

Cat. # 6679

For Research Use

TAKARA

**AAVpro[®] Freeze-Thaw Extraction Buffer
(All Serotypes)**

Product Manual

v202108Da

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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

AAVpro Freeze-Thaw Extraction Buffer (All Serotypes) is a cell suspension solution for extracting AAV particles from AAV-producing cells (living and frozen cells) by the freeze-and-thaw method.

Suspending AAV-producing cells with this product makes it possible to extract AAV particles of various serotypes efficiently with a single freeze-and-thaw procedure. In combination with AAVpro Purification Pack Maxi (All Serotypes) (Cat. #6678), AAV particles from AAV-producing cells can be purified and concentrated to obtain highly-purified AAV particles in about 4 hours without complicated steps such as ultracentrifugation.

III. Component

AAVpro Freeze-Thaw Extraction Buffer (All Serotypes) 20 ml x 2

IV. Storage -20°C

V. Materials Required but not Provided

[Reagents]

- AAVpro Purification Pack Maxi (All Serotypes) (Cat. #6678)
- 0.5 M EDTA (pH 8.0) (e.g. EDTA Buffer Powder, pH8.0 (Cat. #T9191))

[Equipment]

- General equipment necessary for cell culture
- Sterile centrifuge tubes

VI. Protocol

The following protocol describes the purification of AAV particles from producer cells in five T225 flasks. AAVpro Helper Free System (Cat. #6230, 6650 - 6663, 6668 - 6671, 6673) is recommended for preparing AAV-producing cells.

[Preparation of AAV particle extract]

1. Add 1/80 volume of 0.5 M EDTA (pH 8.0) to a culture medium containing AAV-producing cells and mix well.
2. Incubate for 10 min at room temperature, then detach the cells and transfer them to a sterile centrifuge tube.
3. Centrifuge at 1,700 to 2,000g for 10 min at 4°C and discard the supernatant.
4. Centrifuge again at 1,700 to 2,000g for 10 min at 4°C and completely remove the remaining supernatant.
 - Note: Before proceeding to the next step, confirm that the supernatant has been completely removed, as any residual supernatant may affect the later steps.
5. Loosen the cell pellet thoroughly by tapping or vortexing.
 - Note: If the cell pellet has not been loosened completely, the efficiency of extraction may decrease. Confirm that there are no clumps of cells before proceeding to the next step.
6. Add 10 ml of AAVpro Freeze-Thaw Extraction Buffer (All Serotypes).
7. Resuspend the cells by vortexing for 15 sec.
 - Note: Loosen the cell pellet thoroughly.
8. Completely freeze the cell suspension by placing it in a freezer at -80°C for 10 to 20 min or in dry ice-ethanol for 5 to 10 min.
9. Completely thaw the cell suspension by placing it in a water bath or an incubator at 37°C for 5 to 10 min.
10. Resuspend the thawed cell suspension by vortexing for 15 sec.
11. Centrifuge at 9,000g for 10 min at 4°C.
12. Transfer the supernatant (AAV particle extract) to a new sterile centrifuge tube, taking care to ensure that no impurities are transferred along with the supernatant.
 - Note 1: AAVpro Purification Pack Maxi (All Serotypes) (Cat. #6678) can be used to purify AAV particles from this extract. For the details of the protocol, see "VII. Purification of AAV particles from extract with AAVpro Purification Pack Maxi (All Serotypes)".
 - Note 2: The AAV particle extract can be stored at -80°C in a centrifuge tube suitable for frozen storage. For thawing, make sure to thaw rapidly in a water bath at 37°C.
 - Note 3: AAVpro Titration Kit (for Real-Time PCR) Ver. 2 (Cat. #6233) can be used for measuring the titer of the extracted AAV particles.

VII. Purification of AAV particles from extract with AAVpro Purification Pack Maxi (All Serotypes)

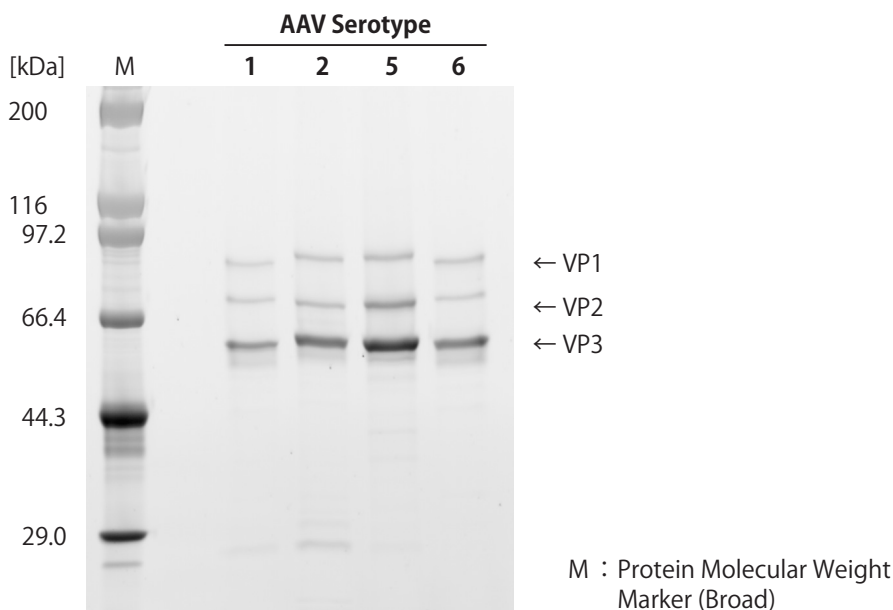
Use swinging bucket rotors for centrifugation in steps VII-5, VII-6, and VII-7.
Reagents marked with an asterisk (*) are components of AAVpro Purification Pack Maxi (All Serotypes) (Cat. #6678).

1. Add 1/100 volume of Cryonase Cold-active Nuclease* (final concentration 200 U/ml) to the supernatant from step VI-12 and incubate at 37°C for 1 hr.
2. Add 1/10 volume of Precipitator A* and vortex for 10 sec to mix. Incubate at 37°C for 30 min, and vortex again for 10 sec.
 - Note 1: 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 it completely at 37°C before use.
 - Note 2: Although a precipitate may form during the reaction, this is not a problem. Proceed to the next step.
3. Add 1/20 volume of Precipitator B* to the solution and then promptly vortex for 10 sec to mix. After centrifuging at 9,000g for 5 min at 4°C, recover the supernatant.
 - Note 1: If the recovered supernatant contains a precipitate, centrifuge it again to eliminate as much of the precipitate as possible.
 - Note 2: The centrifugation conditions differ from those used with the AAVpro Cell & Sup. Purification Kit Maxi (All Serotypes) (Cat. #6676).
4. Filter the supernatant using a Millex-HV 0.45 μ m filter*.
5. Transfer the filtered AAV particle solution to an Amicon Ultra-15, 100 kDa*. Centrifuge at 2,000g for 5 min at 15°C, and confirm that the AAV solution volume is ~1.5 ml.
 - Note 1: To avoid excessively concentrating the AAV particle solution, centrifuge for a short period of time (about 1 to 2 min) and then adjust the centrifugation time after checking the volume of the AAV particle solution.
 - Note 2: If the volume of AAV solution is over ~1.5 ml, centrifuge more time.
6. After removing the filtrate, add 5 ml of Suspension Buffer* to the cup of the Amicon Ultra-15 filter unit and mix by pipetting. Centrifuge at 2,000g for 5 min at 15°C, and then confirm that the volume of the AAV solution in the filter is now 1.5 ml or less.
 - Note: If the amount of AAV solution is over ~1.5 ml, centrifuge again.
7. Repeat step VII-6 four times (for a total of five times). Concentrate the AAV solution in the filter to the desired volume.
 - Note: If AAV solution is concentrated too much, the AAV particles may become insoluble. While performing centrifugal concentration, stop the centrifuge as appropriate and try pipetting the AAV solution to make sure it has not become insoluble.
8. After removing the filtrate, resuspend the solution inside the cup of the Amicon Ultra-15 filter unit by pipetting or vortexing for 30 sec and transfer the AAV solution to a new tube.
 - Note: To avoid repeatedly freezing and thawing the AAV particle solution, we recommend that you aliquot and store the solution at -80°C.

VIII. Experimental Examples

**VIII-1. Data 1: The purity of AAV particles after purification
(When used in combination with AAVpro Purification Pack Maxi (All Serotypes) (Cat. #6678))**

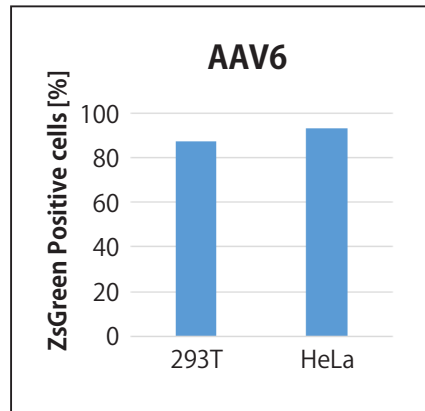
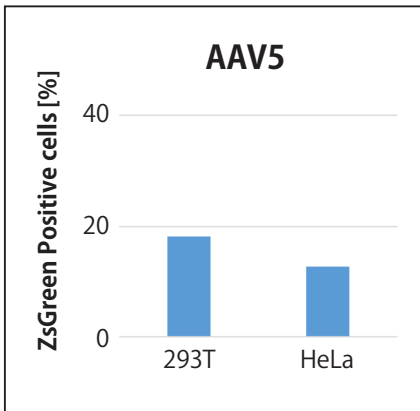
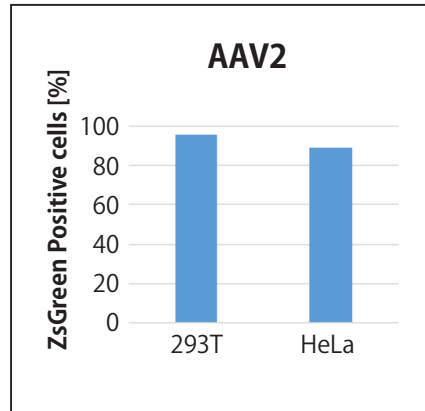
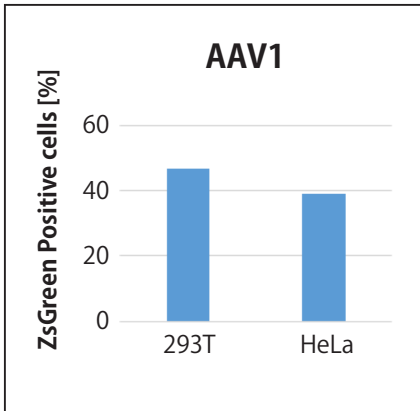
AAV (serotype 1, 2, 5 and 6) particles carrying the fluorescence protein ZsGreen were extracted from producer cells with this product and purified with AAVpro Purification Pack Maxi (All Serotype) (Cat.#6678). The titers of the purified AAV particles were measured using the AAVpro Titration Kit (for Real Time PCR) Ver.2 (Cat. #6233), and SDS-PAGE was performed with 1×10^9 vector genome (vg)/lane. The AAV capsid proteins (VP1, VP2, and VP3) were confirmed to be the major bands present.



VIII-2. Data 2: Infectivity of purified AAV particles

(When used in combination with AAVpro Purification Pack Maxi (All Serotypes) (Cat. #6678))

Using the AAV particles of each serotype obtained in Data 1, the infectious titers were evaluated. Cells were infected with the purified AAV particles at 10,000 vg/cell (AAV1, AAV2) or 100,000 vg/cell (AAV5, AAV6), and flow cytometry analysis was performed after 3 days. It was confirmed that the AAV particles extracted with this product and purified with AAVpro Purification Pack Maxi (All Serotypes) retained their infectivity.



IX. 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)*

pAAV-ZsGreen1 Vector (Cat. #6231)

AAVpro® Packaging Plasmid (AAV1) (Cat. #6672)
AAVpro® Packaging Plasmid (AAV2) (Cat. #6234)
AAVpro® Packaging Plasmid (AAV5) (Cat. #6664)
AAVpro® Packaging Plasmid (AAV6) (Cat. #6665)

CalPhos™ Mammalian Transfection Kit (Cat. #631312)

AAVpro® 293T Cell Line (Cat. #632273)

AAVpro® Purification Kit Maxi (All Serotypes) (Cat. #6666)
AAVpro® Purification Kit Midi (All Serotypes) (Cat. #6675)
AAVpro® Extraction Solution (Cat. #6235)
AAVpro® Cell & Sup. Purification Kit Maxi (All Serotypes) (Cat. #6676)*
AAVpro® Cell & Sup. Extraction/Concentration Pack Maxi (All Serotypes) (Cat. #6677)*
AAVpro® Purification Pack Maxi (All Serotypes) (Cat. #6678)*

AAVpro® Concentrator (Cat. #6674)

AAVpro® Titration Kit (for Real-Time PCR) Ver. 2 (Cat. #6233)

* Not available in all geographic regions. Please check for availability in your area.

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CalPhos is a trademark of Takara Bio USA, Inc.

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