UNLOCK THE POTENTIAL OF AAV

AN INTRODUCTORY GUIDE TO ADENO-ASSOCIATED VIRUS (AAV) GENE DELIVERY

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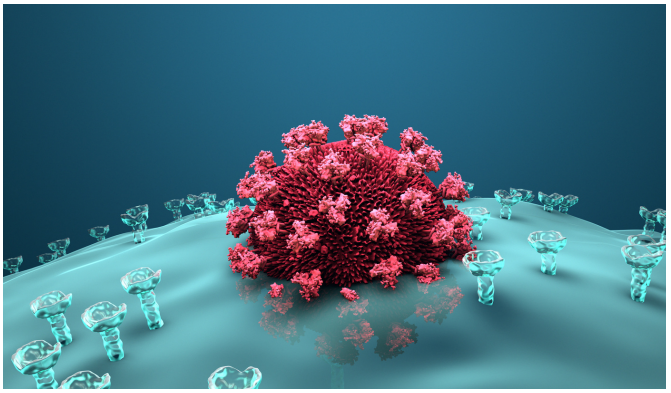
Understanding AAV & Its Significance in Gene Delivery



What is AAV?

Adeno Associated virus (AAV) is a small, non-enveloped virus belonging to the Parvoviridae family, which was first identified in 1965 as a contaminant of human and simian adenovirus preparations[1]. AAV has a single-stranded DNA genome of about 4.7kb in length, flanked by two inverted terminal repeats (ITRs) that serve as origins of replication and packaging signals.

This virus has gained significant attention in the research community due to its ability to deliver genetic material into targeted cells, with minimal immunogenicity and a broad range of infectivity. AAV remains a promising gene delivery vehicle for preclinical and clinical research and in recent years has made advances in a variety of therapeutic applications, such as treating Duchenne's Muscular Dystrophy (DMD).

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How Does AAV Compare to Other Viral Vectors?

Adeno-associated virus (AAV) stands out from other viral vectors like lentivirus and adenovirus in several ways. AAV exhibits a low immune response, enables long-term transgene expression, and carries a low risk of insertional mutagenesis. To delve deeper into the comparisons and contrasts among popular viral vectors used for gene delivery, please refer to the table below.

Virus	Expression	Packaging Capacity	Genome	Virus Size (nm)	Cells Infected	Target Cell Genome Integration	Immune Response
Lentivirus	Stable	<8 Kb	RNA	80-130	Dividing/ Non-dividing	Yes	Low
AAV	Transient or Stable*	~4.5 Kb	ssDNA (linear)	18-26	Dividing/ Non-dividing	No*	Very Low
Adenovirus	Transient	>8 Kb	dsDNA (linear)	105	Dividing/ Non-dividing	No	High
y-Retrovirus	Stable	<8 Kb	RNA	80-130	Dividing	Yes	Moderate

*Recombinant AAV has a low frequency of target cell genome integration

Understanding the characteristics and differences among viral vectors used for gene delivery is crucial in designing effective gene therapy strategies. While each vector has its own advantages and considerations, AAV emerges as a particularly promising choice. By leveraging the unique properties of AAV and other viral vectors, researchers and clinicians can continue to advance the field of gene therapy, bringing us closer to transformative treatments for a wide range of genetic disorders.

Benefits of AAV for Gene Delivery

Adeno-associated virus (AAV) is a promising viral gene delivery method with several key benefits that make it attractive for clinical and research applications. These benefits include:

- **Low immune response:** AAV naturally elicits a mild immune response in the body, which reduces the risk of host rejection or damage. This property of AAV can potentially prolong treatment time and limit the chance of unwanted side effects.
- **Long-term transgene expression:** AAV remains in the episome, therefore its expression is highly dependent on the cell's turnover rate, as this dilutes expression over time. The average time for expression is estimated to be 6-12 months[2]. This characteristic of AAV reduces the frequency of treatments required, providing a long-lasting therapeutic effect.
- **Broad range of infectivity:** Similar to other viral gene delivery methods, AAV can infect both dividing and non-dividing cells. This feature broadens its potential applications, as AAV can target a wide range of cell types in various diseases.
- **Low risk of insertional mutagenesis:** AAV does not integrate into the host genome, eliminating the potential risk of insertional mutagenesis. This significantly reduces the chances of unwanted complications associated with gene therapy treatments.

An illustrative case study highlighting the use of AAV in clinical settings is the development of Sarepta Therapeutics' gene therapy for Duchenne Muscular Dystrophy (DMD). On June 22nd, 2023, The FDA granted accelerated approval for ELEVIDYS™, the first gene therapy for Duchenne Muscular Dystrophy (DMD), which utilizes Adeno-associated Virus (AAV) to deliver mini-dystrophin gene to muscle tissues. This AAV-based therapy represents a significant advancement in gene therapy, showcasing the potential of AAV in treating genetic disorders.

AAV Structure and Gene Delivery Mechanism

Recombinant AAV (rAAV) differs from wildtype-AAV in that it lacks viral DNA. rAAV has been described to act as a protein-based nanoparticle since it is optimized and engineered to traverse the cellular membrane to deliver its DNA to the nucleus[3].

rAAV particles have the ability to form circular concatemers which persist as episomal DNA. Since this episomal DNA does not integrate into host genomes, it has the benefit of maintaining transient expression while avoiding insertional mutagenesis. This makes AAV stand out compared to other popular gene delivery methods such as lentivirus.

The process of AAV particle uptake through endocytosis is currently not fully understood. Among the different AAV serotypes, AAV2 has been extensively studied, providing the most available data on uptake mechanisms. Initially, research indicated that clathrin-mediated endocytosis is involved in the internalization of rAAV particles.

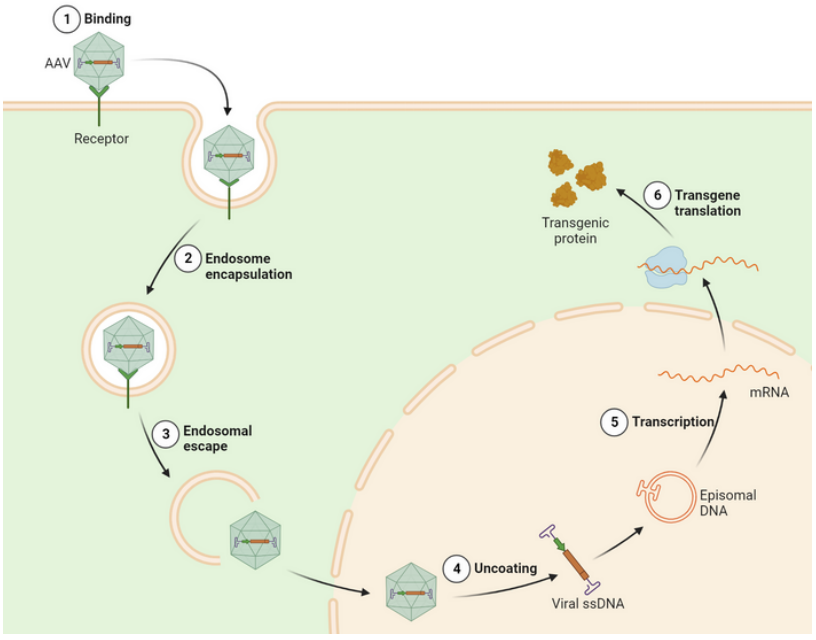

Fig 1. AAV Infection Mechanism

However, other studies have also shown evidence supporting alternative pathways that are independent of clathrin, as well as pathways involving GPI-enriched compartments. The data on endocytic mechanisms varies across different cell types, suggesting that these mechanisms may be dependent on the specific type of cell[4].

Upon entry into the cell (Fig. 1), it is believed that AAV is transported through the Rab5+ early endosomal compartment, as well as the Rab7+ late endosome and Rab11+ recycling endosomes, although this can vary depending on the AAV serotype. After traversing the endomembrane system, AAV undergoes endosomal escape and remains in the cytosol.

The method by which AAV gains entry into the nucleus is still unknown. The variations observed in cell types and serotypes pose a significant challenge in comprehending the transduction mechanisms of AAV.

Once inside the nucleus, AAV undergoes uncoating, and its single-stranded DNA is converted into double-stranded DNA, which then undergoes transcription.

References :
1. Rose JA, Hoggan MD, Shatkin AJ. Nucleic acid from an adeno-associated virus: chemical and physical studies. Proc Natl Acad Sci U S A. 1966 Jul;56(1):86-92. doi: 10.1073/pnas.56.1.86. PMID: 5229859; PMCID: PMC285679.
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3. Naso MF, Tomkowicz B, Perry WL 3rd, Strohl WR. Adeno-Associated Virus (AAV) as a Vector for Gene Therapy. BioDrugs. 2017 Aug;31(4):317-334. doi: 10.1007/s40259-017-0234-5. PMID: 28669112; PMCID: PMC5548848.
4. Berry GE, Asokan A. Cellular transduction mechanisms of adeno-associated viral vectors. Curr Opin Virol. 2016 Dec;21:54-60. doi: 10.1016/j.coviro.2016.08.001. Epub 2016 Aug 18. PMID: 27544821; PMCID: PMC5138113

Key Considerations in the Laboratory

Producing AAV Particles in the Laboratory

AAV viral particles are generated by co-transfecting typically a HEK293T cell line with a recombinant AAV vector carrying the gene of interest (GOI), a Rep/Cap plasmid (encoding AAV Rep/Cap genes), and an adenovirus-derived helper plasmid providing genes (E4, E2A, VA) needed for replication (Fig. 3).

Day 1: Plate HEK293T cells
Day 2: Co-transfect rAAV transfer plasmid, helper plasmids and Rep/Cap plasmid
Day 4-7: Harvest and Purify AAV particles

AAV particles can then be stored for later use at either 4°C for short term (<2 weeks), -80°C for long term (1 year) or immediately titered and used to transduce target cell lines.

What is MOI & Why Does it Matter?

MOI stands for Multiplicity Of Infection and is the number of AAV particles added per cell during infection. Determining the appropriate MOI is important because it helps determine the optimal viral dose required for efficient transduction of your target cells, ensuring sufficient gene delivery and transgene expression levels.

The optimal MOI for each cell line varies significantly. AAV MOI ranges from 10,000 to 500,000 depending on serotype and cell type. The appropriate amount of viruses needed for the infection is crucial to the experimental result. Thus, it is strongly recommended to infect your target cells with an AAV control particle of your desired serotype, in your preliminary study to determine the optimal MOI.

AAV Biosafety

Despite being derived from a non-pathogenic, wild-type virus, it is crucial to emphasize the significance of implementing robust biosafety measures when working with AAV. Understanding and adhering to stringent biosafety protocols is imperative to ensure the safe handling, production, and utilization of AAV for gene delivery in research and clinical settings.

OriGene highly recommends that you treat all AAV particles as BSL-2 organisms and strictly follow published BSL-2 guidelines with proper waste decontamination. AAV carrying harmful or toxic genes (e.g. activated oncogenes) require high levels of protection.

For more information on biosafety levels, please read the NIH Biosafety Guidelines.

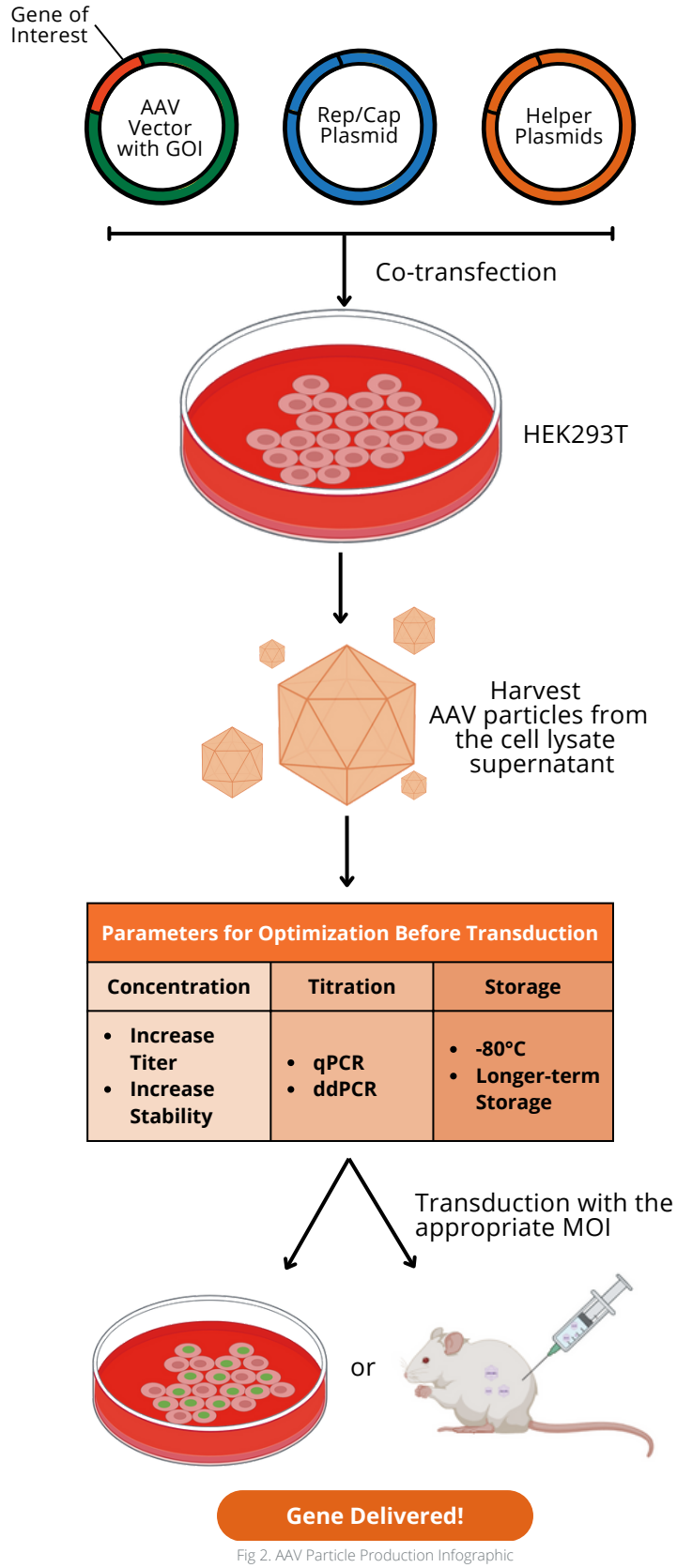


Fig 2. AAV Particle Production Infographic

Serotypes and Tissue Tropism

Compared to other viral gene delivery methods, AAV's serotype-dependent tropism is a unique feature. These serotypes enable AAV to preferentially transduce specific organs or tissues. To date, there have been 12 natural serotypes and more than 100 variants of AAV isolated and studied as gene delivery vehicles[1].

AAV2 is the most commonly used serotype. The natural serotypes include AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10 (AAVrh.10), AAV11, and AAV12.

The table[2] below demonstrates a few AAV serotypes and their various tropisms:

Target Tissue	Adeno-associated virus (AAV) Serotype
CNS	AAV1, AAV2, AAV4, AAV5, AAV7, AAV9, AAV-rh10, AAV11
Liver	AAV2, AAV3, AAV5, AAV7, AAV8, AAV9, AAV-rh10, AAV11
Lung	AAV4, AAV5, AAV6, AAV9, AAV-rh10, AAV12
Muscle	AAV1, AAV2, AAV6, AAV7, AAV8, AAV9, AAV12
Heart	AAV1, AAV4, AAV6, AAV7, AAV8, AAV9
Retina	AAV1, AAV2, AAV4, AAV5, AAV7, AAV8, AAV9, AAV-rh10

Selecting your Serotype

Selecting the appropriate serotype is of the utmost importance since transduction efficiencies can be highly variable. Analyzing the literature is a good way to narrow down to a few targets, followed by testing with a panel of control particles of various serotypes that contain reporter constructs (on a small scale). This allows for visual analysis of transduction efficiencies. Testing kits are commercially available.

References:
1.Li, C., Samulski, R.J. Engineering adeno-associated virus vectors for gene therapy. Nat Rev Genet 21, 255–272 (2020). https://doi.org/10.1038/s41576-019-0205-4
2.Issa, S.S.; Shaimardanova, A.A.; Solovyeva, V.V.; Rizvanov, A.A. Various AAV Serotypes and Their Applications in Gene Therapy: An Overview. Cells 2023, 12, 785. https://doi.org/10.3390/cells12050785

AAV Packaging & Titration

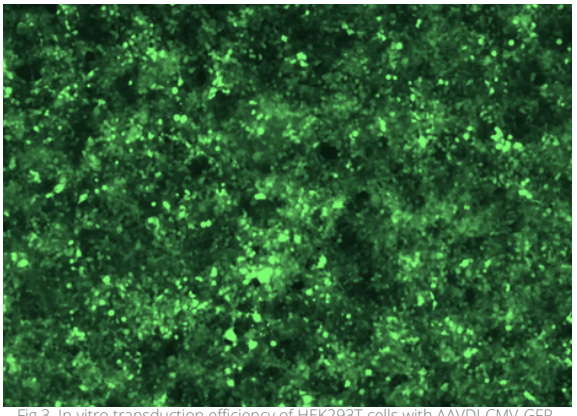


Fig 3. In-vitro transduction efficiency of HEK293T cells with AAVDJ-CMV-GFP (Cat # CV900002S)

What Factors Effect Packaging Efficiency?

While there are many factors that contribute to packaging efficiency of AAV, these are the most common factors:

- **DNA quality:** Potential recombination events can result in lower packaging efficiency and therefore must be tested. Plasmid recombination can be tested by carrying out a diagnostic enzyme digestion and Sanger sequencing.
- **Optimal ratio of plasmids:** Studies report conflicting evidence for the optimal ratio for co-transfecting the required plasmids to produce AAV. OriGene recommends a 1:1:1 ratio[1]. i.e. Rep/Cap plasmid: Helper plasmid: Transfer plasmid
- **Fragment length:** The packaging limit for AAV is around 4.7 kb, larger inserts may lead to lower packaged viral titer.
- **Health of HEK293T cells:** HEK293T cells usually lose packaging efficiency after many passages, therefore, cells should not be used after culturing for 1-2 months. Secondly, cells are happier when seeded the day before transfection, which results in higher transfection efficiency.
- **Transfection Reagent:** Since the Rep/Cap plasmid, Helper plasmid, and Transfer plasmid need to be co-transfected into HEK293T cells to produce viral particles, transfection efficiency is critical for high titer production. A transfection reagent that results in high transfection efficiency in HEK293T is essential for packaging.

> ***Since the packaging limit for AAV is around 4.7 kb, larger inserts may lead to lower packaged viral titer.***

Quantification of AAV Particles

Determining the titer of adeno-associated virus (AAV) is important in the production and characterization of AAV particles. The titer refers to the concentration or number of viral particles per unit volume.

Titering generally comes in two forms: physical or infectious.

Physical titer refers to the concentration of viral particles, also known as virions, in a sample. It quantifies the total number of viral particles, regardless of their infectivity. Physical titer is usually measured by techniques such as electron microscopy, spectrophotometry, quantitative polymerase chain reaction (qPCR), or Droplet Digital PCR (ddPCR).

Although qPCR is currently the most popular method used to quantify AAV, ddPCR can measure DNA copies without having to create a standard curve. The standard procedure for obtaining the physical titer involves lysing the cell and using PCR primers to bind to the ITR regions of the viral genome in the stock[2].

Most commercial suppliers provide qPCR-based AAV titer kits.

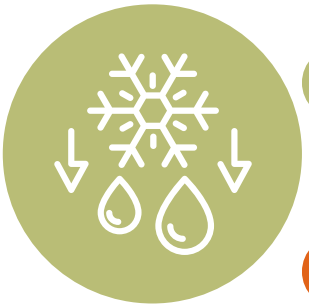
Infectious titer, on the other hand, specifically measures the concentration of infectious viral units in a sample. AAV differs from other viral gene delivery systems, like lentivirus, in that the infectious titer is not reported due to its variability between serotypes and target cell lines.

References :
1. Grieger JC, Soltys SM, Samulski RJ. Production of Recombinant Adeno-associated Virus Vectors Using Suspension HEK293 Cells and Continuous Harvest of Vector From the Culture Media for GMP FIX and FLT1 Clinical Vector. Mol Ther. 2016 Feb;24(2):287-297. doi: 10.1038/mt.2015.187. Epub 2015 Oct 6. PMID: 26437810; PMCID: PMC4817810.
2. Sanmiguel J, Gao G, Vandenberghe LH. Quantitative and Digital Droplet-Based AAV Genome Titration. Methods Mol Biol. 2019;1950:51-83. doi: 10.1007/978-1-4939-9139-6_4. PMID: 30783968; PMCID: PMC7377043.

AAV Particle Storage

What's the Best Way to Store AAV?

In this section, we explore the key factors that influence AAV storage and provide you valuable tips to maintain the integrity and potency of your AAV preparations. Whether you are a seasoned researcher, a clinician working on gene therapy applications, or an industry professional involved in AAV production, these storage tips are essential for maximizing the shelf life and efficacy of your viral stocks.



01

Minimize Free-Thaw Cycles

- Minimize freeze-thaw cycles. While AAV is relatively stable and can tolerate multiple cycles with minimal loss of activity, it is best to avoid unnecessary freeze-thawing.

02

Avoid Drying

- Prevent drying of AAV samples, as it can result in protein denaturation.

03

Prevent Introduction of Air

- Refrain from introducing air into the sample through vortexing, blowing bubbles, or similar operations, as it can lead to protein denaturation.

04

Avoid Exposure to Denaturing Agents

- Avoid exposure to environmental extremes such as pH, chelating agents like EDTA, high temperatures, organic solvents, protein denaturants, and strong detergents as they can denature AAV.

05

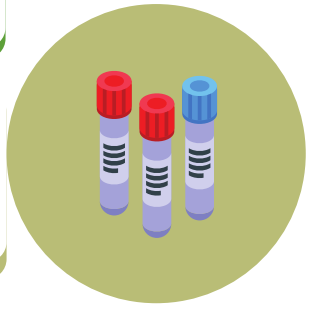
Optimal Long Term Storage

- For long-term storage, aliquot AAV samples and freeze them at -80°C if the virus will not be used within 1-2 weeks. This helps maintain the viability and activity of the AAV over an extended period.

06

Maintain Appropriate Salt Concentrations

- Do not dilute AAV into low salt solutions. Some AAV serotypes, such as AAV-2, may aggregate in low salt environments, resulting in non-infectious aggregates if they are large. Maintain appropriate salt concentrations to prevent aggregation.



07

Use Suitable Containers to Prevent Adhesion

- Minimize AAV's exposure to regular plastics, especially hydrophobic ones such as polystyrene, which can cause loss of activity. Store thawed AAV in siliconized or low protein binding tubes and use matching pipette tips. Add 0.01%-0.1% Pluronic F-68 to the formulation buffer to reduce sticking on regular plastics.

Exploring OriGene's AAV Solutions: Products & Services

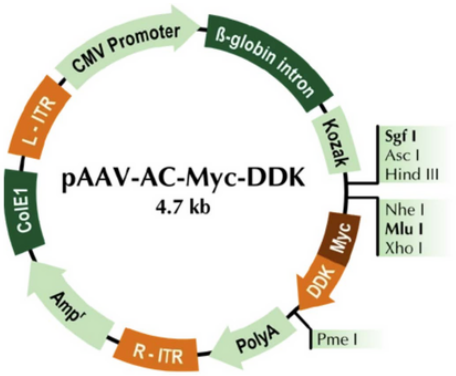
OriGene is committed to supporting and empowering researchers in their pursuit of scientific breakthroughs. Within this section, we showcase OriGene's comprehensive range of AAV solutions tailored to meet the diverse needs of the scientific community. From cutting-edge AAV vectors to personalized production services, OriGene offers an array of resources designed to enhance your research capabilities.

Why Should You Use OriGene's Pre-made AAV-ORF™ Particles?

Our pre-made particles offer an exceptional platform for gene study with a host of advantageous features. With availability for over *30,000 targets*, researchers gain unprecedented access to a diverse array of genes across various species, facilitating comprehensive investigations. Moreover, these particles are packaged with the *AAV-2 serotype*, a highly characterized and widely utilized serotype for gene delivery (options to customize are available). Another key attribute of these particles is their *Myc-DDK tag*, which is small enough to facilitate purification and detection without significantly limiting the gene insertion capacity.

Key Benefits

- **Particles Arrive Transduction-ready:** Save up to 4 weeks of work
- **Ultra-Purified:** Mitigates risk of immune responses due to contaminants
- **High Titers:** >10^{13} GC/mL titer guaranteed for efficient transduction
- **Custom options available:** Flexible titers, serotypes, tags, and more, allowing you cater the AAV particles to your unique experiment



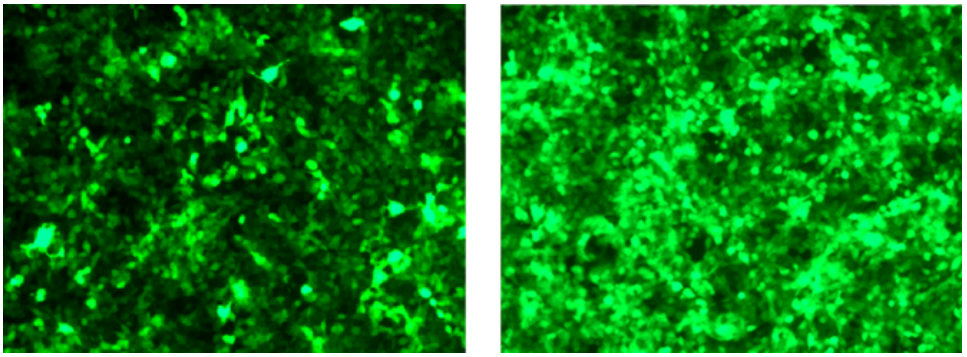
Scan the QR code to browse our collection pre-made AAV particles

Your Research, Your Rules: Custom AAV Packaging Service

Producing high quality, high titer AAV can be difficult and time consuming. Our highly experienced AAV team is ready to tackle complex cloning & packaging projects to save you time and effort (Fig. 4).

The OriGene Advantage

- Wide Serotype Selection: AAV1, AAV2, AAV3, AAV5, AAV6, AAV7, AAV8, AAV9, AAV-DJ, AAV-PHP.eb, AAVrh.10
- High Titers: Guaranteed titers > 10^{13} GC/mL
- Flexibility Tagging Options: Myc-DDK, GFP, & RFP
- Fast Turnaround: 2-3 weeks



AAV 2 **AAV DJ**

Fig 4. Internal QC data of custom AAV2 & AAV-DJ particles using a GFP reporter.

AAV Vectors

Name	Application	Volume	Catalog #
pAAV-AC-Myc-DDK Gene Expression Vector	Over Expression	10ug	PS100089
pAAV-AC-GFP Gene Expression Vector	Over Expression	10ug	PS100090
pAAV-AV-RFP Gene Expression Vector	Over Expression	10ug	PS100091
pAAV-EF1a-tGFP-WPRE Gene Expression Vecor	Over Expression	10ug	PS100127
pGFP-A-shAAV Gene Expression Vector	Knockdown	5ug	TR30034

AAV Control Particles

Serotype	Reporter	Volume	Catalog #
AAV-1	GFP	50ul/100ul	CV900009S
AAV-2	GFP	50ul/100ul	CV900001S
AAV-3	GFP	50ul/100ul	CV900005S
AAV-5	GFP	50ul/100ul	CV900011S
AAV-6	GFP	50ul/100ul	CV900006S
AAV-7	GFP	50ul/100ul	CV900008S
AAV-8	GFP	50ul/100ul	CV900004S
AAV-9	GFP	50ul/100ul	CV900007S
AAV-rh.10	GFP	50ul/100ul	CV900010S
AAV-DJ	GFP	50ul/100ul	CV900002S
AAV-DJ	P53-GFP	50ul/100ul	CV900003S
AAV-PHP.eb	GFP	50ul/100ul	CV900012S

Find out more at www.origene.com/products/cdna-clones/aav-products

OriGene Technologies Inc.
9620 Medical Center Drive, Suite #200
Rockville, MD 20850
USA

Phone: 1-301-340-3188
Fax: 301-340-9254
E-mail: productorders@origene.com
Website: www.origene.com

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