Gator™ AAV9 Probes for Highly Selective AAV9 Quantitation

INTRODUCTION

Adeno associated virus (AAV) capsids are a leading modality for in vivo gene delivery. Complete and precise characterization of capsid particles, including capsid and vector genome concentration, is necessary to safely and efficaciously dose patients. In virus development and production, it is important to determine the virus concentration at different stages of the process, to optimize the clone used as well as the production yields. Bio-layer Interferometry (BLI) using AAV specific biosensors is a rapid label-free method for quantitation of AAV serotypes.

GATOR™ AAV9 PROBES

The Gator™ AAV9 Probes are high specificity nanobody-based biosensors that enable direct capture and quantitation of AAV9 in crude lysates, column eluates, cell lysates and cell culture supernatants. It serves as an alternative to traditional time-consuming analytical methods, such as qPCR, ddPCR, Dot blot and ELISA. The Gator™ AAV9 probe uses proven CaptureSelect™ (Thermo Fisher Scientific) high affinity and high specificity anti-AAV9 nanobody.

PERFORMANCE SUMMARY

- Dynamic range: $3 \times 10^9 - 1 \times 10^{13}$ vp/mL
- Throughput: 8 samples in 4 min, 96 samples in 26 min
- LoD: 1×10^9 vp/mL
- Crude sample tolerant
- Stable over broad pH range
- Cost effective - Reusable at least 10 times

RESULTS
DYNAMIC RANGE

Channel#	Known Conc. (vp/mL)	Binding Rate	Calculated Conc. (vp/mL)	% Accuracy
1	3.00×10^{11}	0.0477	3.11×10^{11}	96.33
2	1.50×10^{11}	0.0144	1.40×10^{11}	93.33
3	7.50×10^{10}	0.00542	6.93×10^{10}	92.40
4	3.75×10^{10}	0.00329	3.78×10^{10}	99.20
5	1.88×10^{10}	0.00136	2.00×10^{10}	93.62
6	9.38×10^9	0.000661	9.93×10^9	94.14
7	4.69×10^9	0.000365	4.69×10^9	100.00
8	0	0	0	NA

Table 1: Shows excellent accuracy and dynamic range for AAV9.

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The AAV9 serotype range was tested using the Gator™ AAV9 Probes. The data shows 4 orders of magnitude dynamic range. Starting from 3×10^{11} vp/mL, two-fold serial dilution of AAV9 capsid quantitation data was obtained at 1000 rpm with Gator™ instrument.
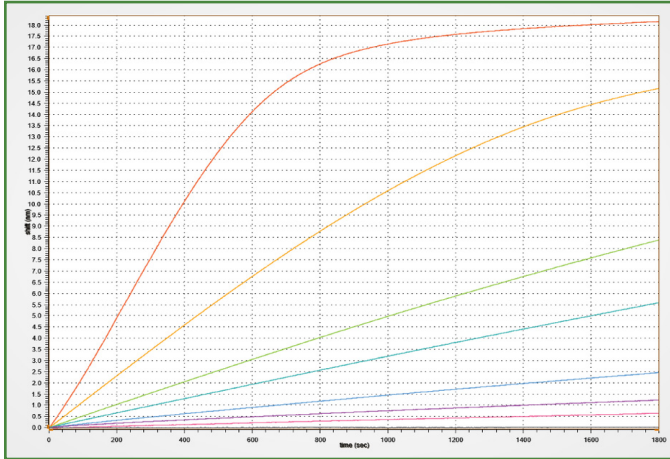

Figure 1: Quantitation of AAV9 using Gator™ AAV9 Probes. AAV9 dose response for concentrations within the dynamic range of Table 1.

ACCURACY AND REPRODUCIBILITY

AAV9 serotype was used to test the accuracy and reproducibility. Table 2 below shows recovery close to 100% and % CV ranging from around 1.5% at medium and high titers to 10.9% at low titer.

Titer Level	Known Conc. (vp/mL)	Average Binding Rate	Average Calculated Conc. (vp/mL)	% Accuracy	% CV (n = 4)
High	1.00×10^{12}	0.17625	1.04×10^{12}	96.0	3.7
Medium	5.00×10^{10}	0.00676	4.66×10^{10}	93.2	5.1
Low	2.50×10^{9}	0.00065	2.49×10^{9}	99.6	4.5

Table 2: Gator™ AAV9 Probes accuracy and reproducibility.

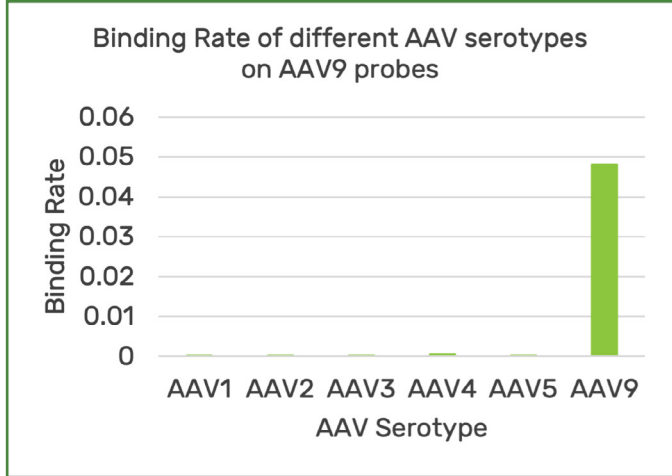# Gator™ AAV9 Probes for Highly Selective AAV9 Quantitation

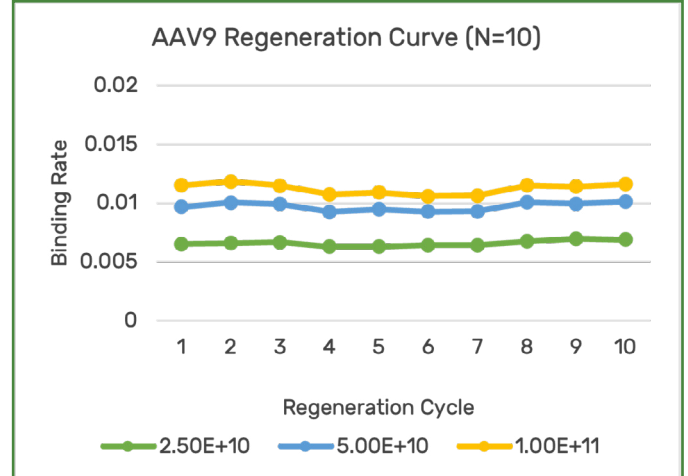Figure 2: AAV9 probes specificity over other serotypes.

Figure 3: AAV9 probes performance through 10 regenerations.

SEROTYPE SPECIFICITY OF AAV9 PROBES

The AAV9 probes are highly selective and bind only to AAV9 serotype. AAV9 probes do not cross react with other AAV serotypes.

RENEGERATION PERFORMANCE

Data above shows performance after 10 regenerations of the same probe. Minimal loss in binding rate observed even after 10 regenerations.
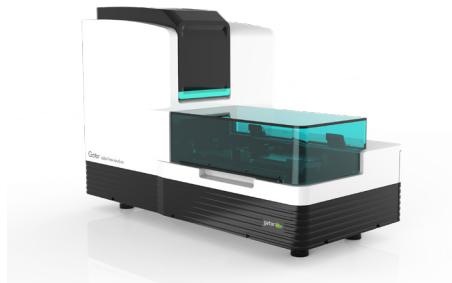
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