Product Manual

ViraBind™ AAV Purification Mega Kit

Catalog Number

VPK-141	2 preps
VPK-141-5	10 preps

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

The viral system includes vectors developed from retrovirus (RV), adenovirus (AdV), adeno-associated virus (AAV), lentivirus (LV), and herpes simplex virus (HSV). AAV belongs to the family of Parvoviridae, a group of viruses among the smallest of single-stranded and non-enveloped DNA viruses. There are eight different AAV serotypes reported to date.

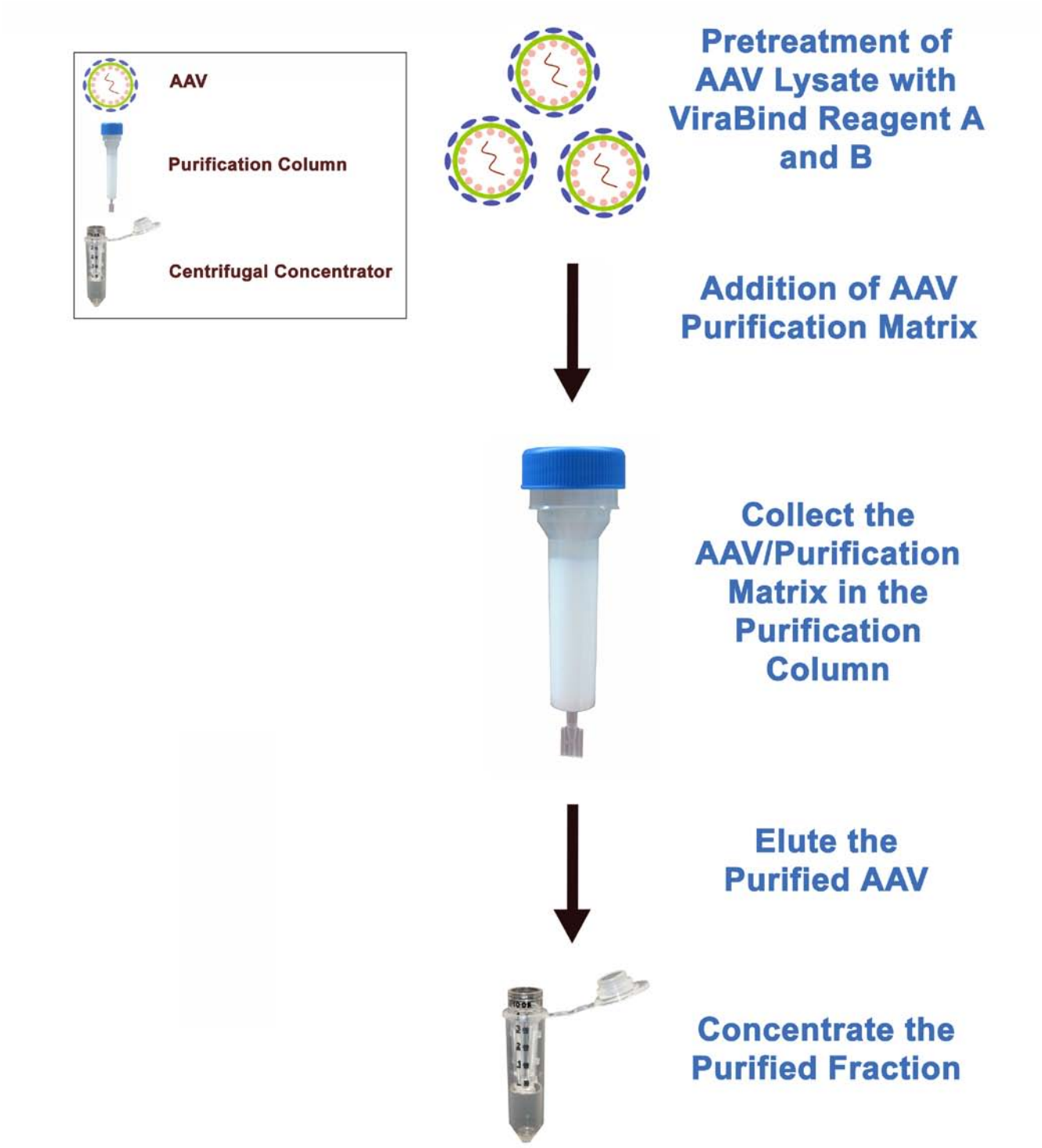
Recombinant AAV-2 is the most common serotype used in gene delivery, and can be produced at high titers with a helper virus or Cell Biolabs' AAV Helper Free System. AAV-2 can infect both dividing and non-dividing cells and can be maintained in the human host cell, creating the potential for long-term gene transfer. Because AAV-2 is a naturally defective virus, requiring provision of several factors in *trans* for productive infection, it is considered the safest viral vector to use. Recently a new vector, AAV-DJ, was developed using DNA family shuffling to create a hybrid capsid from 8 different AAV serotypes, resulting in a vector with significantly higher *in vitro* infection rates across a variety of cells and tissues.

Recombinant AAV-2 and AAV-DJ vectors can be purified by CsCl gradient ultracentrifugation, iodixanol discontinuous gradient ultracentrifugation, and high-performance liquid chromatography (HPLC). The most popular technique, CsCl ultracentrifugation, is time consuming process which may result in poor recovery and quality (nonviral protein contamination and a high ratio of genome copies versus infectious units).

ViraBind™ AAV Purification Mega Kit does not involve ultracentrifugation. AAV-2 or AAV-DJ is first purified from viral supernatant with a single-step column, then further purified/concentrated with a centrifugal concentrator (see Assay Principle below). The entire procedure takes about 3 hours. Each preparation can handle up to 10 x 150 mm dishes, resulting in 100 µL of highly purified AAV-2 (Figure 1) or AAV-DJ with low ratio of genome copies versus infectious units.

ViraBind™ AAV Purification Mega Kit provides an efficient system for quick purification of AAV-2 or AAV-DJ with high recovery (>60%). The highly purified and concentrated viruses can be used in primary cell infections and *in vivo* applications.

Assay Principle



Related Products

1. AAV-100: 293AAV Cell Line
2. AAV-200: ViraDuctin™ AAV Transduction Kit
3. VPK-099: ViraBind™ Adenovirus Miniprep Kit
4. VPK-140: ViraBind™ AAV Purification Kit
5. VPK-145: QuickTiter™ AAV Quantitation Kit

Kit Components

1. ViraBind™ AAV Reagent A (Part No. 314001): Two 0.3 mL sterile tubes.
2. ViraBind™ AAV Reagent B (Part No. 314002): Two 1.5 mL sterile tubes.
3. AAV Purification Matrix (Part No. 314103): One 6 mL bottle.
4. Purification Columns (Part No. 314104): Two empty columns.
5. Purification Buffer (Part No. 314105): One 50 mL bottle.
6. Elution Buffer (Part No. 314106): One 10 mL bottle.
7. Centrifugal Concentrators (Part No. 309505): Pack of 2 units with 4 recovery tubes.

Materials Not Supplied

1. AAV Helper-Free System
2. Transfection Reagent
3. HEK 293 cells and cell culture growth medium
4. Centrifuge (capable of 10,000 x g)
5. 0.45 µm low protein binding filter
6. 50 mL Conical Tubes

Storage

Store ViraBind™ AAV Reagent B, Purification Columns and Centrifugal Concentrators at room temperature and all other kit components at 4ºC.

Safety Considerations

Remember that you will be working with samples containing infectious virus. Follow the recommended NIH guidelines for all materials containing BSL-2 organisms.

Preparation of rAAV-2 or rAAV-DJ Samples

The following procedure is suggested for 10 x 15 cm dishes and may be optimized to suit individual needs. Please refer to the user manual when using Cell Biolabs' AAV Helper-Free System or other packaging systems.

1. Use HEK 293 cells that have been passaged 2-3 times prior to transfection. Culture these cells until the monolayer is 70-80% confluent.
2. Cotransfect cells with the pAAV-RC, pHelper and your expression construct according to manufacturer's manual.
3. After 48-72 hrs, add 0.5 M EDTA to a final of 10 mM to the plate and incubate for 3 min at room temperature. Gently shake the culture plate several times and harvest all media, including cells, in a sterile tube.
   Note: Trypsin may be substituted for EDTA in this step.
4. Centrifuge for 5 min at 3000 rpm to pellet the transfected cells. Resuspend the cell pellet in 2.5 mL of serum-free DMEM per 15 cm dish (25 mL total for 10 x 15 cm dishes).
5. Subject the cell suspension to four rounds of freeze/thaw cycles by alternating the tubes between the dry ice-ethanol bath and the 37ºC water bath.
6. Collect the AAV supernatant by centrifugation at 10,000 x g for 10 minutes. Discard the pellet.
7. (Optional) For AAV samples produced with a helper adenovirus, inactivate the helper adenovirus by incubation the sample at 56ºC for 30 minutes.
8. Filter the supernatant through a 0.45 µm low protein binding filter. The viral supernatant can be stored at -80ºC or immediately purified (see purification instructions below).

Protocol

I. Purification

The following procedure is written for purification and concentration of 25 mL of AAV supernatant. For AAV samples that are less than 25 mL, add serum-free DMEM to the final volume of 25 mL.

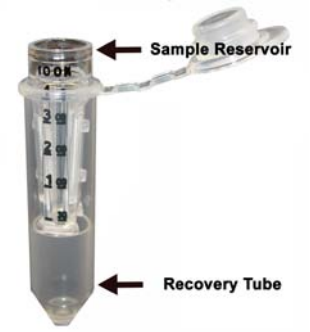
1. Add 0.25 mL of ViraBind™ AAV Reagent A to 25 mL of viral supernatant, mixing well.
2. Incubate for 30 minutes at 37ºC.
3. Centrifuge the AAV supernatant for 15 minutes at 5,000 x g.
4. Carefully collect the supernatant and transfer to a new tube. Discard the pellet.
5. Incubate ViraBind™ AAV Reagent B for 30-60 minutes at 37ºC to ensure Reagent B is dissolved. Add 1.25 mL of ViraBind™ AAV Reagent B to the ViraBind™ AAV Reagent A pretreated 25 mL of viral supernatant, mixing well.
6. Incubate for 30 minutes at 37ºC.
7. Filter the supernatant through a 0.45 µm low protein binding filter.
8. Resuspend the AAV Purification Matrix by inverting and shaking. Add 3 mL of matrix to the filtered supernatant.
9. Mix the supernatant/matrix suspension at **room temperature** for 30 minutes on an orbital shaker. Do not allow more than 30 minutes and proceed immediately to step 11.

10. Remove the break-off tip from the bottom of the Purification Column (by twisting) and place into an empty 50 mL conical tube.

11. Vortex the supernatant/matrix suspension and pour the solution into the Purification Column. AAV Purification Matrix will collect inside the column, with supernatant dripping through. Rinse the AAV supernatant/matrix collection tube with 10 mL of 1X PBS and pour the solution into the Purification Column.
12. Once the supernatant has completely flowed through, and the column stops dripping, wash the collected matrix by add 10 mL of Purification Buffer to the inside the column. Repeat the wash once more.
13. Transfer the column to a clean empty 50 mL conical tube.
14. Elute the purified AAV from the purification matrix by slowly adding 3 mL of Elution Buffer, collecting the flowthrough.

II. Final Buffer Exchange and Concentration

1. Assemble a Centrifugal Concentrator unit by inserting the sample reservoir into a recovery tube.



2. Apply 0.5 mL of the recovered AAV fraction (step 14 above) to the sample reservoir of the Centrifugal Concentrator. Cap the concentrator and place into a tabletop centrifuge (Microfuge). Centrifuge for 5 minutes at 2,000 x g. As the fraction sample is concentrated, top off the concentrator with additional AAV fraction, centrifuging between. Flow through can be discarded.
3. Continue to concentrate the AAV fraction until 100 µL remains in the sample reservoir.

4. Add 400 µL of 1X PBS, or desired final buffer, to the Concentrator and continue to centrifuge until 100 µL remains. Repeat step 4 once more.
5. Finally, concentrate until the desired final volume.
6. Using a clean recovery tube, collect the concentrated AAV sample by inverting the sample reservoir into the tube and briefly centrifuging to collect all of the liquid.

Example of Results

The following figures demonstrate typical purification results. One should use the data below for reference only. This data should not be used to interpret actual results.

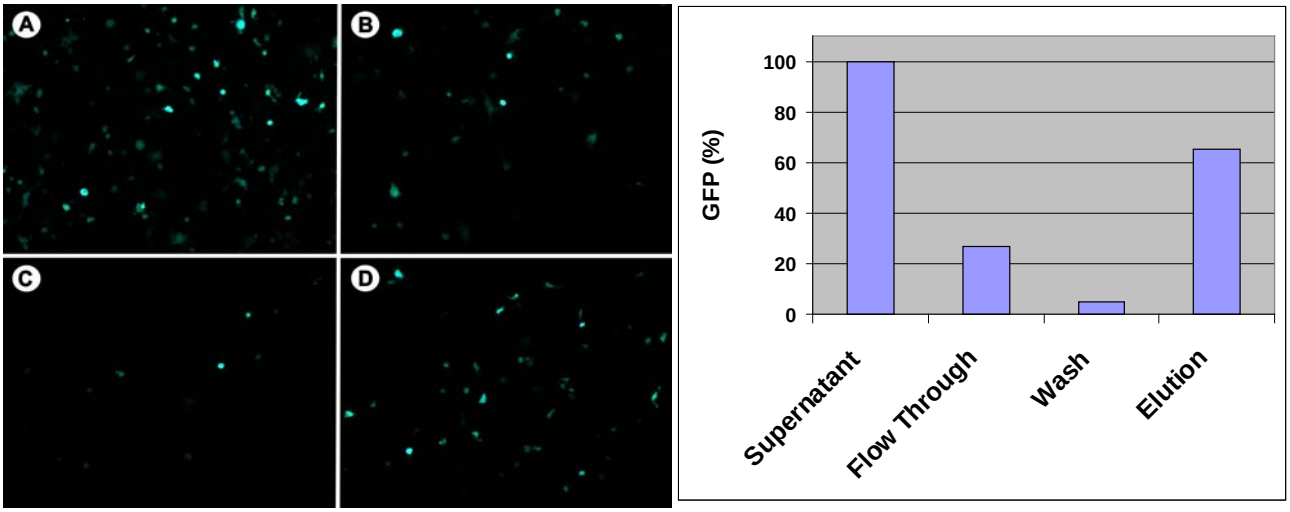


Figure 1: Purification of AAV2-GFP. AAV2-GFP was produced by a helper-free system in 293 cells. AAV supernatant was subjected to the purification steps. Samples from each fraction were used to infect 293 cells, GFP positive cells were scored by counting after three days. A: AAV Supernatant; B: Flow through; C: 1st wash; D: Elution.

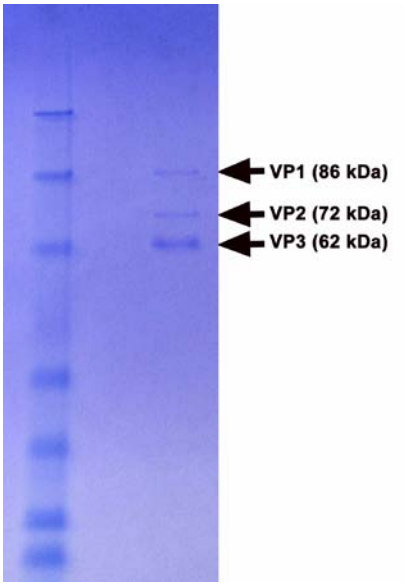


Figure 2: **Electrophoretic Profile of Purified AAV2-GFP.**

References

1. Rabinowitz, J, and Samulski, R. J. (1998) *Curr. Opin. Biotechnol*., **9**, 470-475.
2. Summer ford, C., and Samulski, R. J. (1999) *Nat. Med.*, ***5****, 587-588.*
3. Clark, K., Liu, X., McGrath, J., and Johnson, P. (1999) *Hum. Gene Ther*., **10**, 1031-1039.

Recent Product Citation

Nazari, M. et al. (2014). AAV2-mediated follistatin overexpression induces ovine primary myoblasts proliferation. *BMC Biotechnol*. **14**:87

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com