A streamlined workflow for adeno-associated virus isolation, purification, and titration

Adeno-associated virus (AAV) is a nonenveloped, single-stranded DNA virus that can infect both nondividing and dividing cells. AAV is thought to be nonpathogenic to humans and only replicates in the presence of a helper virus. These features have made AAV a useful tool for gene delivery to a wide variety of cell types and an attractive vector for gene therapy.

Features of recombinant AAV vectors:

- Simple to produce at high titer (helper-free systems)
- Recombinant AAV does not integrate into host genome
- Can be used to transduce both proliferating and non-proliferating cells
- Can impart long-term expression in non-dividing cells
- Do not elicit significant immune responses *in vivo*

AAV workflow

AAVpro workflow overview

The AAVpro series is a suite of products for generating high titers of transduction-ready recombinant AAV particles from preparation and extraction through purification and titration. The AAV2 particles obtained using the AAVpro system can be used for transduction of mammalian cells or individual animals (*in vivo* transduction).

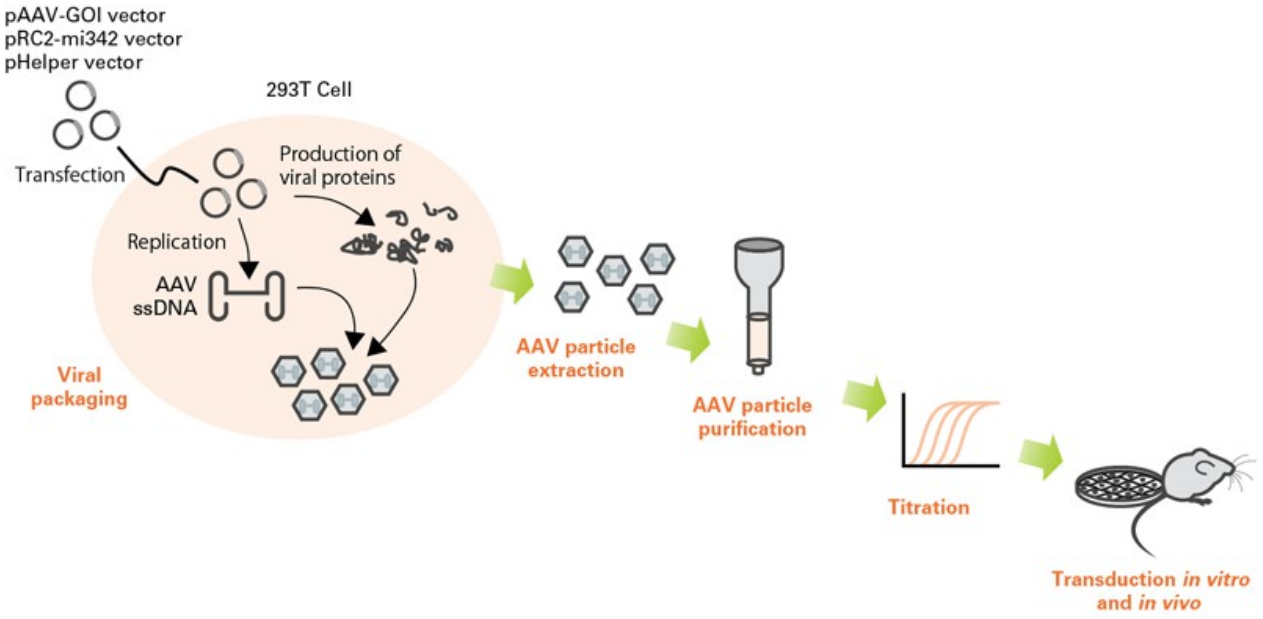

Figure 1. Workflow for AAV particle production.

1 High-titer AAV preparation without a helper virus

The AAVpro Helper Free System (AAV2) is a unique system for the preparation of high-titer AAV2 particles without the use of a helper virus.

- Cotransfect HEK 293T cells with the three plasmids encoding all of the factors necessary to generate recombinant AAV particles.

- Produce AAV particles at very high-titer expression of human microRNA-342 in this system increases titer (Figure 2).

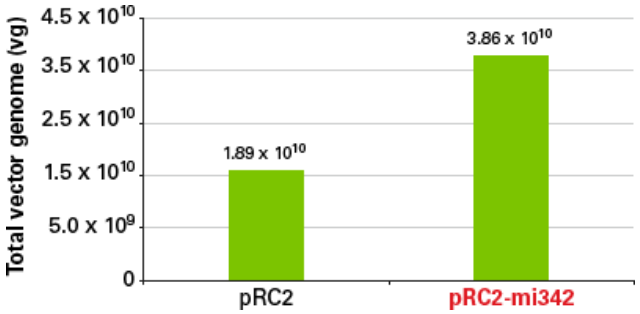

Figure 2. Effect of human miRNA-342 expression on AAV2 titer. AAV2 particles were extracted from virus-producing HEK 293T cells. Viral titer was evaluated using real-time PCR (total vector genome; vg). Expression of miR-342 in this system resulted in a two-fold increase in titer as compared to cells that did not express the microRNA.

2 Easy and efficient AAV particle extraction

Isolation of AAV particles from AAV particle-producing cells is conventionally performed using freeze-thaw or sonication methods. However, these methods are time-consuming and/or require special equipment. The AAVpro Extraction Solution provides a simple and efficient method for AAV particle isolation from AAV particle-producing cells.

- Viral particle recovery increases by at least 3-fold as compared to conventional freeze-thaw methods (Figure 3).
- Resulting viral particle solution contains low amounts of host DNA and protein contamination.
- Extracted AAV particles can be used for cell infection or further purification*.

*For *in vivo* transduction, use the AAVpro Purification Kit.

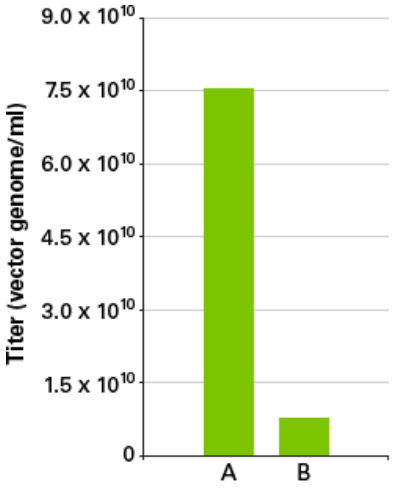

Figure 3. AAV particle extraction. AAV2 particles were extracted with AAVpro Extraction Solution (A) or the freeze-thaw method (B) from virus-producing cells. The titer of the extracted AAV vector prep was evaluated by quantitative PCR.

3 Highly pure AAV particles without ultracentrifugation

The purity of AAV particles is important for achieving high transduction efficiency into individual animals and cultured cells. Ultracentrifugation protocols are commonly used to purify AAV particles, but such methods are time-consuming and require careful technique to obtain high yields. The AAVpro Purification Kit (AAV2) allows simple and fast (~4 hours) AAV2 particle purification from virus-producing cells.

- High purity and high yield of AAV particles using column purification (Figure 4)

- Watch the video protocol »

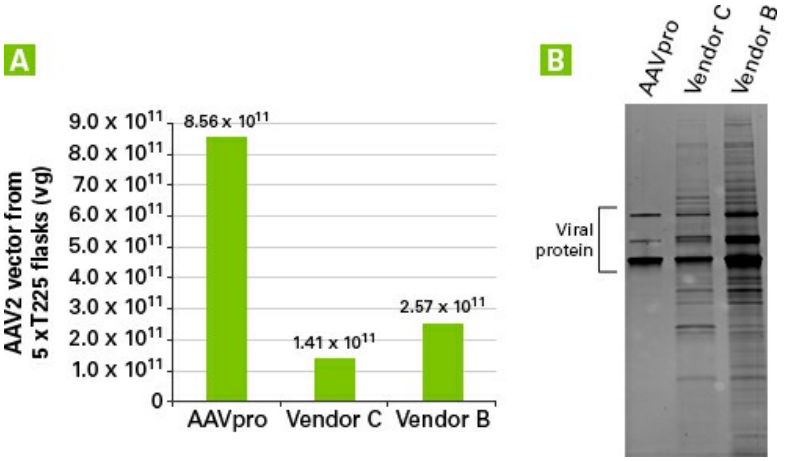

Figure 4. Recovery (Panel A) and purity (Panel B) of purified AAV2 particles. AAV2 particles were purified with kits from two different vendors (Vendor B and Vendor C) purification kit from virus-producing cells. The yield of each purified AAV2 vector was evaluated by quantitative PCR (**Panel A**), and the purity of each purified AAV2 vector was evaluated by SDS-PAGE using 1 x 10^9 vg per lane (**Panel B**).

4 Quick determination of AAV titer by qPCR

The AAVpro Titration Kit (for Real Time PCR) Ver.2 contains all of the reagents necessary to determine the titer of AAV preps using qPCR. AAV particles are extracted from virus-producing cells, and qPCR is used to quantify a viral genomic sequence, resulting in a titer in under 2.5 hours.

- Can be used for any AAV serotype—quantification is based on amplification of the ITR (inverted terminal repeat) of AAV2, a region common to most AAV vectors (Figure 5)
- More precise quantification than conventional DNA blot or ELISA methods

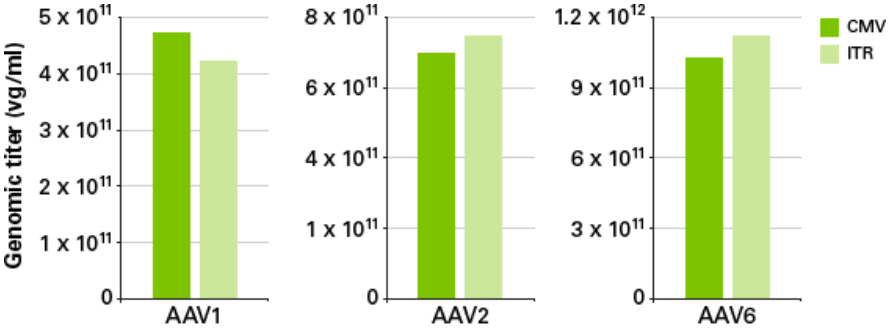

Figure 5. Titer measurement of AAV by qPCR using the ITR and CMV promoter targets. The viral genomic titers of extracted AAV1, AAV2, and AAV6 particles were evaluated using two primer sets (CMV region and ITR region). In all serotypes, there was no significant difference in viral genomic titers.

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