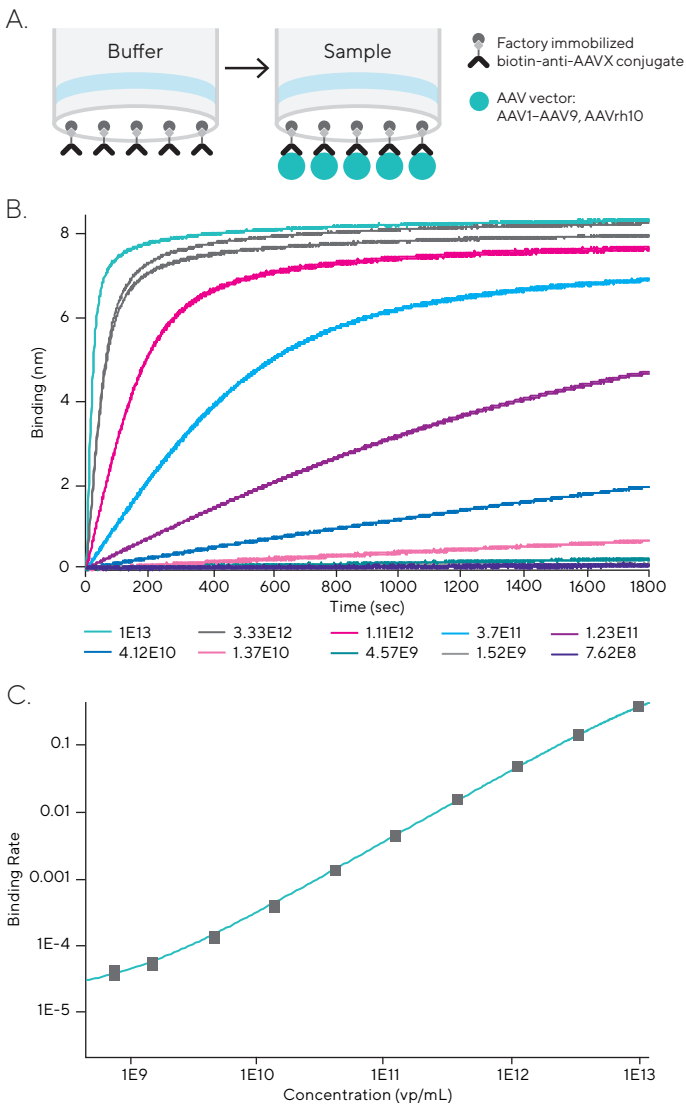
Overview

Octet® AAVX Biosensors offer a rapid, label-free, and high-throughput method for Adeno-Associated Viral (AAV) capsid titer measurement on the Octet® BLI platform. Octet® AAVX Biosensors are pre-immobilized with Thermo Scientific™ CaptureSelect™ Biotin Anti-AAVX Conjugate from Thermo Fisher Scientific Inc., which binds with high affinity and specificity to various native and recombinant AAV serotypes, including AAV1 to AAV9 and AAVrh10. Due to its high specificity, the biosensor can be used to quantitate AAV capsids in both purified and crude cell culture samples with titer in the range of $8.5E8$ – $1.0E13$ vp/mL for most serotypes. The AAV titer can be measured on the Octet® BLI platform in as little as 15 minutes providing an efficient alternative to time-consuming and laborious technologies such as ELISA and HPLC. Finally, the Octet® AAVX Biosensors can be efficiently regenerated up to 10 – 20 times, which makes them an extremely useful and cost-effective solution for high-throughput AAV capsid titer measurement in Process Development and QC.

Quantitation Assay Workflow

The AAV quantitation workflow is outlined in Figure 1. The AAV capsid titer is determined based on the binding rate of AAV particles to the biosensor surface (the binding rates are proportional to AAV titers). To calculate AAV titer in tested samples, the corresponding binding rates are compared to those of a standard calibration curve constructed using the same AAV samples with known capsid titer. An example of AAV9 quantitation results generated using the Octet® AAVX Biosensors is shown in Figure 1 and Table 1.

Figure 1
AAV Quantitation 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) AAV9 serotype dose response for capsid titer within the range of 7.62E8 – 1.0E13 vp/mL. (C) AAV9 capsid titer standard calibration curve fitted using 4PL (weighted Y) model (log scale applied to illustrate calibration curve accuracy at low end).

Table 1

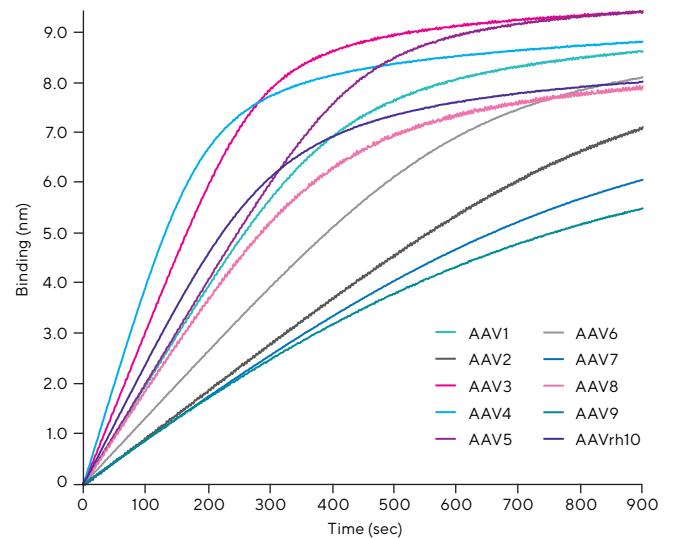
AAV9 Quantitation Assay Results Generated Using the Octet® AAVX Biosensors.

Known Titer, vp/mL	Average Calculated Titer (n=3), vp/mL	Titer %CV (n=3)	% Recovery
1.00E13	1.0E13	1.1%	100%
3.33E12	3.3E12	2.2%	99%
1.11E12	1.13E12	0.4%	101%
3.70E11	3.85E11	0.2%	104%
1.23E11	1.17E11	0.6%	95%
4.12E10	3.9E10	0.5%	95%
1.37E10	1.16E10	2.4%	85%
4.57E9	3.95E9	4.1%	86%
1.52E9	1.32E9	8.9%	87%
7.62E8	7.62E8	8.7%	100%

Binding Specificity

The Octet® AAVX Biosensors bind with high affinity and specificity to various native and recombinant AAV serotypes, including AAV1 to AAV9 and AAVrh10.

Figure 2
Binding of the Various AAV Serotypes to the Octet® AAVX Biosensor.



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

Correlation with ELISA

The AAV capsid titer results generated using the Octet® AAVX Biosensors correlate well with the results generated using labor- and time-consuming ELISA. Examples demonstrating correlation of AAV8 and AAV9 capsid titers measured using Octet® AAVX Biosensors vs. ELISA are shown below.

Figure 3

Correlation of AAV8 Titer Measured Using the Octet® AAVX Biosensors vs. ELISA.

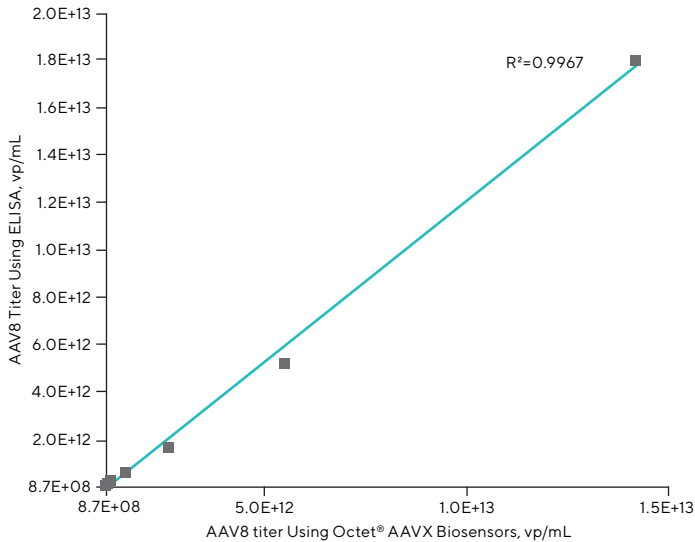


Figure 4

Correlation of AAV9 Titer Measured Using the Octet® AAVX Biosensors vs. ELISA.

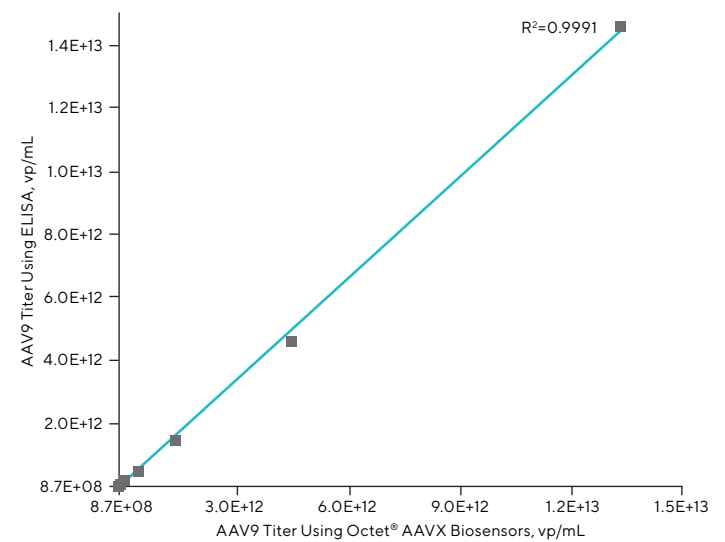


Table 2

Comparison of AAV8 Quantitation Assay Results Generated Using the Octet® AAVX Biosensors vs. ELISA.

AAV8	Capsid Titer Using Octet® AAVX Biosensor, vp/mL	Capsid Titer Using ELISA, vp/mL
Sample 1	1.31E13	1.79E13
Sample 2	4.43E12	5.08E12
Sample 3	1.58E12	1.62E12
Sample 4	5.37E11	5.16E11
Sample 5	1.66E11	1.77E11
Sample 6	4.59E10	6.07E10
Sample 7	1.59E10	2.12E10
Sample 8	4.52E9	7.67E9
Sample 9	1.45E9	2.25E9

Table 3

Comparison of AAV9 Quantitation Assay Results Generated Using the Octet® AAVX Biosensors vs. ELISA.

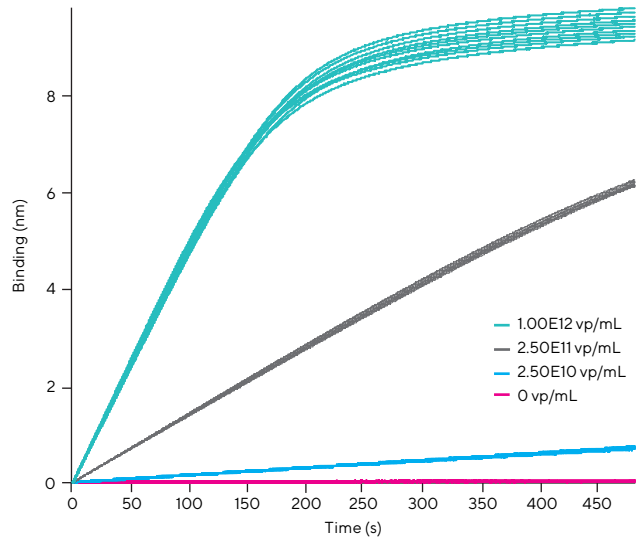
AAV9	Capsid titer Using Octet® AAVX Biosensor, vp/mL	Capsid titer Using ELISA, vp/mL
Sample 1	1.33E13	1.46E13
Sample 2	4.61E12	4.61E12
Sample 3	1.53E12	1.47E12
Sample 4	5.18E11	5.02E11
Sample 5	1.64E11	1.74E11
Sample 6	4.83E10	5.98E10
Sample 7	1.75E10	2.07E10
Sample 8	4.57E9	6.76E9
Sample 9	8.28E8	1.05E9

Efficient Regeneration

The Octet® AAVX Biosensors can be regenerated up to 10 – 20 cycles via a standard, low pH buffer protocol using 10 mM Glycine, pH 1.7 in as little as 2 minutes. Regeneration allows for biosensor re-use and provides a cost-saving solution for generating replicates or for analyzing large number of samples in sequence. An example of AAV5 quantitation assay with 10 regeneration cycles is shown in Figure 5 and results after 10 and 20 regeneration cycles are shown in Table 4.

Figure 5

Overlay of Binding Curves for AAV5 Quantitation Assay After 10 Consecutive Regenerations of the Octet® AAVX Biosensors.



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

Table 4

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	%CV After 10 Regenerations	% Recovery After 10 Regenerations	Average Calculated Titer After 20 Regenerations, vp/mL	%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%

Ordering Information

Part No.	UOM	Description
18-5160	Tray	One tray of Octet® AAVX Biosensors
18-5161	Pack	Five trays of Octet® AAVX Biosensors
18-5162	Case	Twenty trays of Octet® AAVX Biosensors
18-1104	Each	Octet® Sample Diluent Buffer, 50 mL

Germany

Sartorius Lab Instruments GmbH & Co. KG
Otto-Brenner-Strasse 20
37079 Goettingen
Phone +49 551 308 0

USA

Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
Phone +1 888 OCTET 75
Or +1 650 322 1360



For further contacts, visit
www.sartorius.com/octet-support