

## Gator™ AAVX Probes for Rapid and Label-free Quantitation of AAV Serotypes

### 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. Biolayer Interferometry (BLI) using AAV specific biosensors is a rapid label-free method for quantitation of AAV serotypes.

### Gator™ AAVX Probes

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



### Performance Summary

- Dynamic range:  $1 \times 10^9$ – $1 \times 10^{13}$  vp/mL for most AAV serotypes
- Throughput: 8 samples in 10 minutes, 96 samples in 120 minutes
- Limit of detection: typically,  $5 \times 10^8$  vp/mL (serotype dependent)
- Crude sample tolerant
- Stable over broad pH range
- Cost effective - Reusable at least 10 times by regeneration

### Results

#### Dynamic range:

The AAV2 serotype range was tested using the Gator AAVX probes. The data shows 4 orders of magnitude dynamic range. The data was acquired using 1:3 dilution in 10 min at 1,000rpm.

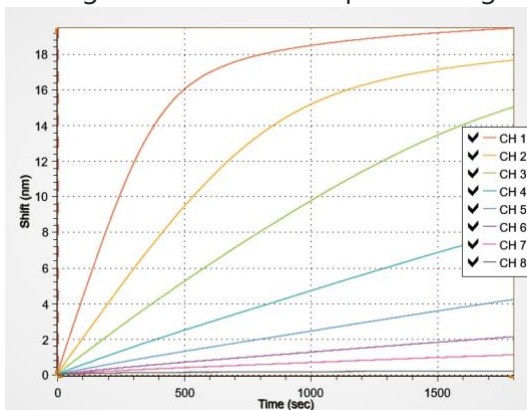


Figure 1: Capture of AAV2 serotype on the

AAV8 Gator AAVx Std. Curve			
Channel #	Known Con. (vg/mL)	Binding Rate	Calc. Conc. (µg/mL)
1	$3.33 \times 10^{11}$	0.069	$3.41 \times 10^{11}$
2	$1.67 \times 10^{11}$	0.0304	$1.69 \times 10^{11}$
3	$8.33 \times 10^{10}$	0.0119	$7.62 \times 10^{10}$
4	$4.17 \times 10^{10}$	0.00779	$5.31 \times 10^{10}$
5	$2.08 \times 10^{10}$	0.0023	$1.87 \times 10^{10}$
6	$1.04 \times 10^{10}$	0.00115	$1.02 \times 10^{10}$
7	$5.21 \times 10^9$	0.000592	$5.55 \times 10^9$
8	0	0	0

Table 1: Example of AAV8 serotype anti-AAVx

standard cuve

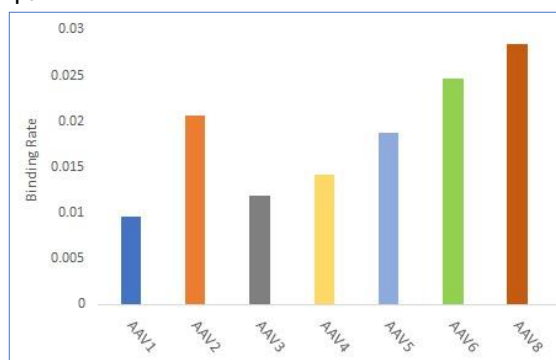
### Accuracy and reproducibility

AAV5 serotype was used to test the accuracy and reproducibility. The table 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 concentration (vp/ml)	% Recovery	% CV (n = 3)
High	$2.00 \times 10^{12}$	0.11069	$2.03 \times 10^{12}$	102	1.50
Medium	$5.10 \times 10^{10}$	0.0045	$5.27 \times 10^{10}$	103	1.81
Low	$8.30 \times 10^8$	0.00010	$8.30 \times 10^8$	100	10.9

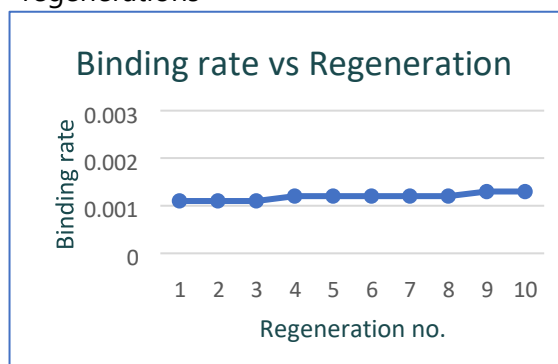
### Binding rate of different AAV serotypes

The binding rate of 7 different serotypes of AAV were studied. The figure below shows binding rates of tested serotypes at  $2 \times 10^{11}$  vp/mL concentration.



### Regeneration performance

Data below shows performance after 10 regenerations of the same probe. No loss in binding rate observed even after 10 regenerations



## Gator Bio Total Solution

Gator™ AAVX Probes



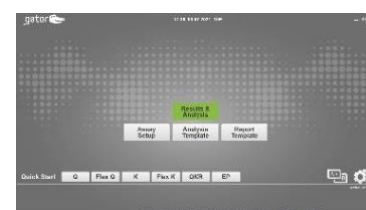
+

Gator™ Prime OR Gator™ Plus



+

Gator Bio Software



### Ordering info

PL168-160017: Gator™ AAVX Probe