Serotype-independent, simple, and efficient purification method for Adeno-Associated Virus (AAV)

- **Simple workflow:** optimized protocol that eliminates the need for freeze-thaw cycles and ultracentrifugation
- **High purity for reliable data:** purified AAV particles are suitable for *in vivo* research
- **Serotype agnostic:** precipitation and filtration protocol that can purify AAV particles from any serotype

Introduction

Adeno-associated virus (AAV) vectors have proven to be highly effective gene delivery tools for therapeutic research thanks to a variety of features, including minimal pathogenicity, sustained viral persistence, and the ability to target specific cells and tissue types with a variety of recombinant AAV serotypes (Büning 2019). In order to make good on this far-reaching potential, AAV vectors must be generated with high purity. However, many currently available schemas for their generation are serotype dependent, requiring separate method development and optimization for each vector. Our AAVpro "All Serotypes" kits solve this issue through the use of a simple precipitation and filtration protocol (Figure 1) that can purify AAV particles with any capsid (i.e., from any serotype).

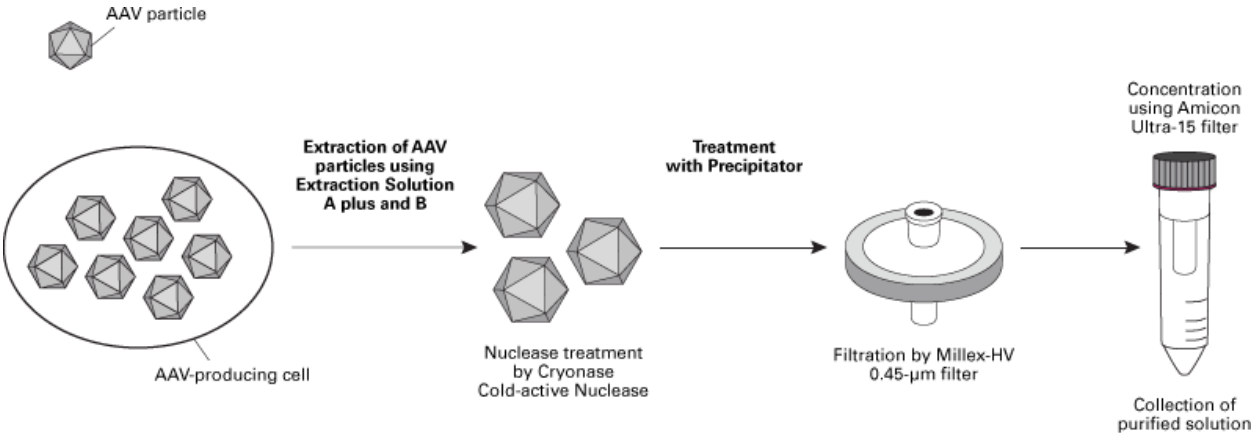

Figure 1. Simple and efficient workflow for AAV particle purification using the AAVpro Purification Kit Maxi (All Serotypes). The purification protocol of AAVpro Purification Kit Midi (All Serotypes) is the same as the workflow shown above, except an Amicon Ultra-4 filter is used in place of the Millex-HV 0.45-µm filter. Both midi and maxi kits can purify AAV particles from all serotypes. The maxi kit can be used to purify four virus preps, each from a culture size of 5 x T225 flasks. Alternatively, the midi version of the kit provides four preps from one T225 flask (or four 10-cm dishes).

Results

High purity for each AAV serotype

In order to test the purity of the system, AAV viral particles of each serotype (AAV1, AAV2, AAV3, AAV5, and AAV6) carrying the fluorescent protein ZsGreen1 were purified from producer cells using the AAVpro Purification Maxi Kit (All Serotypes). Viral titer was measured using real-time PCR analysis of the viral genomic sequence. This was followed by SDS-PAGE for each sample, and all serotypes displayed the AAV capsid proteins (VP1, VP2, and VP3) as the major bands present, which confirmed the purity of the AAV particles.

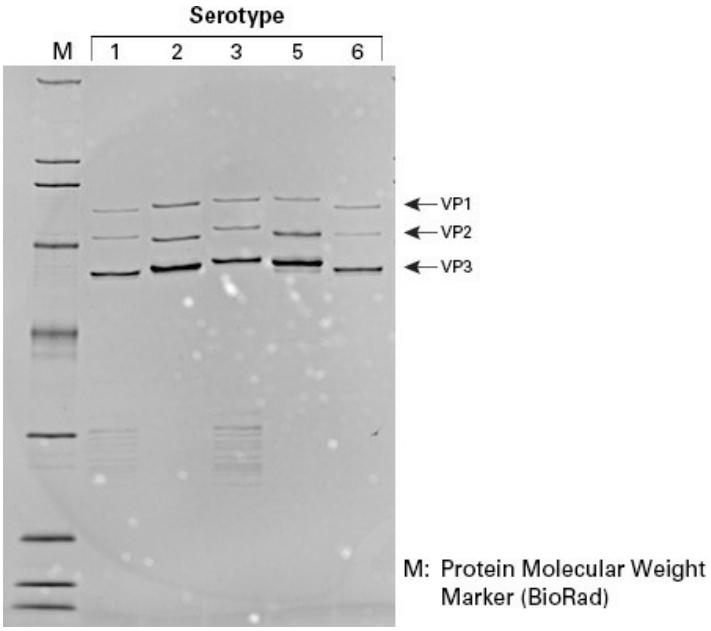

Figure 2. Purity of viral particles collected using the AAVpro Purification Kit Maxi (All Serotypes). Viral particles from serotypes AAV1, AAV2, AAV3, AAV5, and AAV6, each carrying the fluorescent protein ZsGreen1, were purified from producer cells cultured in five T225 flasks according to the the kit's user manual. Viral titer was measured using the AAVpro Titration Kit (for Real Time PCR) Ver.2 (Cat. # 6233). SDS-PAGE was performed on each sample, with 1 x 10^9 vector genomes loaded per lane.

Infectivity of purified AAV particles

Each of the AAV samples purified above were then used to infect three different cell lines: CHO, RD, and HT1080. Three days later, flow cytometry was performed to assess infectivity. The resulting data showed that the AAV particles purified using this kit were able to infect the cell lines tested.

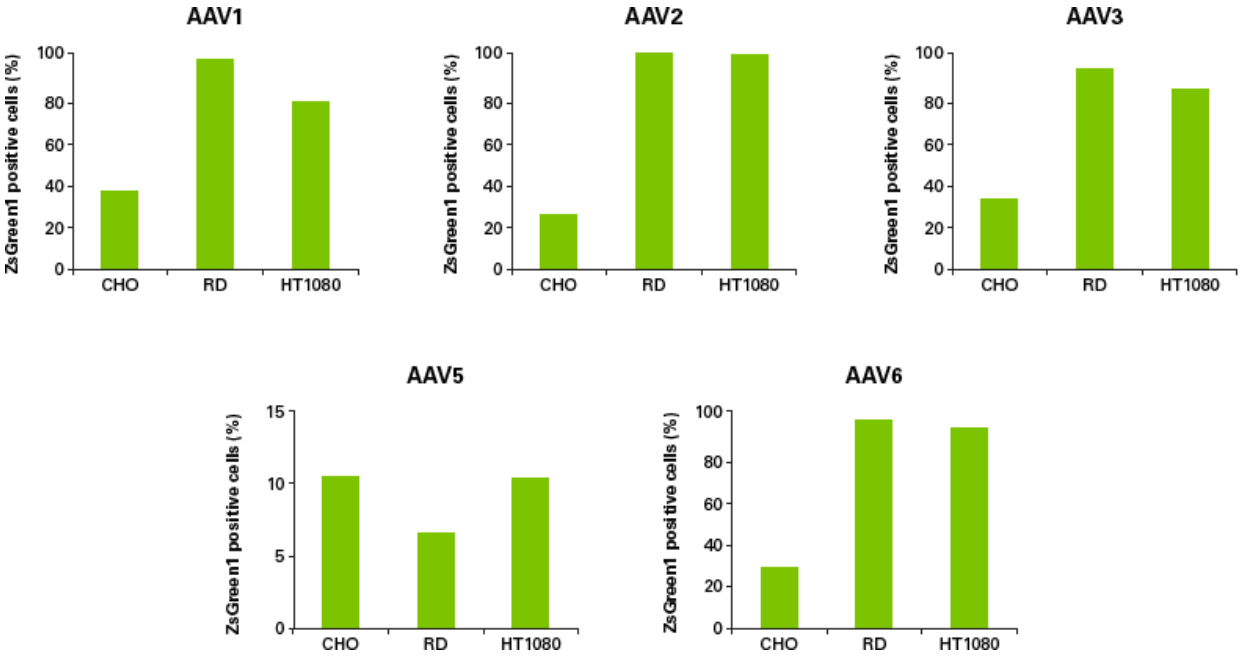

Figure 3. Infectivity of AAV particles purified with the AAVpro Purification Kit Maxi (All Serotypes). Purified AAV particles carrying the fluorescent protein ZsGreen1 were used for infection of three different cell lines (CHO, RD, and HT1080) at 5,000 vector genomes per cell (serotypes 1, 2, 3, and 6) or 50,000 vector genomes per cell (serotype 5). Flow cytometry was performed three days later.

Infection of iPSC-derived cardiomyocytes

We further tested AAV viral particles purified with this kit by looking at their applications with iPSC-derived cardiomyocytes. Once again, viral particles carrying the fluorescent protein ZsGreen1 (serotypes 1, 2, 5, and 6) were purified from producer cells using the AAVpro Purification Kit Midi (All Serotypes), and titer of each purified vector was measured as described above. iPSC-derived cardiomyocytes were infected with either purified AAV1, 2, 5, or 6 vectors. The resulting ZsGreen1 fluorescence showed that the purified particles were indeed effective in this application, although AAV6, 2, and 1 appear more suitable for transducing iPSC-derived cardiomyocytes than AAV5.

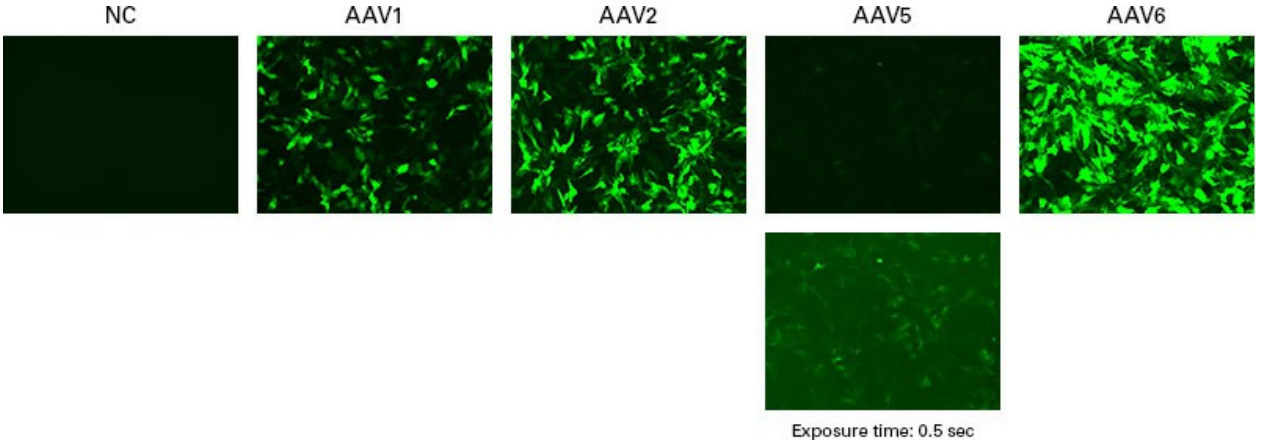

Figure 4. Gene transduction of iPSC-derived cardiomyocytes. AAV1, 2, 5, and 6 vectors carrying the *ZsGreen1* gene were purified from producer cells cultured in five T225 flasks with the AAVpro Purification Kit Midi (All Serotypes) kit. Each purified vector was used for viral transduction of iPSC-derived cardiomyocytes at 500,000 vector genomes per cell. After 72 hr of infection, ZsGreen1 fluorescence was analyzed by microscopy, with an exposure time of 0.2 sec. AAV5 was additionally exposed at 0.5 sec. NC = negative control.

Conclusion

The AAVpro Purification Kit (All Serotypes) can be used reliably for any AAV serotype with high purity and recovery in just four hours. The optimized protocol eliminates the need for freeze-thaw steps and ultracentrifugation, which are otherwise needed for extraction from cells and purification, respectively. We recommend browsing a collection of recent publications (listed below) to supplement your knowledge base for use of these "All Serotypes" kits for various *in vivo* research studies, including as direct injection into mouse brain.

Methods

Purity and infectivity of purified AAV particles

Viral particles from serotypes AAV1, AAV2, AAV3, AAV5, and AAV6, each carrying the fluorescent protein ZsGreen1, were purified from producer cells cultured in five T225 flasks according to the the AAVpro Purification Kit Maxi (All Serotypes) User Manual. Viral titer was measured using the AAVpro Titration Kit (for Real Time PCR) Ver.2 (Cat. # 6233). SDS-PAGE was performed on each sample, with 1 x 10^9 vector genomes loaded per lane.

The purified AAV particles carrying the fluorescent protein ZsGreen1 (above) were used to infect three different cell lines (CHO, RD, and HT1080) at 5,000 vector genomes per cell (serotypes 1, 2, 3, and 6) or 50,000 vector genomes per cell (serotype 5). Flow cytometry was performed three days later.

Infection of iPSC-derived cardiomyocytes

AAV1, 2, 5, and 6 vectors carrying the *ZsGreen1* gene were purified from producer cells cultured in five T225 flasks with the AAVpro Purification Kit Midi (All Serotypes) kit, according to the user manual. Each purified vector was used for viral transduction of iPSC-derived cardiomyocytes at 500,000 vector genomes per cell. After 72 hr of infection, ZsGreen1 fluorescence was analyzed by microscopy, with an exposure time of 0.2 sec. AAV5 was additionally exposed at 0.5 sec. NC = negative control.

References & citations

References

Büning, H. and Srivastava, A. Capsid Modifications for Targeting and Improving the Efficacy of AAV Vectors. *Mol. Ther. Methods Clin. Dev.*

12, 248–265 (2019).

Selected product citations

Correa-Gallegos, D., *et al.* Patch repair of deep wounds by mobilized fascia. *Nature* **576,** 287–292 (2019).

Hashimoto, M. CREG1 stimulates brown adipocyte formation and ameliorates diet-induced obesity in mice. *FASEB J*. **33,** 8,069–8,082 (2019).

Hoshino, Y. The adeno-associated virus rh10 vector is an effective gene transfer system for chronic spinal cord injury. *Sci. Rep.* **9,** 9,844 (2019).

Li, H. Gene suppressing therapy for Pelizaeus-Merzbacher disease using artificial microRNA. *JCI Insight.* **4,** 125052 (2019).

Miyawaki, T., *et al.* Visualization and molecular characterization of whole-brain vascular networks with capillary resolution. *Nat. Commun.* **11,** 1,104 (2020).

Tomono, T., *et al.,* Infectivity assessment of recombinant adeno-associated virus and wild-type adeno-associated virus exposed to various diluents and environmental conditions. *Hum. Gene Ther. Methods.* **30,** 137–143 (2019).

Zhong, G., *et al.* A reversible RNA on-switch that controls gene expression of AAV-delivered therapeutics *in vivo*. *Nat. Biotechnol.* **38,** 169–175 (2020).

Related Products

Cat. #	Product	Size	License	Quantity	Details
6666	AAVpro® Purification Kit Maxi (All Serotypes)	4 Preps		*	

The AAVpro Purification Kit Maxi (All Serotypes) is a four-prep kit that can be used for purifying AAV of any serotype without the need for ultracentrifugation. The purification yields highly pure AAV particles that can be used directly for *in vivo* transduction or transduction of cultured cells. Cryonase Cold-Active Nuclease is included. This product allows for purification of four virus preps, each from a culture size of 5 x T225 flasks.

Documents | Components | You May Also Like | Image Data

6675	AAVpro® Purification Kit Midi (All Serotypes)	4 Preps		*	

Add to Cart

Takara Bio USA, Inc.
United States/Canada: +1.800.662.2566 • Asia Pacific: +1.650.919.7300 • Europe: +33.(0)1.3904.6880 • Japan: +81.(0)77.565.6999