In-Capillary Immunoassay and Total Protein Detection for Adeno-Associated Virus (AAV) Proteins During Purification from Whole-Cell Lysate

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INTRODUCTION

During adeno-associated virus (AAV) manufacture, critical quality attributes must be monitored including the presence, identity, and purity of viral vector proteins. Traditionally, the identity and purity of these proteins is monitored by Western blot using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). However, SDS-PAGE is notoriously challenging and labor-intensive. Additionally, AAVs are limited in sample size as they are difficult to manufacture, and demand outstrips supply.

Here, we have developed a method to monitor the purification of AAV2 using automated capillary electrophoresis followed by immunoassay and total protein detection directly in the capillary, eliminating the need for SDS-PAGE. AAV2 was purified from HEK293 cell lysate using affinity chromatography, and the steps of the purification process (load, flow-through, wash and elution) were monitored by the capillary-based immunoassay. VP1, VP2 and VP3 capsid proteins were resolved and identified either individually or simultaneously, depending on the AAV antibody used for detection, and the total protein assay monitored the presence of impurities. The sensitivity of this assay reduced sample size down to 3 µL of sample, corresponding to approximately 400 pg or $1x10^8$ genomic copies loaded per well. We anticipate that this in-capillary immunoassay and total protein detection can replace traditional SDS-PAGE methods in AAV manufacturing workflows.

METHODS

Simple Western Method

The default Wes sample preparation and assay conditions were followed using the 12–230kDa Wes Separation Module (PN SM-W004) and the Anti-Mouse Detection Module (PN DM-002). For total protein detection, the instructions were followed in the Total Protein Detection Module for Wes (PN DM-TP01).

AAV antibodies

Anti-AAV VP1/VP2/VP3 mouse monoclonal (PN 61058), anti-AAV2 VP1/VP2 mouse monoclonal (PN 61057) and anti-AAV2 VP1 mouse monoclonal (PN 61056) antibodies were obtained from PROGEN.

AAVs:

AAV2 was purchased from Vigene Biosciences (custom order), or internally produced and purified at CGTC.

RESULTS

AAV2 was purified from whole HEK293 cell lysate using affinity chromatography, and the steps of the purification process (load, flow-through, wash and elution) were monitored on Wes. This analysis showed the presence of VP1, VP2 and VP3 proteins in the elution fraction using the total protein assay (**Figure 1A**) and immunoassay (**Figure 1B**). Less dilute samples were loaded for the immunoassay to detect low abundance immuno-reactive species. Closer analysis of the elution fraction revealed that the signal intensity of VP1 and VP2 were approximately equal, while VP3 had a significantly stronger signal (**Figure 2**).

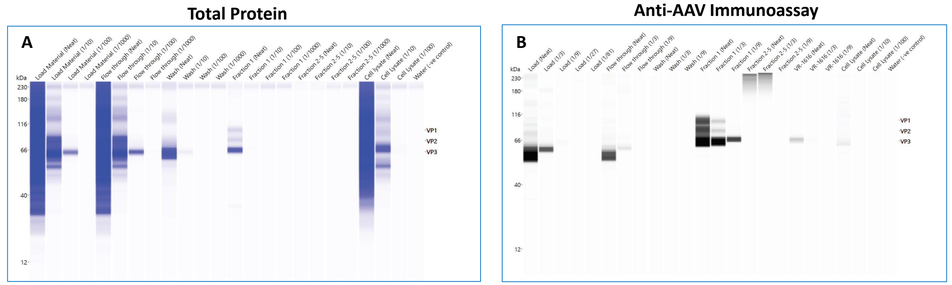

Figure 1. (A) Total protein detection of each fraction during purification from whole-cell lysate. (B) Immunodetection of VP1, VP2 and VP3 proteins during purification. Detection was performed with an anti- AAV VP1/VP2/VP3 mouse monoclonal antibody.
Load: input material loaded onto the columns; flow-through: material not bound on columns; wash: wash buffer from columns; fractions: eluate fractions from columns; VR1616: ATCC purified reference material loaded as a positive control (low titer); cell lysate: HEK293 cell lysate (without virus); water: negative control. Sample dilutions are shown in brackets.

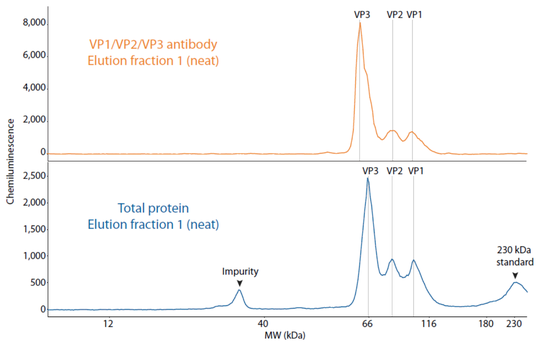

Figure 2. Electropherogram analysis of the elution fraction from the immunoassay (top panel) and total protein analysis (bottom panel). The immunoassay detection was performed with an anti-AAV VP1/VP2/VP3 mouse monoclonal antibody.

The results presented in Figure 1 and Figure 2 leverage a single antibody (anti-AAV VP1/VP2/VP3 mouse monoclonal antibody) that detects all three AAV capsid subunits. Wes is an open platform amenable to any antibody, meaning other targets may be detected simply by swapping in different antibodies as shown in Figure 3.

To detect specific capsid proteins, antibodies targeting AAV capsid proteins VP1/VP2/VP3, VP1/VP2 or VP1 were screened against commercially available AAV2 (**Figure 3**), enabling the identification of individual proteins in addition to all three. Because up to 24 different antibodies may be tested on a single Wes run, screening multiple antibodies is straightforward with Simple Western assays.

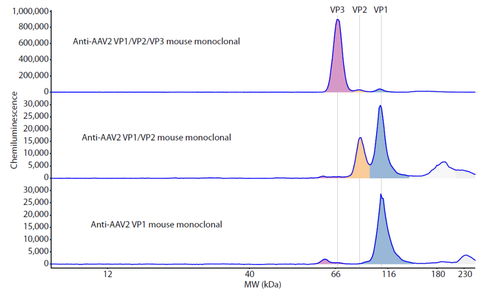

Figure 3. Antibody screen targeting VP1/VP2/VP3 proteins (top panel), VP1/VP2 proteins (middle panel) and VP1 only (bottom panel).

To determine the range of detection, commercially available AAV2 ($1x10^{13}$ GC/mL) was subjected to a 2X dilution series from 1:8 to 1:256 and analyzed on Wes, detected with an anti-AAV VP1/VP2/VP3 mouse monoclonal antibody (Figure 4A). The total peak area of VP1/VP2/VP3 was calculated and plotted at each dilution factor and showed that detection of AAV proteins was possible down to the lowest dilution tested of 1:256 (Figure 4B).

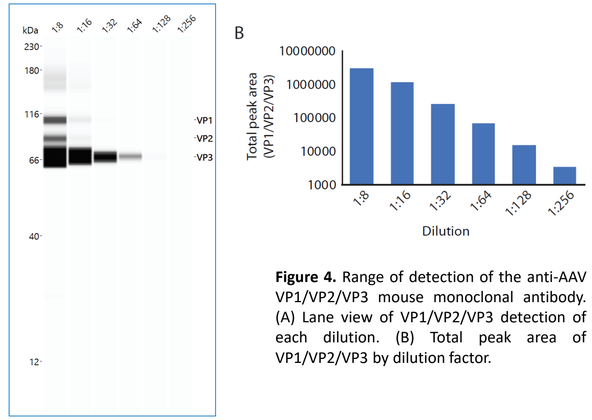

Figure 4. Range of detection of the anti-AAV VP1/VP2/VP3 mouse monoclonal antibody. (A) Lane view of VP1/VP2/VP3 detection of each dilution. (B) Total peak area of VP1/VP2/VP3 by dilution factor.

CONCLUSIONS

- AAV manufacturing requires speed, sensitivity and specificity
- Simple Western can be used to study AAV production, and provides speed and sensitivity, completing a run in 3hrs and detecting AAVs >1:200 diluted sample
- AAV antibodies from PROGEN provide specificity, enabling distinction and characterization of individual VP subunits

References
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