Rapid and easy-to-automate AAV titer assays for cell line and bioprocess development

Annika Roering, Christian Meissner, Karin Mezei, Aris Perrou and Sebastian Giehring

PAIA Biotech GmbH · Köln/Germany · *www.paiabio.com*

Abstract

In the past decade, the use of AAV (adeno-associated virus) as a vector in gene therapy has gained significant popularity, leading to an increase in projects and a pressing need for more efficient bioprocess development. To address this, PAIA Biotech has developed a rapid and robust capsid titer assay based on its proprietary microplate technology. The assay incorporates the proven *Thermo Scientific™ CaptureSelect™ AAVX* products and eliminates several steps from the workflow of traditional microplate-based assays such as ELISA. This innovation allows for the measurement of hundreds of samples within one hour. It is particularly suited for applications in cell line development, enabling fast and reliable screening of AAV-producing cell clones. We present data for AAV serotypes 2, 5 and 8 and demonstrate the robustness of this assay against common matrix components such as cell culture media, salts and detergents.

PAIA Technology

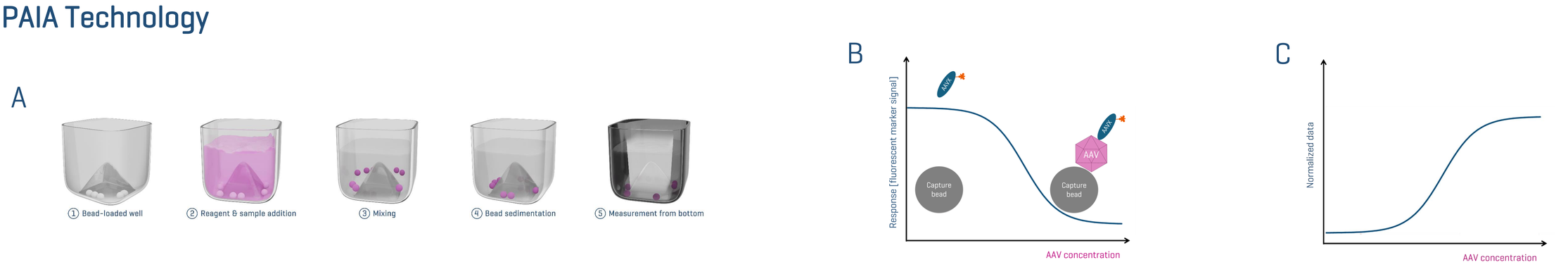

Figure 1. Assay workflow and scheme of signal interpretation

A) The entire assay is performed in the 384-well PAIAplate, pre-filled with dried capture beads [1]. After addition of AAV-specific fluorescent antibodies and sample [2], the plate is shaken for 60 min [3]. The beads are settled on the bottom and thereby out of the measurement area [4]. The plates can be read on a fluorescence plate reader with bottom reading [5]. The reading exclusively detects the fluorescence in solution and therefore samples with high titer will show low fluorescence values. B) AAV particles bind to capture beads and fluorescent markers, forming bead-analyte-marker-complexes resulting in a decrease of fluorescence signal with increasing analyte concentration. C) Subsequently, the fluorescence intensities are normalized to the control lacking AAV. If the measurement value without analyte equals that of the sample, the normalized value is set to 0. The higher the measurement value of the sample, the closer it approaches 1, which would correspond to a marker binding of 100%. This normalization process allows for the comparison of marker binding efficiency across different samples, ensuring accurate and reliable interpretation of the assay results.

Materials and Methods

PA-AAVX titer assays are using *POROS™ CaptureSelect™ AAVX Affinity Resin* as capture beads and *CaptureSelect™ Anti-AAVX Conjugate* in combination with R-Phycoerythrin (R-PE) as fluorescent marker for detection. Different AAV samples (AAV2, AAV5 and AAV8) from various manufacturers were analyzed over a concentration range of 2E+9 to 1E+12 cp/ml.

First, 30 µl of the marker mix was added into the plate containing the dried beads, followed by 10 µl of the analyte or reference, respectively. The microplate was shaken at 1900 rpm on an orbital shaker for 60 minutes. Afterwards the plate was quickly centrifuged at 1000 x g and read on a plate reader in bottom read mode at wavelengths of exc. 565/em. 600 nm.

PA-AAVX Capsid Titer Assay

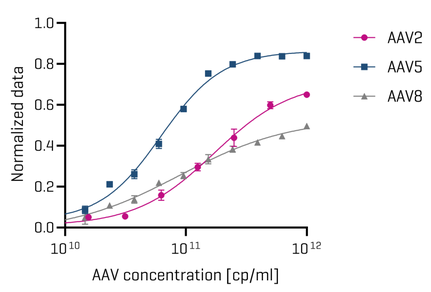

Figure 2. Comparison of different AAV serotypes in PA-AAVX Capsid Titer Assay

Our assay was tested with three AAV serotypes (AAV2, AAV5, and AAV8). The results are in-line with the known affinity of the AAVX ligand towards these serotypes, which results in different assay ranges for the different serotypes.

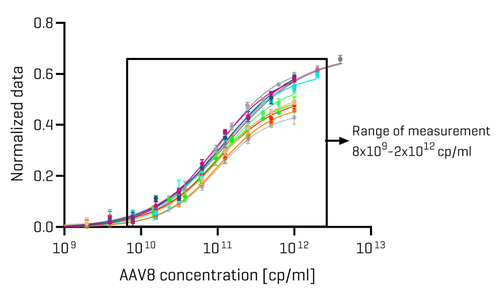

Figure 3. Reproducibility of PAIA's AAV Capsid Titer Assay (PA-AAVX)

The AAV capsid titer assay was conducted over an eight-week period utilizing several AAV8 standards with differing F/E ratios. The outcomes demonstrate excellent reproducibility, with a range of measurement from $8x10^9$ to $2x10^{12}$ cp/ml.

Impact of Different Matrix Components

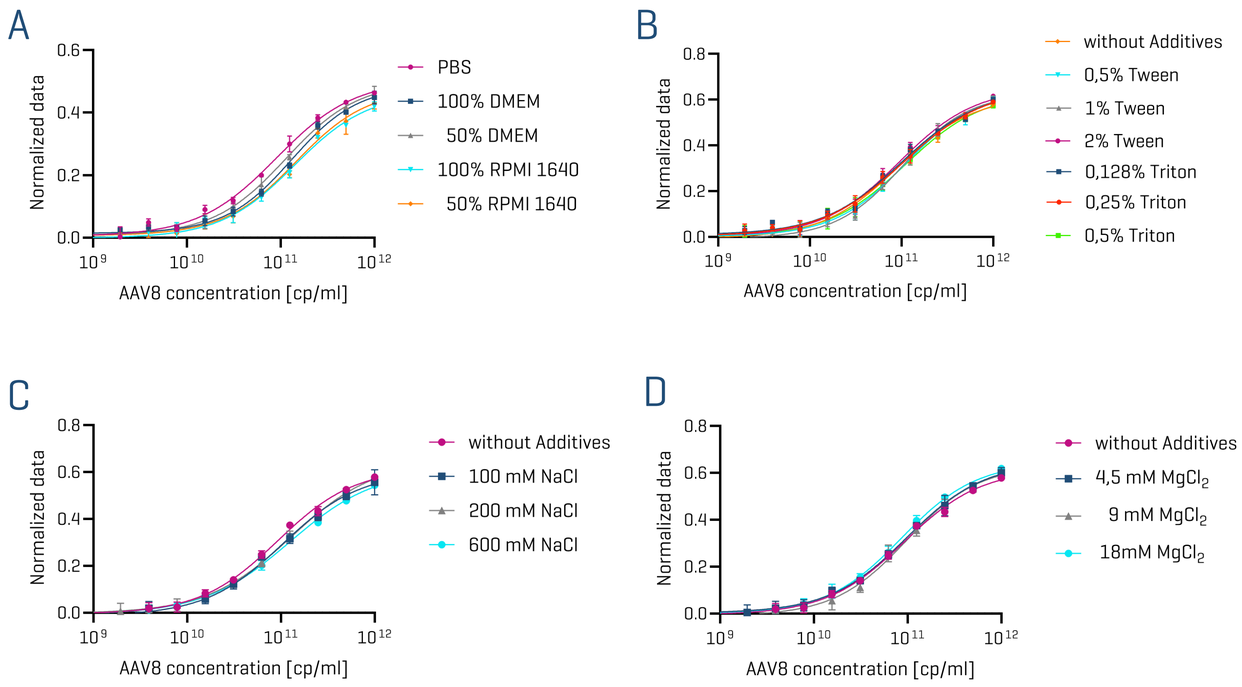

Figure 4. Robustness of PA-AAVX Capsid Titer Assay against Common Matrix Components

The impact of cell culture media, salts, and various detergents on the PA-AAVX capsid titer assay for AAV8 was evaluated. The concentrations shown represent those present in the sample.

A) **Cell culture media**: Undiluted and 1:2 diluted DMEM and RPMI 1640 were tested in comparison to PBS. These components exhibited minimal to negligible effects on marker binding

B) **Detergent effects of Tween and Triton:** Up to 2% Tween and 0.5% Triton did not influence marker binding or the measured AAV concentration.

C) **Salt concentration:** The addition of various NaCl concentrations (100-600 mM) did not affect the assay result.

D) **Magnesium Chloride:** Up to 18 mM $MgCl_2$ in AAV sample showed no influence on marker binding.

Impact of NaCl on AAV2 Binding

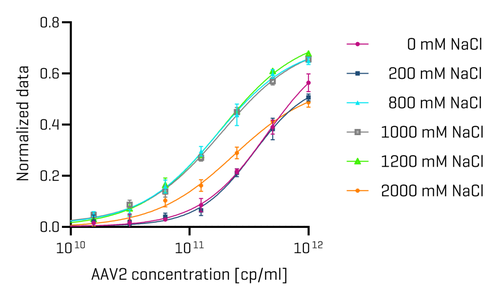

Figure 5. NaCl Concentration Affects the Result of the Titer Assay

AAV2 performs best in a NaCl concentration range of 800–1200 mM. Lower concentrations lead to AAV2 aggregation, while higher concentrations result in decreased marker binding. Both extremes have the same effect: a lower apparent titer than the actual.

- **High throughput screening:** The PA-AAVX assay enables the screening of up to 384 samples within one hour, providing a rapid and efficient method for analyzing large sample sets.
- **User-Friendly:** Our technology streamlines traditional microplate-based assays like ELISA, simplifying the workflow by eliminating several steps. Additionally, it is designed to be easily operated without costly equipment or specialized training, increasing accessibility for researchers.
- **Reproducible:** The PA-AAVX titer assay reliably identifies both full and empty AAV capsids, consistently producing reproducible data. Its resilience to common matrix components, such as salts and detergents, ensures dependable results across experiments.