Cat. # 6235

For Research Use

TaKaRa

AAVpro® Extraction Solution

Product Manual

v201503Da

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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.

Available AAVpro Products

AAVpro® Helper Free System (AAV2)	Cat. #6230
AAVpro® Purification Kit (AAV2)	Cat. #6232
AAVpro® Titration Kit (for Real Time PCR) Ver.2	Cat. #6233
AAVpro® Packaging Plasmid (AAV2)	Cat. #6234
AAVpro® Extraction Solution	Cat. #6235
pAAV-ZsGreen1 Vector	Cat. #6231
AAVpro® Helper Free System (AAV2-CRE Recombinase)	Cat. #6652
AAVpro® Helper Free System (AAV2-LacZ)	Cat. #6655
AAVpro® Tet-One ™ Inducible Expression System (AAV2)	Cat. #634310

I. Description

Adeno-associated virus (AAV) is one of the smallest viruses belonging to the *Parvovirus* family of the *Dependovirus* genus. AAV is a non-enveloped virus with a single-strand DNA genome. There are more than 100 serotypes of AAV, and the host specificity and characteristics of the virus differ among serotypes.

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). When transducing genes into animals using an AAV vector, it is necessary to use a highly-purified vector that does not contain any residual virus-producing cells or impurities from the culture medium. In addition, when transducing cultured cells, purification of AAV vectors eliminates the effects of the aforementioned impurities.

Isolation of AAV particles from AAV particle-producing cells is conventionally performed using the freeze-thaw or sonication methods. However, these methods are time consuming and require special equipment. The AAVpro Extraction Solution is a reagent for the extraction of AAV particles from AAV particle-producing cells. This reagent provides a simple and efficient method for AAV particle isolation; the reagents are added to virus-producing cells and viral particles are recovered by centrifugation. The extracted AAV particle solution contains only a small amount of host protein and nucleic acid contamination. The resulting viral extract is well-suited for cell infection or further purification of viral particles.

Note : When transducing genes into individual animals using an AAV2 vector, purify AAV2 particles purified with the AAVpro Purification Kit (AAV2) (Cat. #6232) to obtain highly-purified particles that do not contain any residual virus-producing cells or impurities from the culture medium.

II. Components

This product is a set of solutions for use in the extraction of viral particles from AAV vector-producing cells.

1. AAV Extraction Solution A	25 ml
2. AAV Extraction Solution B	2.5 ml

III. Storage

Room temperature

IV. Materials Required but not Provided

- Equipment necessary for cell culture
- Sterile centrifuge tubes
- Sterile microtubes
- 0.5 M EDTA (pH 8.0) [EDTA Buffer Powder, pH 8.0 (Cat. #T9191)]

VI. Protocol

A protocol for extraction of AAV particles from AAV particle-producing cells from a single 10-cm dish is provided below. Refer to Table 1. for the necessary volumes of the solution for different types of culture vessels.

1. Add 0.5 M EDTA (pH 8.0) in a volume that is 1/80 of the culture medium to a 10-cm dish containing cultured AAV vector-producing cells*. Allow to stand at room temperature for 10 minutes.
   * Culture cells according to standard procedures.
2. Transfer the detached cells to a sterile 15-ml centrifuge tube.
3. Centrifuge at 1,750x *g* at 4℃ for 10 minutes. Completely remove the supernatant and collect the cell pellet.

   Note : Confirm that the supernatant has been completely removed before proceeding; particle isolation may be affected by residual supernatant.
4. Loosen the cell pellet by tapping or vortexing the tube.

   Note : If the cell pellet is not loosened sufficiently, the efficiency of extraction may decrease. Confirm that there are no clumps of cells before proceeding.
5. Add 0.5 ml of AAV Extraction Solution A.
6. Suspend the cell pellet by vortexing for 15 seconds.
7. Allow to stand at room temperature for 5 minutes. Vortex for 15 seconds.
8. Centrifuge at 2,000 - 14,000x *g* at 4℃ for 10 minutes.

   Note : If the titer of the recovered AAV vector is low, the efficiency may be increased by repeating steps 6 - 8.
9. Collect the supernatant in a new sterile centrifuge tube and add 50 μl of AAV Extraction Solution B.

   Note 1: The mixture can be stored at -80℃. Thaw quickly in a 37℃ water bath before use.

   Note 2: The supernatant may change to a pink color after AAV Extraction Solution B is added.

Titration of the extracted AAV particles can be performed using the AAVpro Titration Kit (for Real Time PCR) Ver.2 (Cat. #6233).

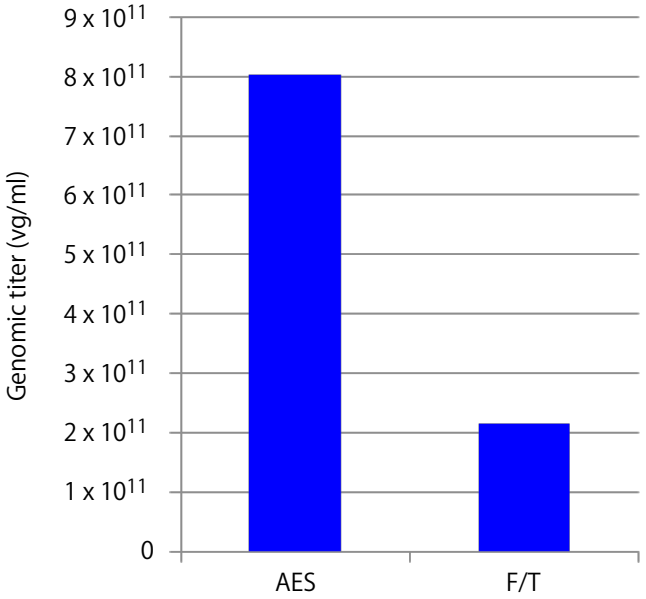
Table 1. Amount of solutions required for various culture vessels.

	Volume of Media	0.5 M EDTA (pH 8.0)	AAV Extraction Solution A	AAV Extraction Solution B
6-cm dish	4 ml	50 μl	200 μl	20 μl
10-cm dish	10 ml	125 μl	500 μl	50 μl
15-cm dish	26 ml	325 μl	1,300 μl	130 μl
T25 flask	4 ml	50 μl	250 μl	25 μl
T75 flask	13 ml	162.5 μl	650 μl	65 μl
T225 flask	40 ml	500 μl	2,000 μl	200 μl

VI. Reference Data

VI-I. Comparison of AAV Extraction Solution with the Freeze-Thaw Method (1)

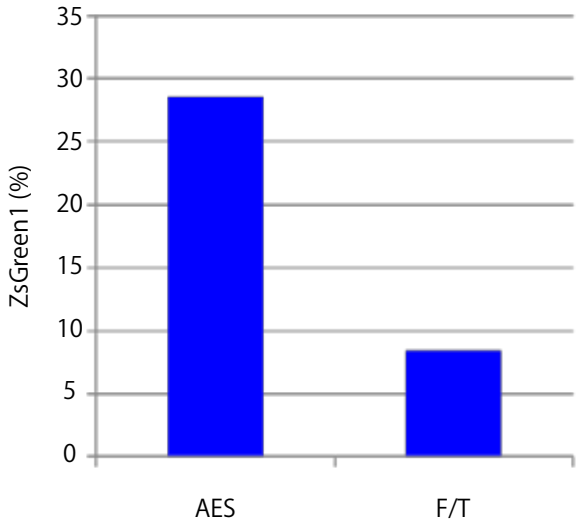
HEK293 cells producing AAV2 particles with the pAAV-ZsGreen1 vector (Cat. #6231) were generated using the AAVpro Helper Free System (AAV2) (Cat. #6230). AAV2 particles were extracted from the cells using either AAV Extraction Solution or the freeze-thaw method. A vector genome assay (real-time PCR) and biological titer measurement with HT1080 cells (ZsGreen1 expression) were performed on the viral extract solutions to assess the titer (Figures 1A and 1B). The titer was higher when AAV Extraction Solution was used for viral particle isolation as compared to the freeze-thaw method.



AES: AAVpro Extraction Solution
F/T: Freeze-Thaw Method

＊vg: Vector Genome

Figure 1A. Vector genome assay.

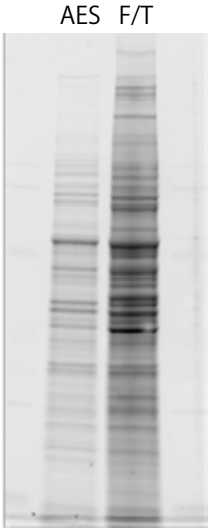


AES: AAVpro Extraction Solution
F/T: Freeze-Thaw Method

Figure 1B. Biological titer measurement.

VI-II. Comparison of AAV Extraction Solution with the Freeze-Thaw Method (2)

AAV2 particles from AAV2-producing cells in five T225 flasks were extracted using Extraction Solution A and B or the freeze-thaw method. The vector genome in the AAV2 extract solutions was determined by real-time PCR. Then, the equivalent of 1 x 10^9 vg of the AAV2 extract solution was analyzed by SDS-PAGE to evaluate the amount of protein impurity (Figure 2A). In addition, residual dsDNA content was assayed using the intercalation method (Figure 2B). The results indicate that the use of the AAV Extraction Solutions clearly reduced the amount of protein impurities and dsDNA in comparison with the freeze-thaw method.



AES: AAVpro Extraction Solution
F/T: Freeze-Thaw Method
(1 x 10^9 vg/lane)

Figure 2A. SDS-PAGE of AAV2 particle extract solutions.

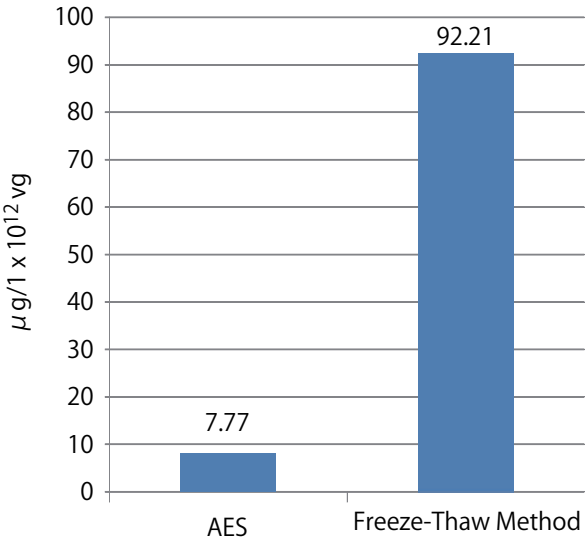


Figure 2B. dsDNA in AAV2 particle extract solutions.

VI-III. Extraction of Serotype 1, 2, and 6 AAV Vectors

HEK293 cells producing AAV1, 2, and 6 vectors were prepared. AAV particles were extracted from cells using either the AAVpro Extraction Solution or the freeze-thaw method. The AAVpro Titration Kit (for Real Time PCR) Ver.2 (Cat. #6233) was used to measure the titer of the AAV solutions. The results indicate that the AAVpro Extraction Solution can be used to efficiently extract AAV serotypes 1, 2, and 6.

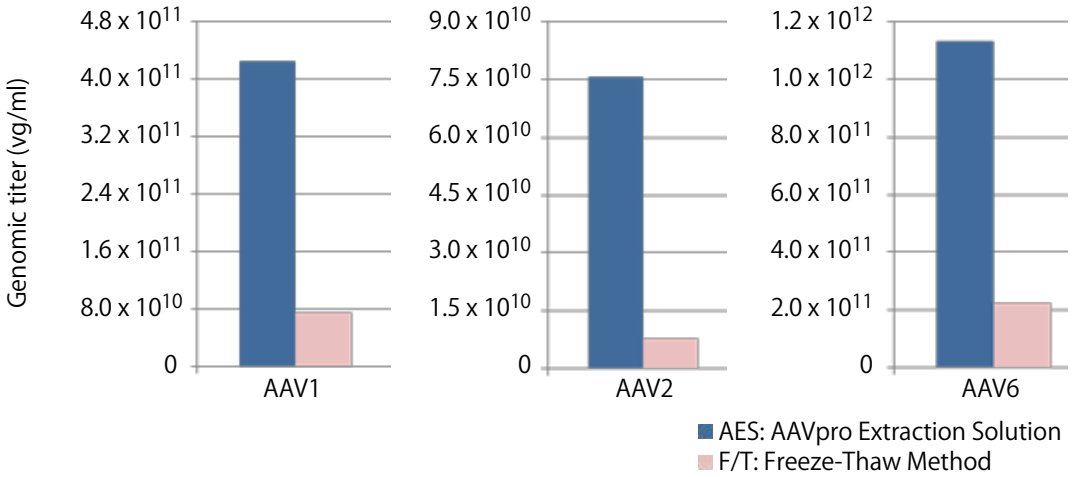


Figure 3. Titer of AAV particles prepared with AAVpro Extraction Solution or the freeze-thaw method.

VII. Related Products

AAVpro® Helper Free System (AAV2) (Cat. #6230)
pAAV-ZsGreen1 Vector (Cat. #6231)
AAVpro® Purification Kit (AAV2) (Cat. #6232)
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AAVpro® Tet-One™ Inducible Expression System (AAV2) (Cat. #634310)

NOTE : This product is for research use only. It is not intended for use in therapeutic or diagnostic procedures for humans or animals. Also, do not use this product as food, cosmetic, or household item, etc.

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