Accurate and Easy AAV Titer and Empty/Full Ratio Determination in Upstream and Downstream Samples

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INTRODUCTION

AAV production for gene therapy requires accurate, precise, and high throughput determination of titer and empty versus full ratio to ensure efficacy, safety and quality.

Gator Bio's Next Gen biolayer interferometry (BLI)-based AAV solutions determine AAV capsid titer and empty versus full content without relying on additional techniques (e.g., ELISA, qPCR, ddPCR, AUC, and TEM) to obtain data on different AAV critical quality attributes (CQA).

Here, we present data from the Gator AAV analytics for Titer and E/F Ratio. Our solutions generate accurate and reproducible data and are suitable for both upstream and downstream samples.

FEATURES OF GATOR'S AAV ANALYTICS SOLUTIONS

- Automated and robust titer in the range of 1.00E+07 to 1.00E+13 vp/mL
- High throughput, automated empty versus full ratio determination
- Kinetic characterization of AAV antibodies

HIGH SENSITIVITY "DIP AND DILUTE" TITER OF UPSTEAM SAMPLES

1. HS AAV kit enables titer of AAV samples down to 1.00E+07 vp/mL for all AAV serotypes.
2. Enhanced sensitivity due to patented signal amplification technology
3. Platform provides automation, accuracy and high precision

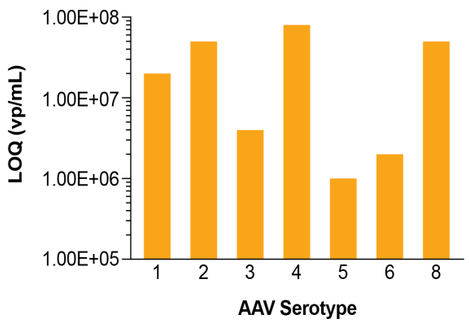


Figure 1. LOQ for AAV1-8. Serotypes are purchased from www.virovek.com.

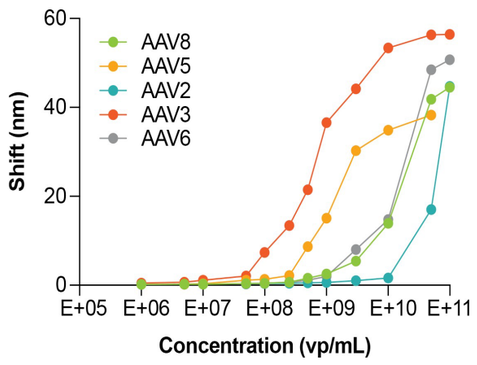


Figure 2. HS AAV dynamic range for different AAV serotypes.

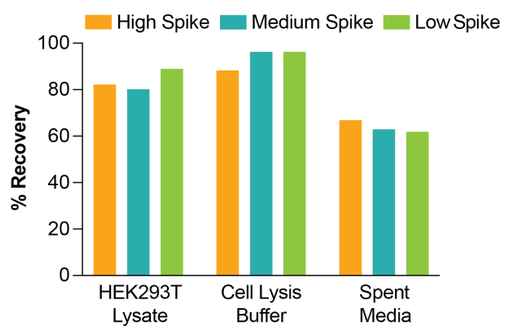
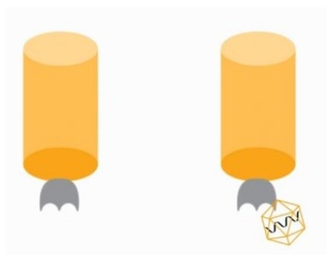


Figure 3. Recovery of AAV spiked at high (1.00E+08 vp/mL), medium (5.33E+07 vp/mL), and low (1.00E+07 vp/mL) concentrations in various sample matrices.

	HS AAV	HS AAV9
Dynamic Range (vp/mL)	1E+7 – 1E+10	1E+7 – 1E+9
LoQ	1E+7	1E+7
Assay time (8 samples)	35 minutes	35 minutes
Precision	< 10%	< 10%

DIRECT BINDING TITER OF DOWNSTREAM SAMPLES

1. Titer is based on the binding rate of the AAV capsid of interest to the AAVX the probe surface.
2. Probes are reusable at least 10 times with regeneration and without a loss in performance.



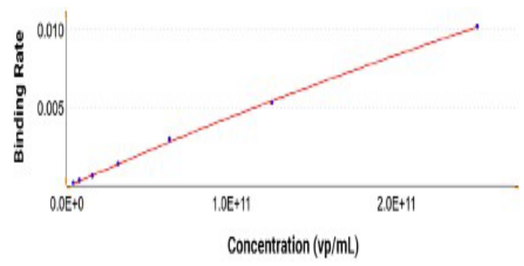


Figure 4. Standard curve for AAV2 generated using AAVX probe.

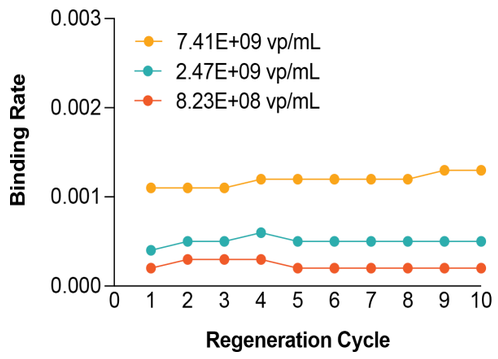


Figure 5. There is no loss in performance after 10 regenerations using the same AAVX probe.

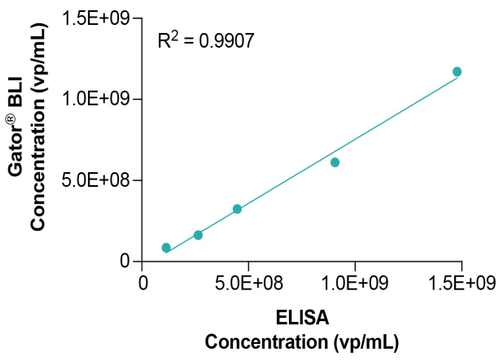
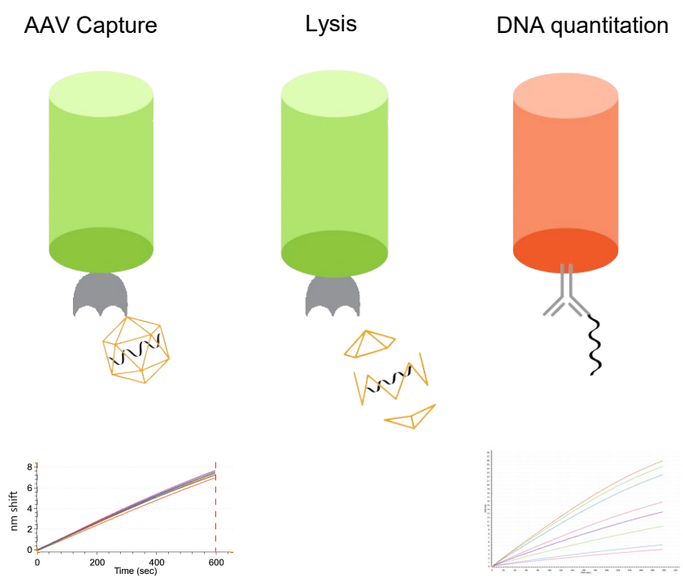


Figure 6. Correlation between the quantification of AAV9 obtained with AAVX probes versus ELISA.

	AAVX Probe	AAV9 Probe
Dynamic Range (vp/mL)	1E+9 – 1E+13	3E+9 – 1E+13
LoQ	1E+9	1E+9
Assay time (8 samples)	4 minutes	4 minutes
Precision	< 10%	< 10%

EMPTY/FULL AAV RATIO DETERMINATION

1. Assay involves three steps: (i) AAV capture using AAVX probes, (ii) lysis for releasing ssDNA, and (iii) capture of ss DNA on ss DNA probes.
2. % full ratio measurement is based on the quantitation of ss DNA released from the AAV capsids
3. Kit provides ease of use with a single assay for empty/full ratio determination without the use of other methods.



EMPTY/FULL AAV RATIO DETERMINATION

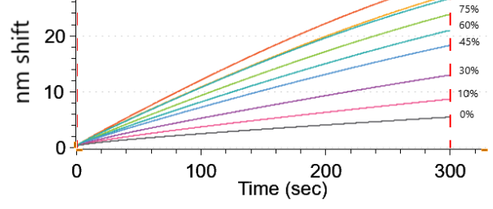


Figure 7. Sensogram showing ss DNA binding to DNA binding probe. The nm shift is proportional to amount of DNA

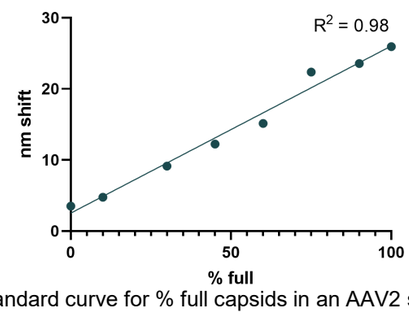
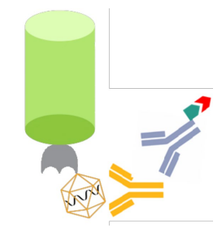


Figure 8. Standard curve for % full capsids in an AAV2 sample.

Range	5 – 100%
Resolution	10%
Assay time (8 samples)	40 - 120 minutes
Precision	< 10%

TOTAL AAV ANTOBODIES IN SERUM

1. AAVX Probes can be used to detect total AAV antibodies in serum sample using a signal amplification format.
2. Simple and automated workflow.



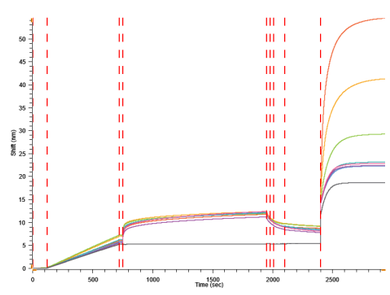


Figure 9. Human serum(1:50) diluted in Q spiked ADK9 antibody

GATOR® AAV ANALYTICS SOLUTIONS GUIDE

Gator® Probe/Kit	Function	Application	Dynamic Range (vp/mL)
AAVX	Binds AAV1-8, 10, and chimeric	Titer, Kinetics	1E+09 – 1E+ 13
AAV9	Binds specifically to AAV9	Titer, Kinetics	3E+09 – 1E+13
High Sensitivity AAV	High sensitivity quantitation of AAV1-8, 10, and chimeric	Titer	1E+07-1E+10
High Sensitivity AAV9	High sensitivity quantitation of AAV9	Titer	1E+07-1E+9
Empty vs Full	% Full Determination	Ratio Determination	5-100%

SUMMARY

Gator® AAV probes and kits combined with BLI technology provide a total AAV analytics solution for gene therapy products.

Advantages of Gator® AAV analytics include:

- Wide dynamic range of 1.00E+07 to 1.00E+13 vp/mL
- Matrix compatibility ideal for upstream analysis
- Single assay for empty/full AAV ratio determination
- Kinetics characterization of AAV neutralizing antibodies
- Qualitative and quantitative determination of antibodies to AAV serotypes in plasma and serum
- Automation
- Suitable for integration into manufacturing processes
- Cost savings from a single technology for AAV titer and content ratio determination