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 AAV ELISA.

AAV serotype quantitation goal

- Total capsid quantitation for serotypes AAV1-10
- Dynamic range up to 1 x 10^10 vp/mL (for most serotypes)
- Less than 1 hr. analysis time
- LOD – 1 x 10^8 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 AAVX 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

Recovery of spiked samples in different matrices

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

- % Recovery in different buffer and media for AAV9
- Good recovery (90%) with low CV (≤15%) above 1 x 10^8 vp/mL was detected across different buffer systems of AAV9 sample diluted in different buffers

Comparison with other methods

Gator™ - Wider dynamic range, ready to use probes, much less dilution
Progen – Highly sensitive, too many steps, multiple kits needed and multiple dilutions

AAV9 concentration - Gator™ AAVX probes versus Progen AAV9 ELISA

- Sample correlation: Day 1
- Sample correlation: Day 2
- Sample correlation: Day 3

Conclusions

The Gator™ AAVX probe used on the GatorPlus™ system is capable of:
- Total AAVX capsid determination for serotypes AAV1-10
- Dynamic range: 1 x 10^8 to 1 x 10^13 vp/mL (for most serotypes)
- 26 min assay/96 samples
- LOD: 5 x 10^8 vp/mL (serotype-dependent)
- Good correlation with Progen AAV9 Xpress ELISA kit
- Handles multiple buffer and media
- Cost effective and reusable ≥10x
- Plug and play with little hands-on time