Cat. # 6674

For Research Use

TaKaRa

AAVpro® Concentrator

Product Manual

v201909Da

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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. 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). However, it is necessary to prepare highly concentrated AAV vectors for transduction, since the amount of AAV vector injected into an individual animal depends on the administration method and target animal, and the injection volume for commonly used animals such as mice is limited due to their small body size.

AAVpro Concentrator contains reagents that allow easy and efficient concentration of AAV vectors. It can be used for various applications, such as concentration of AAV vectors in culture supernatant with or without serum, and buffer exchange of AAV vector solutions. Figure 1 on Page 6 illustrates the protocol for preparing and purifying AAV vectors, which involves concentration and buffer exchange.

Advantages of this product

- Applicable for various serotypes
- High recovery rate
- Simple protocol
- Includes all buffers needed to concentrate AAV vectors
- Concentration is possible in the presence or absence of serum

II. Components

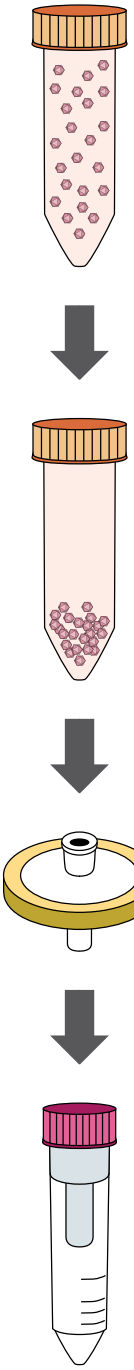
1.	Concentrating solution A	16 ml
2.	Concentrating solution B	80 ml
3.	Dissolving solution	16 ml
4.	Washing solution	30 ml x 2
5.	Millex-HV 0.45 μm	4
6.	Amicon Ultra-15, 100 kDa	2

III. Storage

Concentrating solution B	4℃
Other components	Room temperature

IV. Materials Required but not Provided

- General equipment required for cell culture
- Locking syringe
- Vortex mixer



Add Concentrating solution A followed by B into a solution containing AAV vectors and incubate at 4℃ overnight.

Precipitate AAV vectors by centrifugation.

Resuspend AAV vector pellet using Dissolving solution.

Filtration by Millex-HV 0.45 μm

Purification and concentration using Amicon Ultra-15

Collection of AAV vectors

Figure 1. AAV vector concentration protocol using AAVpro Concentrator

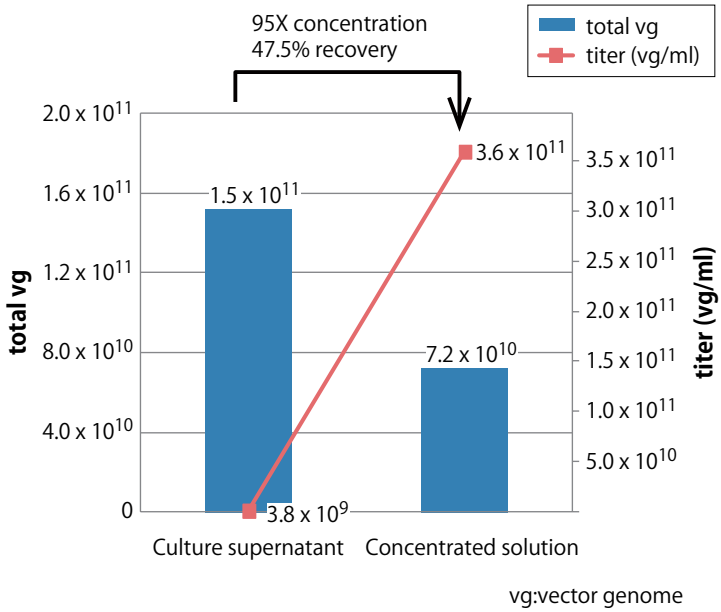
V. Protocol: Concentration from 150 ml of AAV Vector Suspension

When starting with less than 150 ml of AAV vector suspension, add proportionally smaller amounts of the reagents in Steps 1 and 2. (For example, when starting with 75 ml of AAV vector suspension, add half the indicated volume of Concentrating solutions A and B.)

1. Add 8 ml of Concentrating solution A to 150 ml of AAV vector suspension (~20X dilution) and mix well using a vortex mixer.
   [Note] If a large-volume centrifuge is not available, divide the total amount into smaller volumes that can be centrifuged and proceed to Step 2.
2. Add 40 ml of Concentrating solution B and mix well using a vortex mixer; then transfer the mixture to a centrifuge tube and incubate at 4℃ overnight.
3. Make sure that there is a white precipitate at the bottom of the centrifuge tube following Step 2.
   [Note] When an AAV vector suspension contains EDTA, a white precipitate may not be observed in some cases. If so, still proceed to Step 4.
4. Centrifuge at 2,000*g*, 4℃ for 50 minutes, then carefully remove the supernatant.
5. Add 8 ml of Dissolving solution to the pellet and resuspend by pipetting. Transfer the suspension to a new centrifuge tube.
6. After vortexing for 15 sec, place at room temperature for 15 minutes, and then vortex for an additional 15 sec.
7. Centrifuge the suspension at 2,000*g*, 4℃ for 15 minutes, then transfer the supernatant (containing AAV) to a new centrifuge tube, and centrifuge again at 2,000*g*, 4 ℃ for 15 minutes. Then carefully collect the supernatant and filter it using a Millex-HV 0.45 μm syringe filter unit.
   [Note] In some cases, one filter unit may not be enough to filter the entire solution due to clogging. If so, use another new Millex-HV 0.45 μm syringe filter unit to complete the filtration.
8. Transfer the filtered AAV vector suspension from Step 7 to an Amicon Ultra-15, 100 kDa centrifugal filter unit and centrifuge at 2,000*g*, 15℃ for 10 minutes. Confirm that the volume of the AAV vector solution is 1.5 ml or less.
   [Note 1] Using a swinging bucket rotor is recommended. If a fixed angle rotor is used, place the Amicon filter unit in a bucket with the membrane panel facing up.
   [Note 2] If the volume of the AAV vector solution is not 1.5 ml or less, resuspend the AAV vector solution that remains in the Amicon filter unit by pipetting and repeat the centrifugation.
9. After removing the filtrate, add 10.5 ml of Washing solution to the Amicon filter unit. Mix the solution by pipetting and centrifuge at 2,000*g*, 15℃ for 10 minutes. Confirm that the volume of the AAV vector solution is 1.5 ml or less.
   [Note 1] Using a swinging bucket rotor is recommended. If a fixed angle rotor is used, place the Amicon filter unit in a bucket with the membrane panel facing up.
   [Note 2] If the volume of the AAV vector solution is not 1.5 ml or less, resuspend the AAV vector solution that remains in the Amicon filter unit by pipetting and repeat the centrifugation.
10. Repeat Step 9 two more times to concentrate the sample down to the desired volume.
11. After removing the filtrate, mix the AAV vector solution by pipetting or vortexing for 30 sec and transfer it from the Amicon filter unit to a tube.

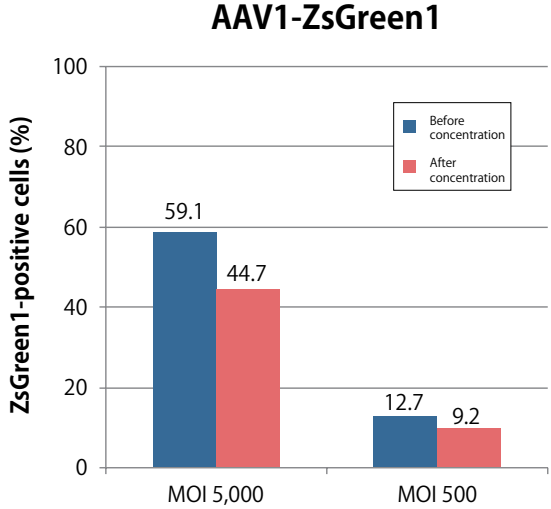
VI. Experimental Example 1: Concentration of AAV Vectors from Culture Supernatant without Serum

Cell culture supernatant containing AAV1 vectors which express ZsGreen was concentrated from 40 ml (one T225 flask) to 200 μl using this kit. The virus titer before and after concentration was measured using the AAVpro Titration Kit (for Real Time PCR) Ver.2 (Cat. #6233) in order to determine the viral recovery rate.



VII. Experimental Example 2: Biological Titer Measurement of Concentrated AAV Vector

Flow cytometric analysis was used to compare ZsGreen expression in HT1080 cells infected with AAV1-ZsGreen vectors before and after they were concentrated using this kit. AAV vectors were infected at 5,000 and 500 vg/cell, and the analysis was performed after 3 days. The concentrated AAV vectors were able to infect the cells.



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® 293T Cell Line (Cat. #632273)
AAVpro® Titration Kit (for Real Time PCR) Ver.2 (Cat. #6233)
AAVpro® Purification Kit (All Serotypes) (Cat. #6666)
AAVpro® Purification Kit (AAV2) (Cat. #6232)
AAVpro® Tet-One™ Inducible Expression System (AAV2) (Cat. #634310)
AAVpro® CRISPR/Cas9 Helper Free System (AAV2) (Cat. #632608)
AAVpro® CRISPR/Cas9 Vector System (Cat. #632609)

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

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