Cat. # 6675
6675S

For Research Use

TaKaRa

AAVpro® Purification Kit Midi (All Serotypes)

Product Manual

v202001Da

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Safety & Handling of Adeno-Associated Virus Vectors

The protocols in this User Manual require the handling of adeno-associated virus vectors. It is imperative to fully understand the potential hazards of and necessary precautions for laboratory use of these vectors.

Viruses produced with AAV-based vectors could, depending on your gene insert, be potentially hazardous. Similar vectors have been approved for human gene therapy trials, attesting to their potential ability to express genes *in vivo*. For these reasons, due caution must be exercised in the production and handling of any recombinant viruses.

Follow all applicable guidelines for research involving recombinant DNA. Take appropriate safety measures when producing or handling recombinant adeno-associated viruses, including working in a biological safety cabinet and wearing protective laboratory coats, face protection, and gloves.

I. Introduction

Adeno-Associated Virus (AAV) is a non-enveloped virus that belongs to the *Parvovirus* family of the *Dependovirus* genus. There are more than 100 serotypes of AAV, and the host specificity and characteristics of the virus differ among serotypes. AAV is not thought to be pathogenic to humans and only replicates in the presence of a helper virus, such as adenovirus or herpesvirus. The AAV genome is a linear, single-strand DNA molecule of approximately 4.7 kb.

Adeno-associated virus vectors (AAV vectors) exploit the properties of AAV for transduction of genes to cells and organisms. AAV vectors are used as research tools and also as vectors for gene therapy. In addition, AAV vectors are generally considered safer than adenoviral and retroviral vectors. AAV vectors can be used to transduce genes into both proliferating and non-proliferating cells and can impart long-term expression in non-dividing cells. In addition, AAV has little immunogenicity and is suitable for the transduction of genes into animals (as an *in vivo* transduction tool).

II. Description

The purity and titer of AAV particles are important factors for obtaining efficient and stable gene transfer in cultured cells and individual animals. Cesium chloride density gradient centrifugation and iodixanol ultracentrifugation are conventional methods used to purify AAV particles. However, these methods are time consuming, and require specialized equipment and advanced techniques.

The AAVpro Purification Kit Midi (All Serotypes) can be used to easily purify AAV particles from AAV-producing cells in about 4 hours:

Note: This kit is for purifying AAV vector from producing cells in single T225 flask. The other kit, AAVpro Purification Kit Maxi (All Serotypes)（Cat. #6666）can be used to purify AAV vector from five T225 flasks. Both kits, Cat. #6675 and #6666, contain the components for 4 purifications.

- Can be used for any AAV serotype
- High purity and recovery
- No complicated techniques, such as ultracentrifugation
- Contains all buffers required to purify AAV particles from producer cells

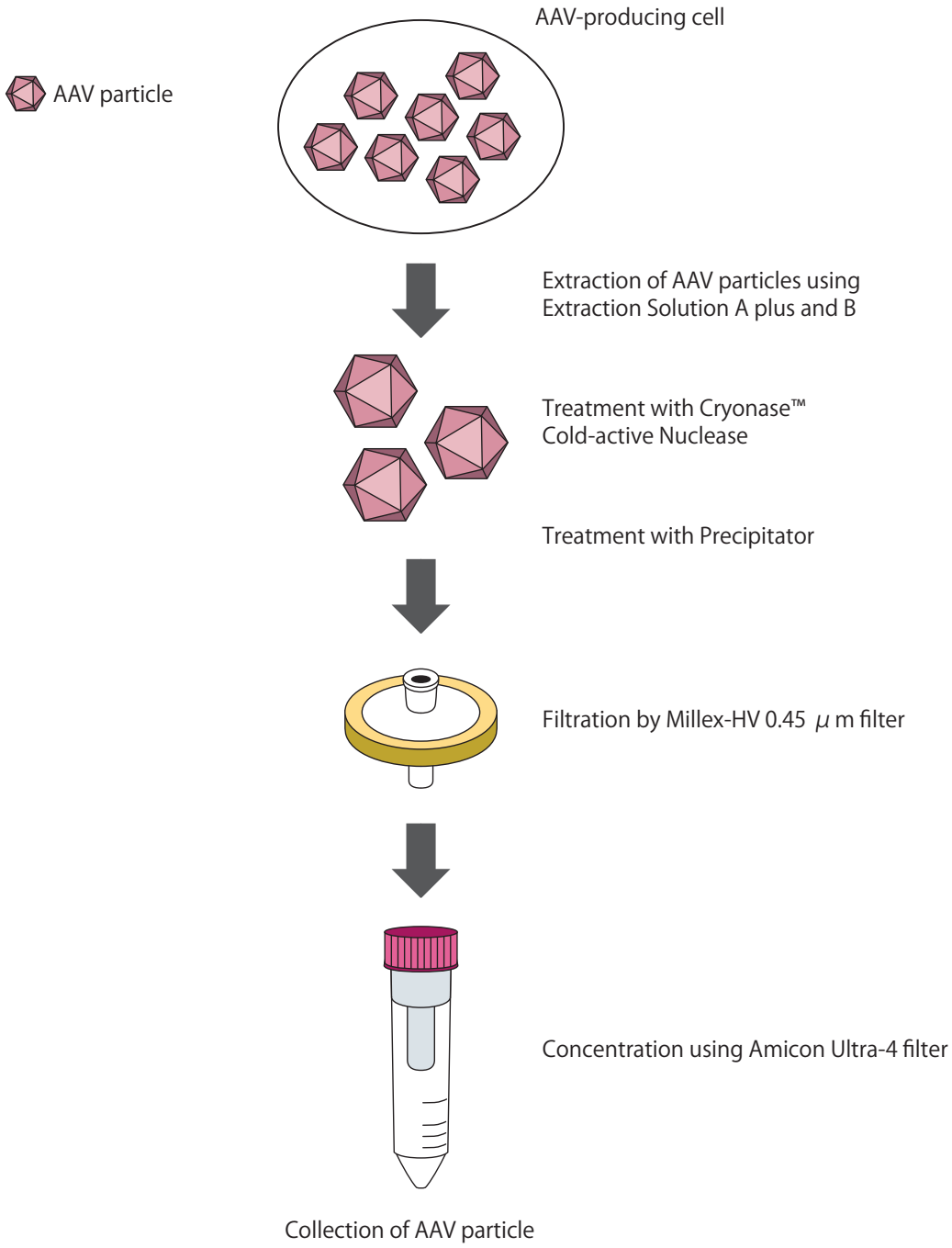


Figure 1. Purification of AAV particles using AAVpro Purification Kit Midi (All Serotypes)

III. Components

This product contains buffers and columns to perform four AAV purifications from packaging cells in one T225 flask.

	Cat. # 6675	6675S
1. AAV Extraction Solution A plus	4.4 ml x 2	2.2 ml x 2
2. AAV Extraction Solution B	0.9 ml	0.45 ml
3. Cryonase™ Cold-active Nuclease	100 µl	50 µl
4. Precipitator A*	1 ml	0.5 ml
5. Precipitator B	0.5 ml	0.25 ml
6. Millex-HV 0.45 µm	4	2
7. Amicon Ultra-4, 100 kDa	4	2
8. Suspension Buffer	12 ml x 2	6 ml x 2

* Precipitator A may produce a white precipitate at low temperatures; however this does not affect the quality or performance of this reagent. If a precipitate is present, dissolve completely at 37℃ before use.

IV. Storage

Cryonase Cold-active Nuclease:	-20℃
All other components:	Room temperature

V. Material Required but not Provided

- General equipment required for cell culture
- Syringe
- 0.5 M EDTA (pH 8.0) [e.g., EDTA Buffer Powder, pH 8.0 (Cat. #T9191)]

VI. Protocol

The protocol below describes purification of AAV particles from producer cells in one T225 flask or four 10 cm dishes.
We recommend using the AAVpro Helper Free Systems (Cat. #6230, 6650-6663, 6668-6671, 6673*) for the preparation of AAV-producing cells.

*Not all products are available in all regions. Please confirm availability in your area.

VI-1. Preparation of AAV extract solution

1. Detach the AAV-producing cells by adding 1/80 volume of 0.5 M EDTA (pH 8.0) to the culture medium; incubate at room temperature for 10 min.
2. Collect the cells in a centrifuge tube from the flask.
3. Centrifuge at 1,700 - 2,000*g* for 10 min at 4℃ and discard the supernatant.
4. Centrifuge again at 1,700 - 2,000*g* for 1 min at 4℃ and remove the supernatant completely.
   Note: Be sure to completely remove the supernatant, because remaining supernatant could impair virus purification.
5. Loosen the cell pellet well by tapping or vortexing.
   Note: If the cell pellet is not loosened sufficiently, the purification efficiency can be decreased. Make sure there are no cell clumps before proceeding to the next step.
6. Add 2 ml of AAV Extraction Solution A plus.
7. Resuspend by vortexing for 15 sec.
   Note: Continue to vortex until there are no remaining cell clumps.
8. Incubate for 5 min at room temperature and then vortex again for 15 sec.
9. Centrifuge at 4,000 - 9,000*g* for 10 min at 4℃
   Note: In some cases, the virus recovery can be improved by repeating steps 7 - 9.
10. Transfer the supernatant in a new sterile centrifuge tube using a pipet to avoid contamination. Add 1/10 volume of AAV Extraction Solution B to the supernatant.
    Note 1: The virus suspension can be stored at -80℃. Alternatively, promptly proceed to step VI-2-1. If the virus suspension is stored at -80℃, thaw in a 37℃ incubator before using. Be sure to use a tube that is resistant to freezing and centrifugation when a virus suspension is stored at -80℃.
    Note 2: When AAV Extraction Solution B is added, the color of the solution may turn pink in some cases; this does not affect performance.

VI-2. Purification and concentration of AAV particles

Note: Use swing bucket rotors for steps VI-2-5, VI-2-6, and VI-2-7.

1. Add 1/100 volume of Cryonase Cold-active Nuclease to the virus suspension at step VI-1-10, and then incubate at 37℃ for 1 hr.
2. Add 1/10 volume of Precipitator A, vortex for 10 sec, incubate at 37℃ for 30 min, and vortex again for 10 sec.

   Note 1: Precipitator A may produce a white precipitate at low temperature, however this does not affect the quality or performance of this reagent. If a precipitate is present, dissolve it completely at 37℃ before use.

   Note 2: Although a precipitate may form during the incubation, this is not a problem. Proceed to the next step.
3. Add 1/20 volume of Precipitator B to the mixture at the step above, vortex quickly for 10 sec, and then centrifuge at 5,000 - 9,000*g* for 5 min at 4℃.

   Note: A precipitate may be formed after adding Precipitator B, but proceed to centrifugation.
4. Filter the supernatant using Millex-HV 0.45 μm.
5. Transfer the filtrate containing AAV vector into a filter device of Amicon Ultra-4, 100 kDa. Centrifuge at 2,000*g* for 5 min at 15℃, and then confirm that the AAV solution in the filter device is <0.4 ml.

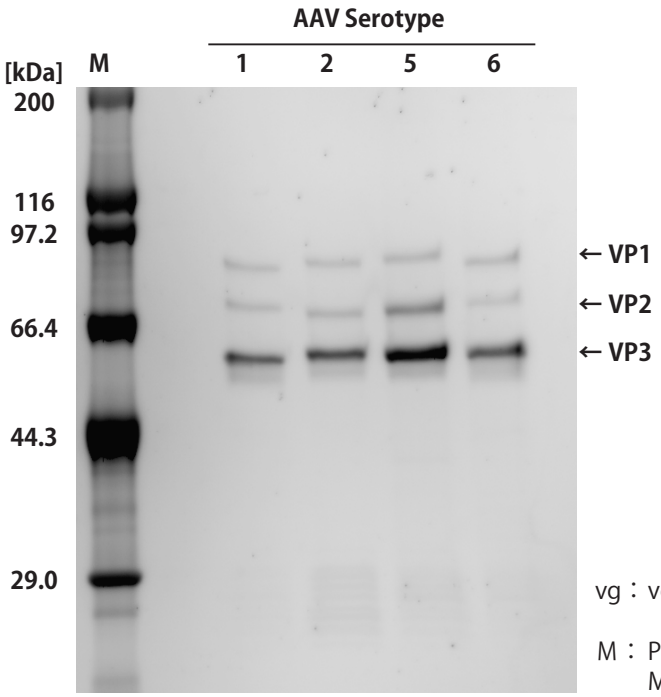
   Note: If the volume of the solution is >0.4 ml, continue to centrifuge.
6. After removing the filtrate, add 1 ml of Suspension Buffer in the filter device of the Amicon Ultra-4 and mix the solution uniformly by pipetting. Centrifuge at 2,000*g* for 5 min at 15℃, and then confirm that the AAV solution inside the filter device device is <0.4 ml.

   Note: If the volume of the solution is >0.4 ml, continue to centrifuge.
7. Repeat step VI-2-6 4 times (total 5 times) to obtain an appropriate volume of solution.
8. After discarding the filtrate, resuspend the solution inside of the cup of the Amicon Ultra-4, 100 kDa filter device by pipetting or vortexing for 30 sec and transfer the virus suspension to a new tube.

VII. Experimental Examples

VII-1. Purity of each AAV serotype after purification

AAV particles carrying the fluorescent protein ZsGreen1 were prepared from producer cells in a T225 flask. The AAV solution was purified using this product, and then virus titer was measured using the AAVpro Titration Kit (for Real Time PCR) Ver.2 (Cat. #6233). SDS-PAGE was performed using 1 x 10^9 vector genome (vg) per lane. The AAV capsid proteins (VP1, VP2, and VP3) were confirmed to be the major bands present.

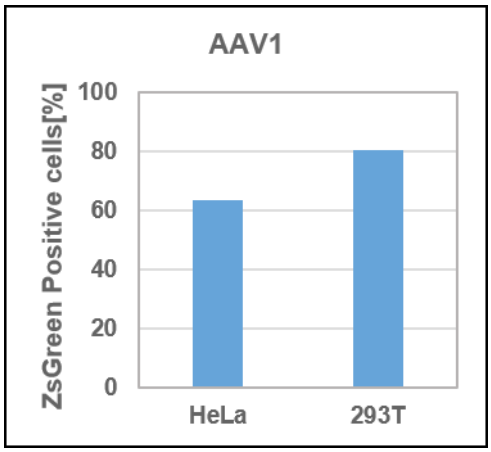


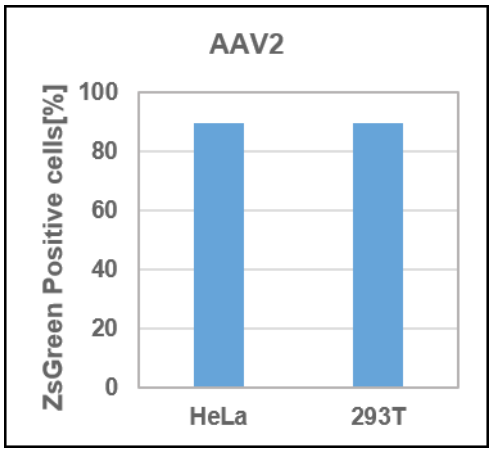
vg：vector genome

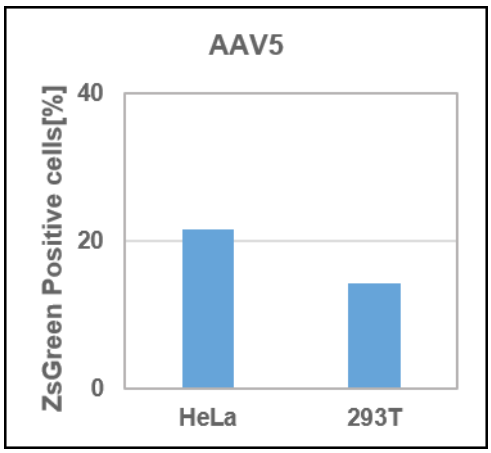
M：Protein Molecular Weight Marker (Broad)(Cat. #3452)

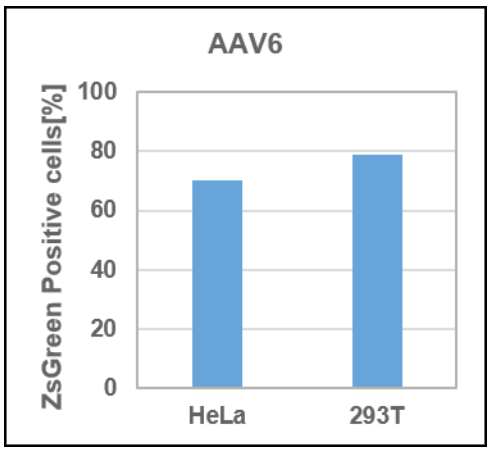
VII-2. Infectivity of purified AAV particles

The infectivity of the AAV particles obtained above was evaluated. Cells were infected with purified AAV vectors at 5,000 vg/cell (serotypes 1, 2, and 6) or 50,000 vg/cell (serotype 5). Flow cytometry analysis was performed 3 days later to determine infection efficiency. The AAV particles purified using this kit were able to infect cultured cell lines.









VIII. Related Products

AAVpro® Helper Free System (AAV1) (Cat. #6673)
AAVpro® Helper Free System (AAV2) (Cat. #6230)
AAVpro® Helper Free System (AAV5) (Cat. #6650)
AAVpro® Helper Free System (AAV6) (Cat. #6651)
AAVpro® Helper Free System (AAV1-CRE Recombinase) (Cat. #6668)
AAVpro® Helper Free System (AAV2-CRE Recombinase) (Cat. #6652)
AAVpro® Helper Free System (AAV5-CRE Recombinase) (Cat. #6653)
AAVpro® Helper Free System (AAV6-CRE Recombinase) (Cat. #6654)
AAVpro® Helper Free System (AAV1-LacZ) (Cat. #6669)
AAVpro® Helper Free System (AAV2-LacZ) (Cat. #6655)
AAVpro® Helper Free System (AAV5-LacZ) (Cat. #6656)
AAVpro® Helper Free System (AAV6-LacZ) (Cat. #6657)
AAVpro® Helper Free System (AAV1-U6-ZsGreen1) (Cat. #6670)
AAVpro® Helper Free System (AAV2-U6-ZsGreen1) (Cat. #6658)
AAVpro® Helper Free System (AAV5-U6-ZsGreen1) (Cat. #6659)
AAVpro® Helper Free System (AAV6-U6-ZsGreen1) (Cat. #6660)
AAVpro® Helper Free System (AAV1-2xU6) (Cat. #6671)
AAVpro® Helper Free System (AAV2-2xU6) (Cat. #6661)
AAVpro® Helper Free System (AAV5-2xU6) (Cat. #6662)
AAVpro® Helper Free System (AAV6-2xU6) (Cat. #6663)
AAVpro® Packaging Plasmid (AAV1) (Cat. #6672)
AAVpro® Packaging Plasmid (AAV2) (Cat. #6234)
AAVpro® Packaging Plasmid (AAV5) (Cat. #6664)
AAVpro® Packaging Plasmid (AAV6) (Cat. #6665)
pAAV-ZsGreen1 Vector (Cat. #6231)
AAVpro® Purification Kit Maxi (All Serotypes) (Cat. #6666)
AAVpro® Titration Kit (for Real Time PCR) Ver.2 (Cat. #6233)
AAVpro® Concentrator (Cat. #6674)
AAVpro® 293T Cell Line (Cat. #632273)
EDTA Buffer Powder, pH 8.0 (Cat. #T9191)