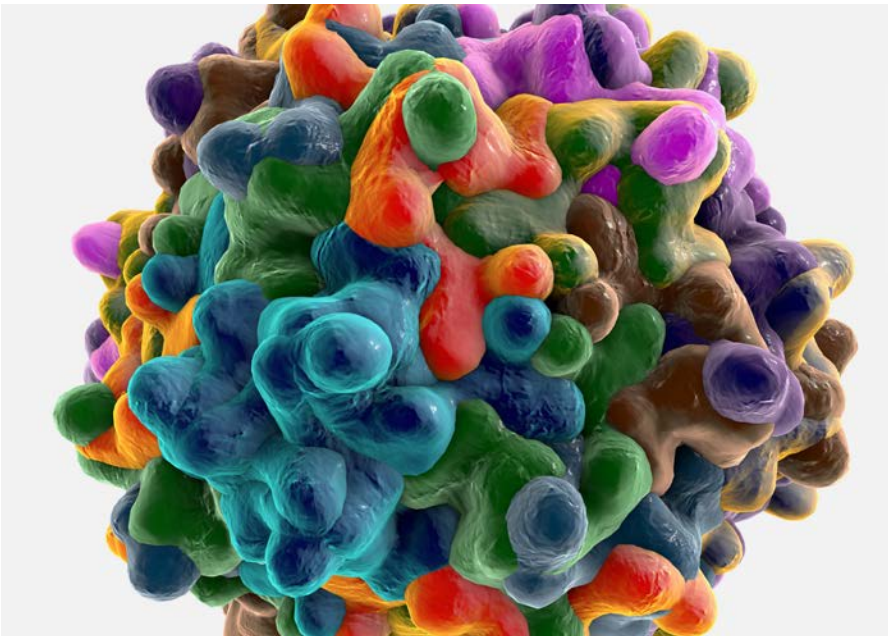

APPLICATION NOTE

AAV Workflow Solutions for Neuroscience Research

Several methods exist that aim to provide a safe vehicle and targeted route into the various tissues of the human body, including chemical carriers, synthetic or natural lipid-, peptide- or polymer-based biodegradables, lentiviruses, et al.

The Utility of Adeno-Associated Viruses (AAVs) in Therapeutic Approaches for Monogenic Neurological Diseases

Gene therapy is a very powerful tool that is currently being explored to combat disorders with underlying genetic causes. Within the field of neurological diseases, there is great interest looking at rare diseases of monogenic origin with the hope of developing disease-modifying gene therapies, as opposed to treatments for symptom management. Though *ex vivo* gene therapies have shown recent advances – namely, genetically modifying cells such as those from stem cells *ex vivo* and returning them to the body – research has yet to sufficiently resolve the proficiency of successful transplant or functional grafts back into a functioning nervous system. Therefore, using relatively tunable systems like recombinant AAVs (rAAVs), scientists are also exploring *in vivo* gene delivery in parallel. With the recent approval of Zolgensma (onasemnogene abeparvovec-xioi), a great deal of attention has been generated on AAV vector-based gene therapies. AAV9 (serotype 9) has been shown to display tissue tropism by more easily overcoming the blood-brain barrier (BBB) to deliver therapeutics across the CNS and is considered a powerful method in transducing neurons and other cells.[1] As a result, promising proof-of-concept and clinical successes have encouraged research into spinal muscular atrophy (SMA), Huntington's disease (HD), monogenic amyotrophic lateral sclerosis (ALS), and Frederich's ataxia (FA) where research is being conducted on gene editing approaches.

Both the therapeutic agent and the delivery method need to be properly considered to maximize efficacy and potency. The therapeutic agent can be DNA that codes for functional genes to replace defective ones or supplement low abundance endogenous genes, shRNAs that can be processed into silencing defective mRNAs or – more recently – the popular CRISPR/Cas9 system that attempts to edit the endogenous gene. Other less permanent genetic manipulations that also holds therapeutic potential focuses on modulating transcription or translation include miRNA, siRNA or antisense oligonucleotide (ASO) therapies. The decision on which transgene to use is often associated with the monogenic disease of interest.

Another big challenge is delivering the transgene safely into the host system. Several methods exist that aim to provide a safe vehicle and targeted route into the various tissues of the human body, including chemical carriers, synthetic or natural lipid-, peptide- or polymer-based biodegradables, lentiviruses, et al. Among these, the rAAV delivery system is the most widely adopted vector for transgene delivery due to its flexibility of housing DNA/RNA therapeutic transgenes, low immunogenicity, and relatively successful profiles of bioavailability, dosing, and pharmacokinetics in past studies.

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For the Better

The AAV delivery system is a popular therapeutic research modality due to its relative non-pathogenicity in eliciting an immune response, and with improved vector design, scientists are overcoming the innate immunity to AAVs. Through research and improvements to the capsid and its assembly, along with modifications to packaged genetic content and smart workarounds to the limitations in insert size, labs are working to optimize the gene transfer to the proper target cells and improve therapeutic efficiency.

PerkinElmer offers robust rAAV solutions for assessing in-process identity and purity for various steps of manufacturing and packaging, as well as tools to help explore and accelerate research of these potential rAAV therapies.

PerkinElmer Instrument and Reagent Solutions

Automated Protein Characterization

LabChip® GXII Touch™ Protein Characterization System

AAVs are encapsulated by a protein shell formed by three viral proteins – VP1, VP2, and VP3 – encoded by the Cap gene, which is part of the 4.7 kb viral genome. Its assembly can be complex, and reproducing viable viral particles requires many steps. Therefore, its characterization to assess identity and purity during these steps are important.

Figure 1. LabChip GX and GXII Touch Instruments.

The LabChip GXII Touch protein characterization system is a microfluidic capillary electrophoresis technology in the presence of SDS (CE-SDS), and it can offer a high-throughput and highly sensitive solution to manual SDS-PAGE. The LabChip system can propel AAV characterization as it simplifies the potential sources of error associated with SDS-PAGE gels or other, more manual procedures. The platform has sufficient resolution to detect shifts in profiles for AAV glycosylation studies as well as discern partial capsid profiles for monomers, dimers, etc. Contaminating proteins can also be assessed, as described in Figure 1. For high-throughput labs needing quick yet reliable characterization, the instrument supports a throughput of 96 samples per run at a speed of 65 second per sample analysis.

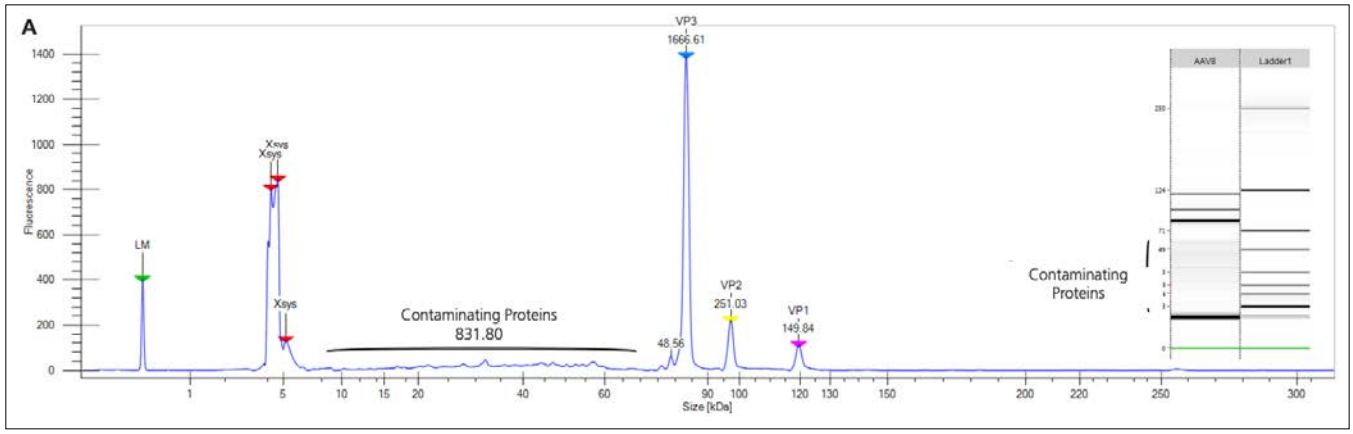

Figure 2. Representative electropherogram of a fully denatured AAV. X-axis is expressed as size (kDa), and Y-axis is fluorescence (RFU) and corresponds to concentration. LM is lower marker while Xsys are system peaks. VP3, VP2, and VP1 correspond to the blue, yellow, and pink labels respectively. Major peaks are additionally labeled with concentration (ng/µL). Contaminating proteins represented 28% of the total protein concentration and were evident as small peaks in a rough baseline and as indiscriminate low molecular weight bands in the virtual gel (inset).

Assessment of Metrics for Therapeutic Efficacy in *In Vitro* Models

Multimode Plate Reader, Microplates, Reagents, and Applications

Different methods exist to assess rAAV infectivity *in vitro*, such as observing rAAV viral titers and performing titration studies. Most methods rely on a plate reader that facilitates generation of accurate results, without sacrificing high-throughput capabilities. PerkinElmer offers a suite of readers – EnVision®, EnSight®, and VICTOR® Nivo™ – offering catered solutions for lab-specific throughputs.

In addition to these well-accepted applications looking at classic features of virulence, immunoassays can also be used to assess efficacy of the therapy on its intended targets using cell or tissue lysates. Reporter gene assays, such as the Britelite™ plus system, allow researchers to determine transduction efficiency of AAVs in developing next-gen capsids with more potent tropism for challenging organs protected by the BBB such as the CNS.[2] Reliable technologies such as homogenous time-resolved fluorescence (HTRF®) and AlphaLISA® assay technology have enabled researchers to detect aberrant protein behaviors that may be associated with pathogenic phenotypes in numerous models of neurological disorders with higher throughput, simplified, and no-wash workflows that use smaller sample volumes than legacy technologies like Western blots or classic ELISAs. Applications can range from assessment of kinase function, G-protein signaling, receptor binding, cytokine response, phospho-protein levels, or protein-protein interactions relevant to your research.

Figure 3. Multimode Plate Reader.

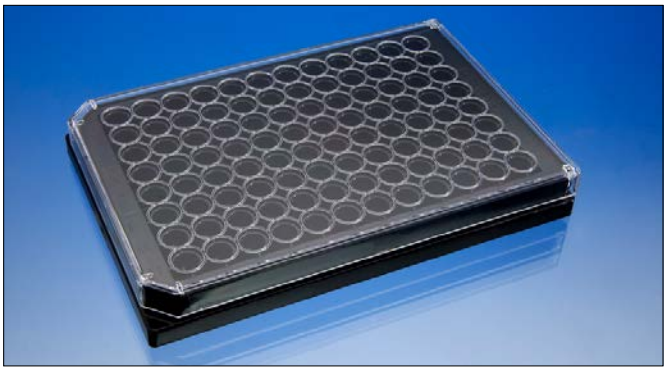

Figure 4. Microplates.

Figure 5. Reagents.

Figure 6. brite**lite** plus System.

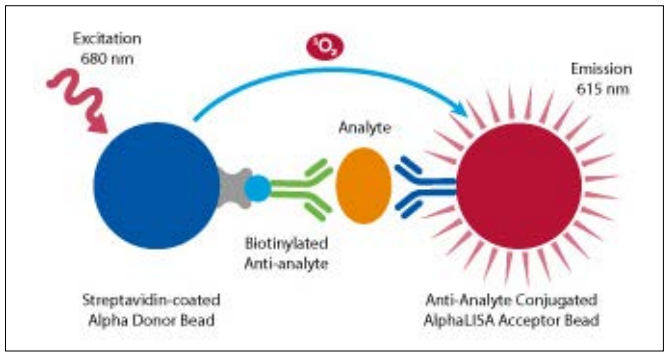

Figure 7. AlphaLISA® assay technology.

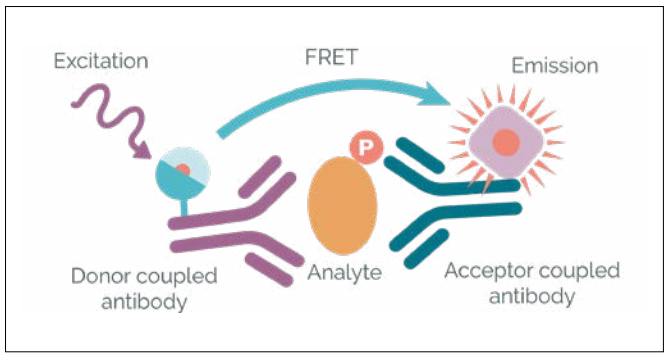

Figure 8. HTRF assay technology.

In Vitro Therapeutic Efficacy and Risk Assessment Using Pre-clinical Imaging

IVIS® Lumina & IVIS® Spectrum Optical Series, and Quantum GX2 microCT Imaging Systems

rAAV vectors are excellent genetic tools for inducing relatively stable transgene expression in transduced cells and exist as various serotypes that tend to exhibit different tropisms, or a tendency to infect different cell types. These features make rAAVs popular vehicles of delivery for eliciting *in vivo* gene transfer for potential therapeutic applications. There are many *in vitro* assays that can assess important criteria for rAAV efficiency, including the determination of the optimal MOI. It is understood, however, that non-invasive technologies such as molecular imaging are important in assessing viral activity in *in vivo* settings for a more comprehensive understanding of the mechanisms that lead to efficient delivery and cell/tissue transduction. Several key publications have explored and characterized novel vector systems such as Anc80L65 or exosome-associated AAVs for more efficient transduction to the CNS using bioluminescence and fluorescence *in vivo* imaging.[3,4] Questions such as time of arrival at a target organ and efficiency or duration of expression can be better studied using strategies that involve multi-modality imaging with anatomical resolution and visualization of potential unintended systemic effects, if any, as well. Metrics such as biodistribution and pharmacokinetic principles of viral gene therapy are active fields of rAAV research today.

Figure 9. IVIS Lumina and IVIS Spectrum Optical Series.

Figure 10. Quantum GX2 MicroCT.

Characterization of Guide RNAs and Assessment of Potential Off-target Genetic Effects

NEXTFLEX® Small RNA-Seq Kit v3

Small RNA-sequencing can provide sequence length and purity information for all small RNAs contained within a sample, including RNAi components and gRNAs. This is critical information to have prior to packaging into the rAAVs. As well as using CRISPR/Cas9 models to directly elicit genetic change to DNA, RNAi technology can be used to assess the efficiency of siRNAs or miRNAs that target specific transcriptional events or mRNA targets. Small RNA-seq is also a popular choice to assess efficiency of gene suppression, as well as understanding potential off-target effects on other unintended miRNAs or mRNAs that have highly similar sequences or isoforms that could be undetected by less sensitive technologies like qPCR.

Several publications report using small RNA-seq in evaluating off-target effects of their designed RNAi or miRNAs that have been packed into AAV particles. This has included assessing the impacts of off-target effects on in vitro or animal models for disease-causing genes for neurological disorders such as amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Huntington's disease (HD), spinocerebellar ataxia type 3 (SCA3) or Machado-Joseph disease (MJD).

To learn more about any of these solutions, please contact your local representative or visit PerkinElmer.com.

Selected Publications on rAAV for Neurological Disease Research

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