Detection of AAV structure: capsid proteins and nucleic acid composition on LabChip® GXII Touch™ Platform

Natalia Rodionova, Zhiyong Peng, Erik Miller, Michael Nilsson
PerkinElmer, 68 Elm St., Hopkinton, MA 01748, USA

1 Introduction

For over fifty years, adeno-associated virus (AAV) has remained an elusive object in virology studies and gene therapeutics due to its non-pathogenicity. Many therapeutic companies are currently involved in recombinant AAV production with needs of viral particle characterization in terms of capsid and nucleic acid content to estimate therapeutic dosages. AAV is a protein shell encapsulating a single-stranded DNA genome of 4.8 kb, which is dependent on co-infection with other viruses to replicate. Its genome contains Rep (Replication), Cap (Capsid), and Assembly genes. The Aap gene encodes four proteins, which are required for genomic replication and packaging, while Cap gene produces capsid proteins (VP1/VP2/VP3) to form protective outer shell and to perform cell binding. All VPs are the product of the same genome which evolves gradually to form a more rigid capsid structure and gain extra functionality. The PerkinElmer LabChip® GXII Touch™ HT platform is an instrument to perform protein and nucleic acid analysis with high sensitivity and reproducibility. The instrument can distinguish differences of protein mass as low as 1kDa, which can be crucial to characterize recombinant capsid proteins. Additionally, the fast sample run times from multi-well plates allow for high throughput experiments and can open opportunities to monitor kinetic processes such as breakdown of virus particles or their interactions with receptors.

2 ProteinExAct™ Assay to evaluate AAV capsid proteins

Because of the increased interest in AAVs in gene therapies, there exists a need for a method to properly characterize AAV capsid proteins (VPs) to accurately benchmark viral infectivity and potency. The PerkinElmer ProteinExAct™ assay for the LabChip® GXII Touch™ protein characterization system utilizes IntelliChip™ assay optimization technology to enable high resolution and reproducibility of protein content and composition analysis of a sample to enable the utmost of accuracy in AAV characterization for recombinant viral production. AAVs vary in stability (temperature at which capsid breaks) and have a similar ratio of capsid protein composition known as VP1:VP2:VP3 = 1:1:10. The ProteinExAct™ kit can be used to confirm this property of recombinant viruses to conserve optimum infectivity. Empty capsid particles of several serotypes (AAV2, AAV5, and AAV9) have been used for these experiments. To break apart capsid particles, AAV samples were repeatedly heated in the presence of detergents. As different AAVs have varying stability, the temperatures were individually chosen for each serotype. As shown in Figure 1, fully broken AAV particles have only three peaks with sizes of approximately 80, 90 and 115kDa molecular weights.

3 Observation of AAV kinetics (Stability)

To investigate the time scales of AAV breakdown, kinetic experiments have been performed with each recombinant construct. The LabChip® GXII Touch™ HT protein characterization system is able to collect up to 300 experimental points every 30-50 seconds, which gives additional opportunity to investigate the full kinetics of capsid breakdown.

4 AAV Glycosylation

Some investigators observed well-distinguished “shoulders” on the main VP3 peaks during experiments on LabChips® GXII Touch™ protein characterization system. Recently, Sara Murray et al. reported certain glycosylation of AAV2 capsid proteins and theoretically estimated the ability of capsid protein surfaces to have four possible locations for glycosylation. To investigate this hypothesis of caps glycosylation, we performed de-glycosylation of AAV2, AAV8 and AAV9 particles using enzymatic treatment (digestion by PNGase F), previously developed in our laboratory; the data in Figure 4 show AAV profiles before and after that treatment. In Figure 4, VP3 peaks were detected to have a shifted molecular weight of 79kDa, compared to 81kDa in the untreated VP3. The same change of about 2kDa can be observed for VP2 (92 vs. 94kDa) and VP1 (113 vs. 115kDa). The observation agrees with the average molecular weight of glycans attached to capsid proteins. Additionally, each of the AAV dimers, shown in enlarged Figure 2b, have up to three shoulders, supporting the hypothesis of multiple glycosylation of viral proteins.

5 Detection of genomic DNA from AAV capsids

Additionally, to accurately protein analysis, PerkinElmer’s DNA assay for the LabChip® GXII Touch™ platform is enable to measure of genomic DNA concentration of broken viral sample with high resolution and reproducibility. Established limit of detection of LabChip® GXII Touch™ for DNAs and proteins allows to estimate the percentage of empty capsids important for dose determination.

6 Citations


7 Summary

• Presented data on PerkinElmer’s ProteinExAct™ assay for the LabChip® GXII Touch™ protein characterization system with AAV samples demonstrate the ability of the platform in providing a full analysis of viral proteins.
• The assay provides a robust method for serotype identification of clinical vectors.
• The highly reproducible and accurate measurements of capsid composition can be a novel method for the characterization of recombinant viral particles, a crucial step in gene therapy applications.
• High-resolution verification of capsid protein glycosylation further benefits production of therapeutically important components.

PerkinElmer, Inc., 940 Winter Street, Waltham, MA USA (800) 762-4000 or (+1) 203 925-4602
www.perkinelmer.com