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Integration of Affinité SPR platform in Adeno-Associated Virus (AAV) bioproduction for gene therapy

Introduction

Adeno-associated viruses (AAV) are increasingly being produced due to their tremendous potential in gene therapy. These viruses have shown promising results in delivering therapeutic genes to target cells and tissues, making them a valuable tool in treating a wide range of genetic disorders. However, as the demand for AAV-based therapies continues to grow, there is a need for consistent quality in scale-up production.

At the research level, small quantities of AAV are produced for proof-of-concept studies. These studies help researchers understand the efficacy and safety of AAV-based therapies in preclinical models. However, when it comes to large-scale production, challenges arise in terms of slow quality control characterization. Conventional methods such as enzyme-linked immunosorbent assay (ELISA) and droplet digital polymerase chain reaction (ddPCR) are commonly used for AAV characterization such as viral genome count, total viral particles and purity. However, especially in the case for ELISA, it can be time-consuming and taking days to perform in centralized labs. This delay in obtaining characterization data can hinder the timely assessment of product quality at different stages of production (Figure 1).

To address this challenge, surface plasmon resonance (SPR) technology offers a faster and simpler alternative to ELISA. Affinité lensless SPR technology has the potential to revolutionize AAV analysis by enabling label-free and real-time analysis at the bench. By leveraging the principles of SPR, this technology allows quantitation of total AAV capsids without the need for labels or extensive sample preparation. This not only saves time but also provides valuable insights into the quality of AAV at each step of the process.

In this application note, the lack of characterization can be effectively addressed using the Affinité SPR platform. This aims to highlight the potential of using the portable 4-channel static SPR system, known as P4SPR or flow-based SPR, P4PRO for the analysis of AAV in bioprocess production. Our compact and versatile systems can be conveniently used at the bench, and have potential for in-line, at-line integration.

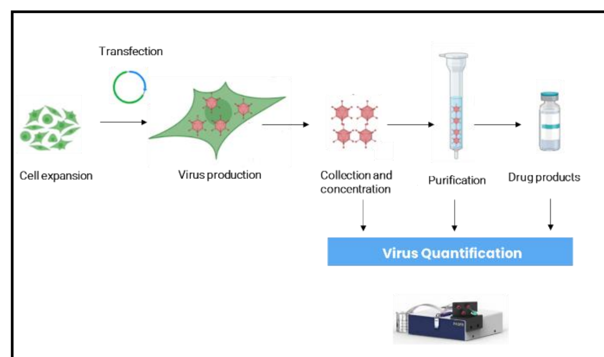

Fig. 1. AAV production workflow and integration of P4SPR at different stages of production.

AAV Total capsid titer

Currently, the measurement of AAV total capsid titer is done using ELISA. However, Surface Plasmon Resonance (SPR) can be a more efficient and cost-effective alternative. In fact, the SPR method does not require additional reagents like a secondary antibody or substrates for color development.

To produce different AAV serotypes, triple transfection was performed in HEK-293 cells that were adapted to serum-free media for suspension growth. The total capsid in purified samples was then analyzed. For titer analysis and surface regeneration, Affinité's biotin sensor and streptavidin kit were used to immobilize Biotin-Anti-AAVx CaptureSelect™ (Figure 2). The accuracy of the titer measured by SPR in viral genome/mL was compared to the titer obtained from ddPCR, assuming each viral particle typically contains one viral genome. The results showed a correlation of 91-111% between the two methods.

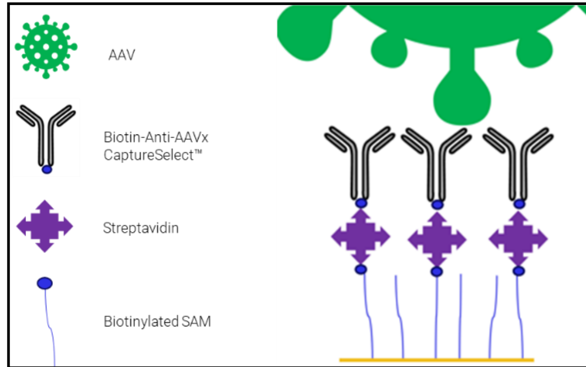

Fig. 2. Schematic illustration of AAV titer analysis by SPR.

Furthermore, detection limit has been determined to be in the 10^8 vg/mL for both the static and flow-based mode. This was determined using serotype AAV2 with a 1/1000 titer (Figure 3).

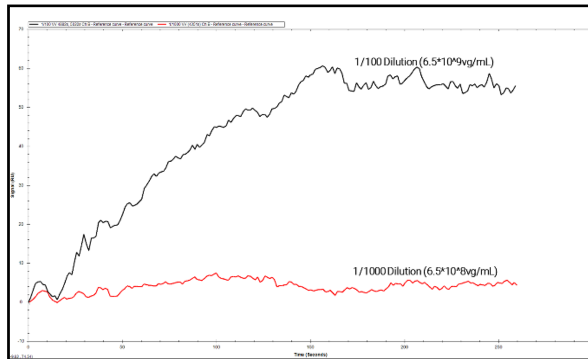

Fig. 3. Flow-based detection of AAV2 in viral buffer. Control response subtracted.

In addition, using purified AAV standards, calibration curve was established in different ranges with $R^2 > 0.98$ for different serotypes.

Serotype	Dynamic range (vg/mL)	R^2
AAV9	6.3×10^{10} to 1×10^{12}	0.980
AAV2/5	8.7×10^{9} to 8.7×10^{10}	0.998
AAV2	6.7×10^{9} to 4×10^{10}	0.986

Table 1. Calibration curves using different AAV serotype standards.

By incorporating reliable AAV titer information, SPR can significantly streamline the quality control process, resulting in improved efficiency. The 4-channel system offers a control channel and three sample channels, allowing for triplicate measurements which enhances data reliability.

When it comes to titer analysis, the static SPR system with easy setup proves to be a highly efficient and time-saving approach. Analysts can rapidly determine the concentration of AAV, enabling them to obtain crucial titer information in a timely manner. Notably, An entire titer analysis experiment including immobilization, 5 titer levels with surface regeneration steps in between can be completed in less than 2 hours, highlighting the speed and efficiency of the static system. This expedited analysis process is particularly advantageous in time-sensitive situations or when quick decision-making is required.

In addition to its speed and efficiency, the SPR workflow has also been validated for analyzing in-process samples. Preliminary results indicate that the same SPR workflow can be applied to analyze crude samples, providing valuable insights into the titer levels during different stages of production. This flexibility is again highly beneficial as it allows for real-time monitoring and adjustment of the production process, ensuring consistent and optimal AAV production.

Moreover, the developed assay has been tested with additional AAV serotypes, including AAV5, phpEB, and CapB10. This testing demonstrates the robustness and versatility of the assay, as it has potential to analyze multiple AAV serotypes. This is particularly important as different serotypes may have varying characteristics and requirements, and the ability to analyze them accurately ensures the reliability of the assay across different AAV variants.

In-line, at-line integration

The compact footprint of the Affinité SPR makes it suitable for use in BSL level 2, where it does neither interrupt the BSL workflow or the SPR analysis. Both at-line and in-line SPR testing offer significant advantages in terms of efficiency and convenience. Implementing at-line testing reduces the need to access the QC lab with its lengthy turnaround time and complex equipment, thus saving time and reducing the risk of cross-contamination. In-line SPR can be installed in each viral vector clean room, providing real-time results without the need to take samples out of the clean room and send them to the QC lab. This streamlined process eliminates the potential for

cross-contamination and enables timely decision-making based on timely data.

Fig. 4. P4SPR operated into a BSL 2 environment.

Summary

The application of SPR technology in AAV analysis has the potential to significantly improve product quality. By providing faster and more accurate characterization data, informed decisions can be made during the production process, ensuring the consistency and effectiveness of AAV-based therapies.

In summary, the SPR instrument developed by Affinité can be used to quantify various AAV serotypes. The assay is efficient and reproducible, offering several benefits in streamlining the quality control process. Its ability to provide triplicate measurements, analyze in-process crude samples, and rapidly determine concentration all contribute to improved efficiency, accuracy, and decision-making. The versatility of the SPR workflow, combined with its successful testing with various AAV serotypes, further enhances its reliability and applicability in AAV production and research.

Acknowledgements

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