

Accurate and reliable quantitation of AAV serotypes using novel AAVX biosensors and the Gator™ system

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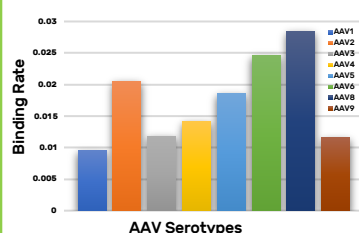
Introduction

AAV vectors have become a preferred gene therapy delivery modality. Different AAV serotypes target different organs and tissues. Commonly used serotypes include AAV2, AAV5, AAV8, and AAV9.

Accurate quantification of purified AAV particle preparations represents a critical step for clinical applications. Determination of total capsid titer is one of the critical quality attributes, hence, accurate and reproducible quantification of AAV titers is essential for the safe and effective use of AAVs in gene therapy.

Here, we present data on the accuracy, dynamic range, robustness, and inter- and intra-assay variability in quantitation using GatorPrime™ and AAVX probes for different serotypes. Furthermore, we compare data with Progen AAV9 ELISA.

Binding rates of different AAV serotypes

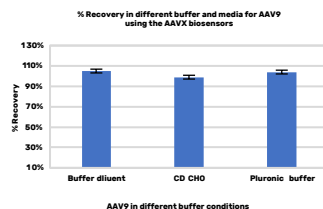


The binding of different AAV serotypes to Gator™ AAVX probes were compared at 2×10^{11} vp/mL in buffer diluent

Recovery of spiked samples in different matrices

Matrix	Tolerated concentration
Pluronic F-68	0.05%
MgCl ₂	3 mM
Tween 20	0.1%
NaCl	200 mM
BSA	2%
Spent CD CHO media	100%

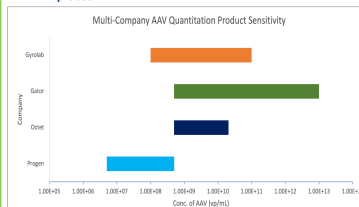
Matrix effects of different additives in an AAV9 quantitation assay using Gator™ AAVX probes



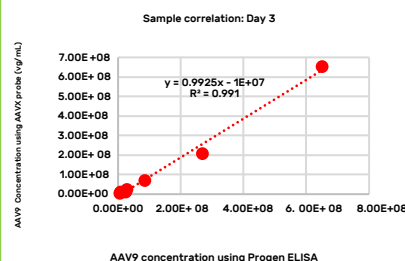
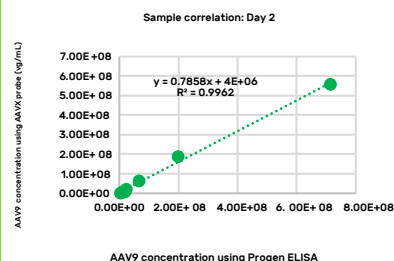
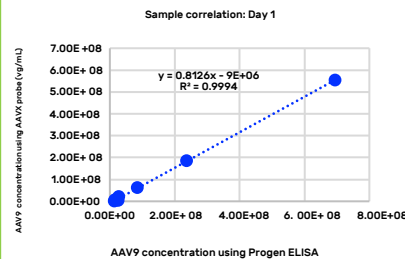
Good recovery (>90%) with low CV (<15%) above 1×10^9 vp/mL was detected across different buffer systems of AAV9 sample diluted in different buffers

Comparison with other methods

Gyrolab – Wide dynamic range but indirect measurement with multiple steps
Gator – Wider dynamic range, ready to use probe, much less dilution
Octet – Application note shows narrow range and self prepare the probes
Progen – Highly sensitive, too many steps, multiple kits needed and multiple dilutions

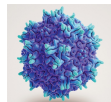


AAV9 concentration – Gator™ AAVX probes versus Progen AAV9 ELISA



AAV serotype quantitation goal

- Total capsid quantitation for serotypes AAV1-10
- Dynamic range up to 1×10^{14} vp/mL (for most serotypes)
- Less than 1 hr. analysis time
- LOD – 1×10^9 vp/mL
- Crude sample tolerant
- Stable over broad pH range
- Cost effective
- Easy to use with little hands-on time

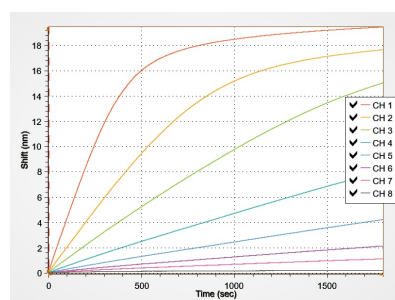


AAVX biosensors and workflow

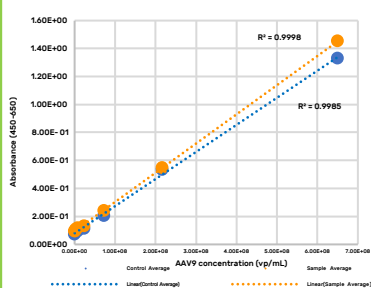
- The probe uses CapSelect™ AAVX nanobody as a ligand to enable direct measurement
- Samples containing AAV viral particles are pipetted in the reaction plate and captured on the probe
- The total virus capsid concentration determined using rate of binding of the AAV serotype of interest to the probe
- Different AAV serotypes bind at different rates
- The software calculates the binding rates from standards with known concentrations to generate a standard curve
- The binding rate of each standard is proportional to concentration
- Concentration of samples is calculated by comparing the binding rate standard curve created for standards



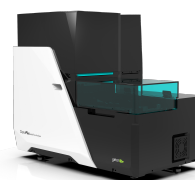
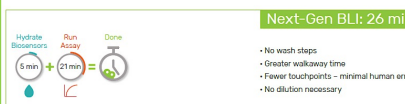
Capture of the AAV8 serotype on Gator™ AAVX probes



Verification of Progen Xpress AAV9 ELISA linearity



Gator™ and ELISA workflow comparison



Conclusions

The Gator™ AAVX probe used on the GatorPlus™ system is capable of:

- Total AAVX capsid determination for serotypes AAV1-10
- Dynamic range: 1×10^9 to 1×10^{13} vp/mL (for most serotypes)
- 26 min assay/96 samples
- LOD: 5×10^8 vp/mL (serotype-dependent)
- Good correlation with Progen AAV9 Xpress ELISA kit
- Handles multiple buffer and media
- Cost effective and reusable $\geq 10x$
- Plug and play with little hands-on time