Description

Preclinical and clinical successes of Adeno-associated virus (AAV)-mediated gene therapy have established AAV as an ideal and safe therapeutic vector. Moreover, these successes have motivated research in both discovering and engineering novel AAV capsids that are more selective and clinically desirable than existing capsids.

When injecting AAV particles into animals, it is necessary to use highly purified particles that do not contain any residual AAV host cells. Isolating AAV particles from the AAV host cells is conventionally conducted using freeze-thaw or sonication methods. However, these methods are time consuming and carry significant amounts of proteins from the host cells.

The AAV ONE-Extract™ Solution is a reagent for quickly extracting AAV particles from AAV host cells. This reagent provides a simple and efficient method for isolating the AAV particles and is suitable for all AAV serotypes. The resulting viral particles are well-suited for cell infection or further purification.

Applications

Quickly and efficiently isolate AAV particles from AAV host cells

Supplied Materials

Catalog #	Name	Amount	Storage
	AAV ONE-Extract Solution A	30 ml	Room temperature
	AAV ONE-Extract Solution B	6 ml	Room temperature

Materials Required but Not Supplied

0.5 M EDTA (pH 8.0)

Storage Conditions

Reagents will perform optimally for up to 6 months from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. Overall, this product should be considered hazardous and harmful by inhalation, in contact with skin or eyes, and if swallowed. If contact occurs, wash thoroughly.

General Assay Protocol

Below is a protocol for extracting AAV particles from AAV host cells cultured in a single 10 cm dish. Refer to **Table 1** for the necessary volumes to use with different types of culture vessels.

1. To detach the AAV-producing cells, add 0.5 M EDTA (pH 8.0) in a 1:80 ratio to the cell culture medium. Incubate at room temperature for 10 minutes.

2. Carefully collect the mixture of medium and detached cells and centrifuge at 2000 rpm at 4°C for 10 minutes.

3. Completely remove the supernatant and keep the cell pellet.

4. Centrifuge one more time at 2000 rpm at 4°C for 2 minutes. Remove all residual supernatant from the cell pellet.

Note: *Confirm that the supernatant has been completely removed before proceeding; particle isolation may be affected by the presence of any residual medium.*

5. Loosen the cell pellet by tapping the tube.

   Note: *Extraction may be less efficient if the cell pellet is not loosened sufficiently.*

6. Add **0.5 ml** of AAV ONE-Extract **Solution A**.

7. Resuspend the cell pellet by vortexing for 15 seconds and incubate at room temperature for 10 minutes.

8. Vortex again for 15 seconds and centrifuge at 4000 rpm at 4°C for 15 minutes.

9. Collect the supernatant in a new sterile centrifuge tube and centrifuge one more time at 4000 rpm at 4°C for 5 minutes.

   Note: *There will be a white layer on top of the supernatant; tilt the centrifuge tube while aspirating to carefully aspirate the clear supernatant while avoiding the white layer as much as possible.*

10. Collect the supernatant into a new sterile centrifuge tube.

11. Under continuous mixing, add **100 µl** of AAV ONE-Extraction **Solution B** to the supernatant, dropwise.

    Notes:
    - *The mixture can be stored at -80°C. Thaw quickly in a 37°C water bath before using.*
    - *Filter the final AAV suspension using a Millex-HV 0.45 µm if you will transduce cells in vitro.*
    - *Iodixanol discontinuous gradient centrifugation is highly recommended for in vivo studies to remove empty capsids and contaminant proteins from the final AAV suspension.*

Table 1: Suggested volumes to use with various culture vessels.

Vessel	Volume of Media	0.5 M EDTA (pH 8.0)	AAV ONE-Extract Solution A	AAV ONE-Extract Solution B
6 cm dish	4 ml	50 µl	200 µl	40 µl
10 cm dish	10 ml	125 µl	500 µl	100 µl
15 cm dish	25 ml	313 µl	1,200 µl	240 µl
T25 flask	4 ml	50 µl	200 µl	40 µl
T75 flask	13 ml	163 µl	650 µl	130 µl
T175 flask	25 ml	313 µl	1,200 µl	240 µl
T225 flask	35 ml	438 µl	2,000 µl	400 µl

Comparison of AAV ONE Extract Solution with the Freeze-Thaw Method

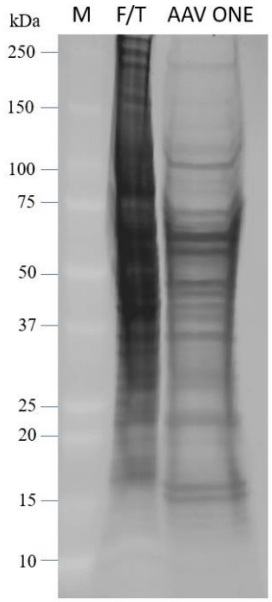

Figure 1. Crude AAV2 particles from AAV producing cells in two T225 flasks were extracted using the freeze-thaw method or the AAV ONE Extract Solution.
The vector genome copies in the AAV2 extract solutions was determined by real-time PCR. Then, the equivalent of 2 x 10^9 VG of the AAV2 particles were analyzed by SDS-PAGE to evaluate the particle impurity. M: Molecular standard, F/T: Freeze-Thaw method, AAV ONE: AAV ONE Extract.

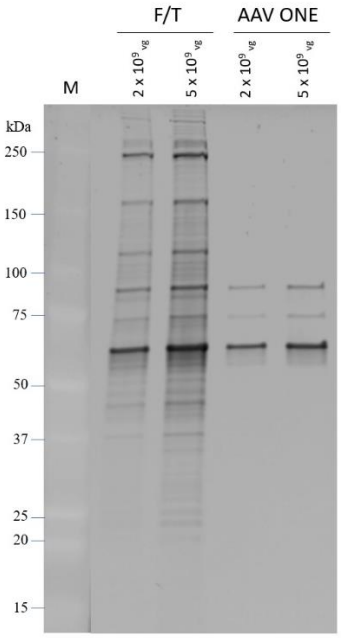

Figure 2. Purified AAV2 particles from Crude AAV2 particles.
The crude AAV2 particles were then purified by iodixanol discontinuous gradient ultracentrifugation, the equivalent of 2 x 10^9 VG and 5 x 10^9 VG were analyzed by SDS-PAGE to evaluate the amount of protein impurity, M: Molecular standard, F/T: Freeze-Thaw method, AAV ONE: AAV ONE Extract.

Troubleshooting Guide

Visit bpsbioscience.com/lentivirus-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
AAV2 ZsGreen	78444	50 µl x 2
AAV2 Luciferase-eGFP	78462	50 µl x 2
AAV2 Luciferase-mCherry	78471	50 µl x 2
AAV2 SaCas9	78480	50 µl x 2
AAV2 Luciferase	78453	50 µl x 2