Rapid and Easy Viral and Lipid-based Vectors Analytics using BLI

Vivienne Lee, Indrani Chakraborty, Harsha Agarwal, Wai Choi, Samuel Yang, Katie Trieu, Pu Li, Robert Zuk
Gator Bio, Inc, Palo Alto, CA

Introduction

Biolayer Interferometry (BLI) is a label-free analytical technique based on optical interference. An additional layer of biomolecules modifies the phase of light as it passes through. This generates an interference pattern that provides real-time information on binding affinity and interaction kinetics.

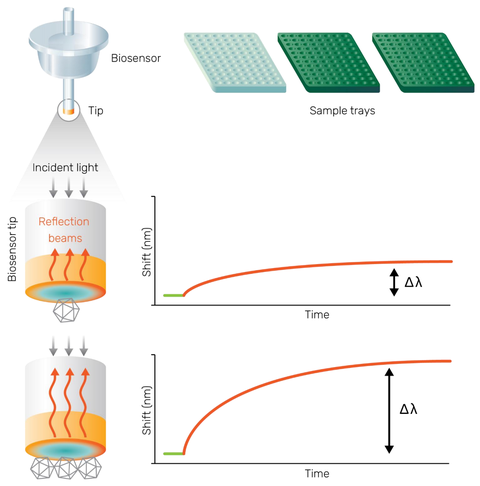


Figure 1. Principle of BLI.

Ensuring the quality and consistency of vectors is critical to the success of gene therapy, but several challenges remain, including:

- Accurate titer, % full and partial determination in crude samples
- Use of multiple techniques to characterize viral and lipid particles
- Easy rank ordering of titers and % full during purification in manufacturing

Here, we present easy, accurate and automated method that meets the above challenges. The **BLI assays** are:

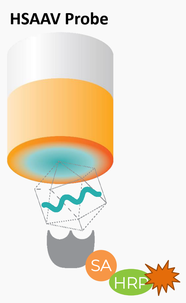
- **Highly accurate and precise**
- **Compatible with various matrices**
- **Minimal hands-on time**
- **Can be easily implemented from harvest to final product titer and % full QC**

AAV Analytics

AAV CAPSID TITER

AAVX probe and HS AAV kit capture various serotypes of AAV with a **dynamic range of 1E+07 to 1E+13 vp/mL**.

CRUDE SAMPLE TITER USING HIGH SENSITIVITY AAV KIT



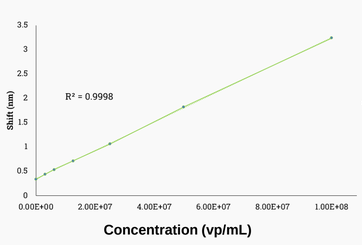


Figure 2. Standard curve for AAV5 generated using HS AAV probe.

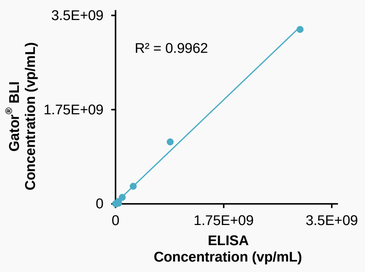
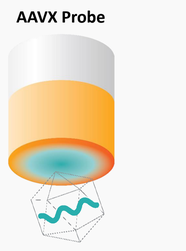


Figure 3. Correlation between HS AAV9 and ELISA on quantification of AAV9.

Titer	5.33E+07 of *AAV5*	1.00E+09 of *AAV8*
Matrix Interference	**% Recovery**	
HEK293T Lysate* (~100 mg/mL of intracellular proteins from 1E+08 cells/mL of HEK293T cell suspended in PBS, 1:10 dilution in buffer)	**80.18%**	**115%**
Cell Lysis Buffer (1X PBS, 100mM NaCl, 0.001% Pluronic)	**96.31%**	**119%**
Spent Media (DMEM media, 10% FBS, 2mM L-Glutamine)	**84.08%**	**110%**

Table 1. HS AAV Kit performance in different matrices.

PURIFIED SAMPLE TITER USING AAVX PROBES



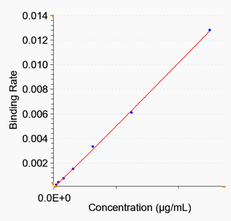


Figure 4. Standard curve for AAV2 generated using AAVX probe.

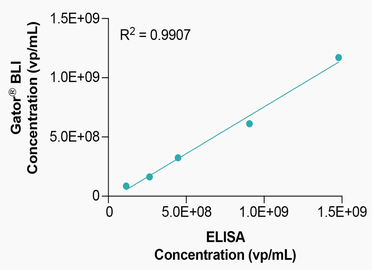


Figure 5. Correlation between AAVX and ELISA on quantification of AAV9.

DETERMINATION OF AAV EMPTY TO FULL RATIOS

AAV Ratio Kit determines the AAV empty to full ratios through AAV capture, lysis and DNA quantitation. This format works for both crude and purified samples.

% Full	% Difference between expected and measured values							
	Buffer 1	Buffer 2	Buffer 3	Buffer 4	Buffer 5	Buffer 6	Buffer 7	Buffer 8
90	9%	4%	2%	14%	13%	18%	6%	10%
75	12%	13%	3%	24%	18%	17%	8%	19%
60	2%	1%	15%	28%	4%	3%	13%	17%
45	5%	12%	6%	19%	10%	0%	19%	19%
30	2%	22%	9%	12%	8%	8%	11%	12%
10	27%	33%	40%	5%	9%	11%	38%	3%

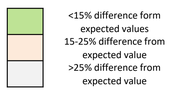


Table 2. AAV Ratio Kit % full data in crude samples.
Complexity of the buffers reduces from Buffer 8 to Buffer 1.

DETERMINATION OF AAV EMPTY TO FULL RATIOS

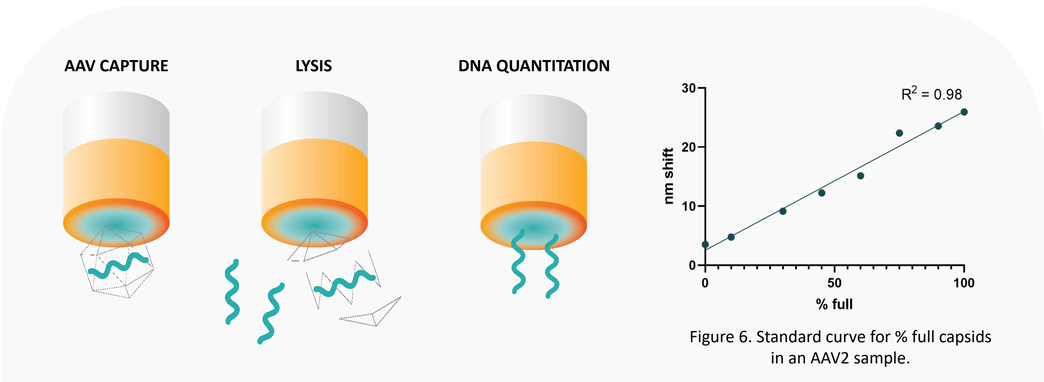


Figure 6. Standard curve for % full capsids in an AAV2 sample.

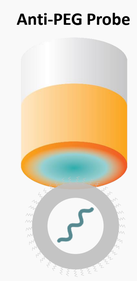
	Crude sample compatibility	Assay time	Automated
Gator AAV Ratio Kit	Yes	**24 samples in 120 minutes**	Yes
Analytical Ultracentrifugation (AUC)	**No**	6 hours	No
Transmission Electron Microscopy (TEM)	**No**	3-6 hours	No
DLS-UV/vis (Stunner)	**No**	96 samples in 1 hour	Yes
Mass Photometry (Refyn)	**No**	5 minutes per sample	Yes
Simple Western (Protein Simple)	**sample prep required**	24 samples in 5 hours	Yes
Charge Detection Mass Spectrometry (CDMS)	**No**	2 hours	No
Size Exclusion Chromatography - Multi Angle Light Scattering (SEC-MALS)	**No**	30 mins per sample	Yes

Table 3. Gator Bio AAV Ratio Kit compared to existing methods.

Analyzing Non-Viral Vectors

KINETICS OF PEGYLATED LIPID NANOPARTICLES

Anti-PEG probe captures and quantifies lipid nanoparticles (LNPs). Serum proteins can also be immobilized onto probe to study their kinetic interactions with LNPs.



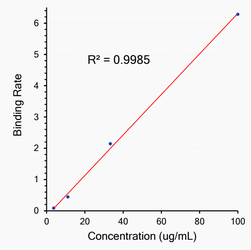


Figure 7. Standard curve for PEGylated LNPs generated using Anti-PEG probe.

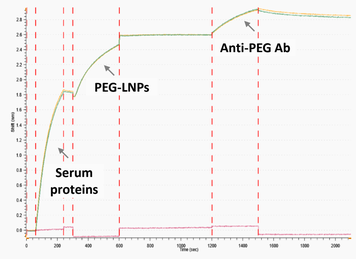
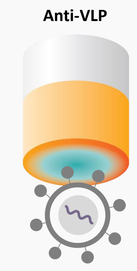


Figure 8. Sensogram showing serum proteins capturing LNPs.

VIRAL-LIKE PARTICLES

Probes are customized to target surface markers, such as biotin or flag tag, to capture viral-like particles (VLPs). The immobilized VLPs can then be used for screening antibodies or ligands against biomolecules embedded in the VLPs.



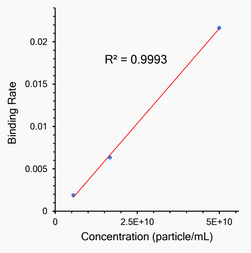


Figure 9. Standard curve for VLPs generated using Anti-VLP probe.

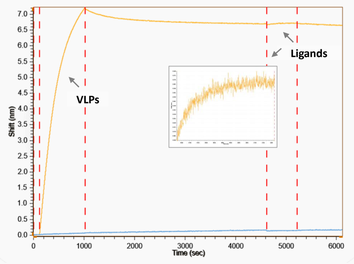


Figure 10. Sensogram showing capture of VLPs and ligands.

Summary

The BLI-based solutions address the challenges faced in vector production:

- Accurate, precise and wide dynamic range in crude and clean matrices
- Accurate empty to full ratios enabling rank ordering during purification
- All AAV assays – compatible with various matrices
- Accurate and easy kinetics platform for LNPs and VLPs