Product Manual

ViraBind™ AAV Purification Kit, Trial Size

Catalog Number

VPK-140-T **2 preps**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

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.

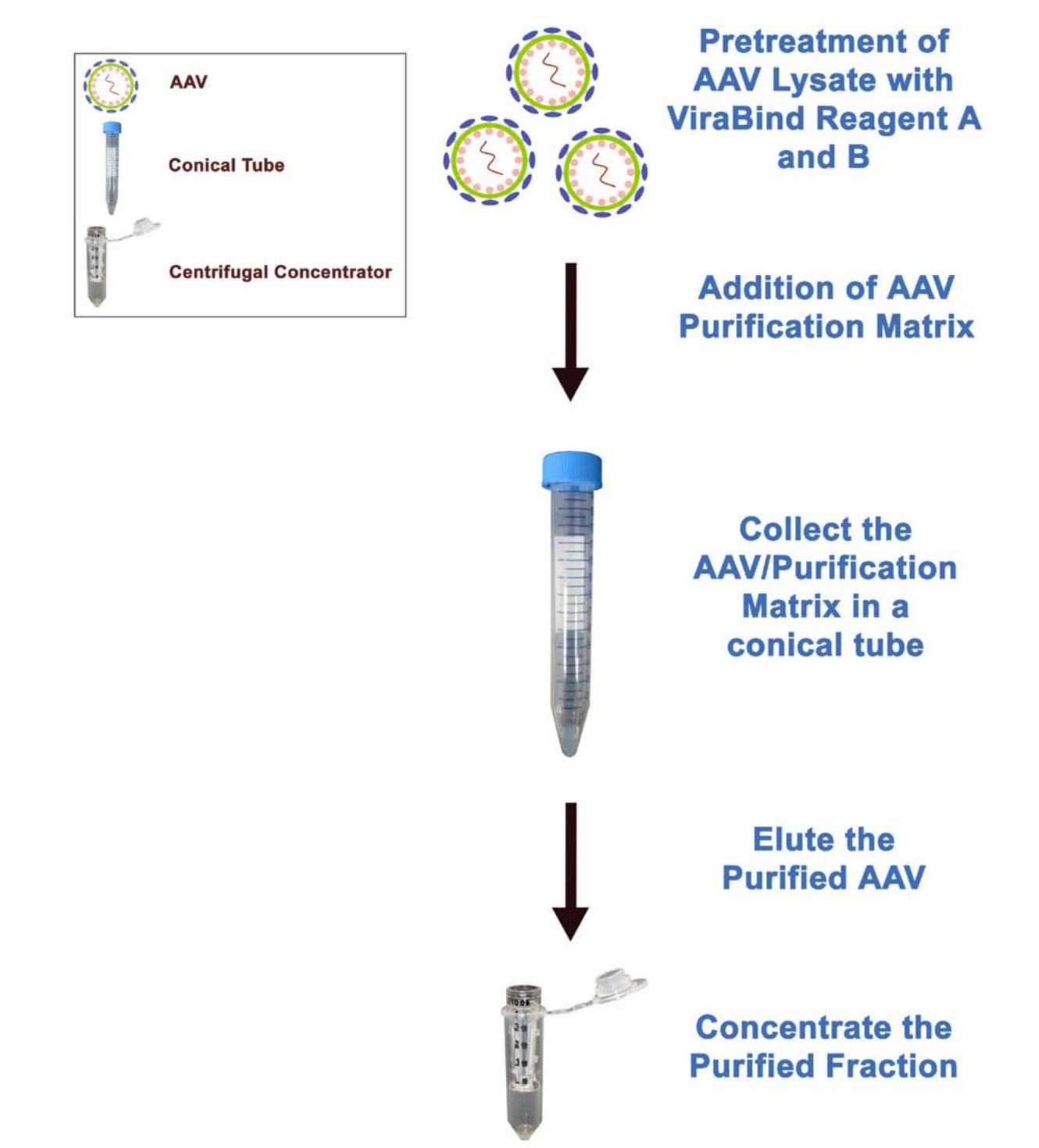
Recombinant AAV-2 is the most common serotype used in gene delivery, and can be produced at high titers with a helper virus or Cell Biolabs' AAV Helper-Free System. AAV-2 can infect both dividing and non-dividing cells and can be maintained in the human host cell, creating the potential for long-term 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, or 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 Kit does not involve ultracentrifugation. AAV-2 or AAV-DJ is first purified from viral supernatant with a single-step purification matrix, then further purified/concentrated with a centrifugal concentrator (see Assay Principle below). The entire procedure takes about 3 hours. Each preparation can handle one 150 mm dish or two 100 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 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-141: ViraBind™ AAV Purification Mega Kit
4. VPK-145: QuickTiter™ AAV Quantitation Kit
5. VPK-099: ViraBind™ Adenovirus Miniprep Kit

Kit Components

1. ViraBind™ AAV Reagent A (Part No. 314001-T): One 60 µL sterile tube.
2. ViraBind™ AAV Reagent B (Part No. 314002-T): One 300 µL sterile tube.
3. AAV Purification Matrix (Part No. 314003-T): One 1 mL sterile tube.
4. Purification Buffer (Part No. 314105-T): One 10 mL bottle.
5. Elution Buffer (Part No. 314106-T): One 2 mL tube.
6. 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. 15 mL conical tubes
5. Centrifuge (capable of 10,000 x g)

Storage

Store ViraBind™ AAV Reagent B 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 one 15 cm dish or two 10 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.
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. Collect the AAV supernatant by centrifugation at 10,000 x g for 10 minutes. Discard the pellet.
8. The viral supernatant can be stored at -80ºC or immediately purified (see purification instructions below).

Protocol

I. Purification

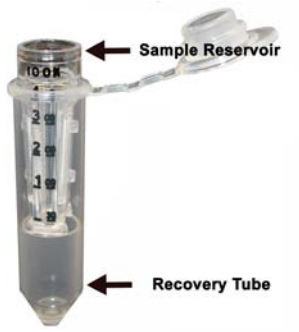
The following procedure is written for purification and concentration of 2.5 mL of AAV supernatant. For AAV samples that are less than 2.5 mL, add serum-free DMEM to the final volume of 2.5 mL.

1. Add 25 µL of ViraBind™ AAV Reagent A to 2.5 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 125 µL of ViraBind™ AAV Reagent B to the ViraBind™ AAV Reagent A pretreated 2.5 mL of viral supernatant, mixing well.
6. Incubate for 30 minutes at 37ºC.
7. Collect the AAV supernatant by centrifugation at 10,000 x g for 10 minutes. Discard the pellet.
8. Transfer the AAV supernatant to a clean 15 mL conical tube.
9. Resuspend the AAV Purification Matrix by inverting and shaking. Add 300 µL of matrix to the centrifuged supernatant.

10. 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.
11. Pellet the Purification Matrix by centrifugation for 10 minutes at 1,000 rpm.
12. Carefully remove the supernatant and wash the Purification Matrix with 2.5 mL of Purification Buffer.
13. Repeat steps 11-12 once more.
14. Carefully remove the final wash.
15. Elute the purified AAV from the purification matrix by adding 0.5 mL of Elution Buffer.
16. Mix at 4ºC for 10 minutes on an orbital shaker.
17. Collect the elution fraction by centrifugation for 10 minutes at 1,000 rpm.
18. Carefully remove the elution supernatant.

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 18 above) to the sample reservoir of the Centrifugal Concentrator. Cap the concentrator and place into a tabletop microcentrifuge. Centrifuge for 5 minutes at 2,000 x g. Flow through can be discarded.
3. 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.

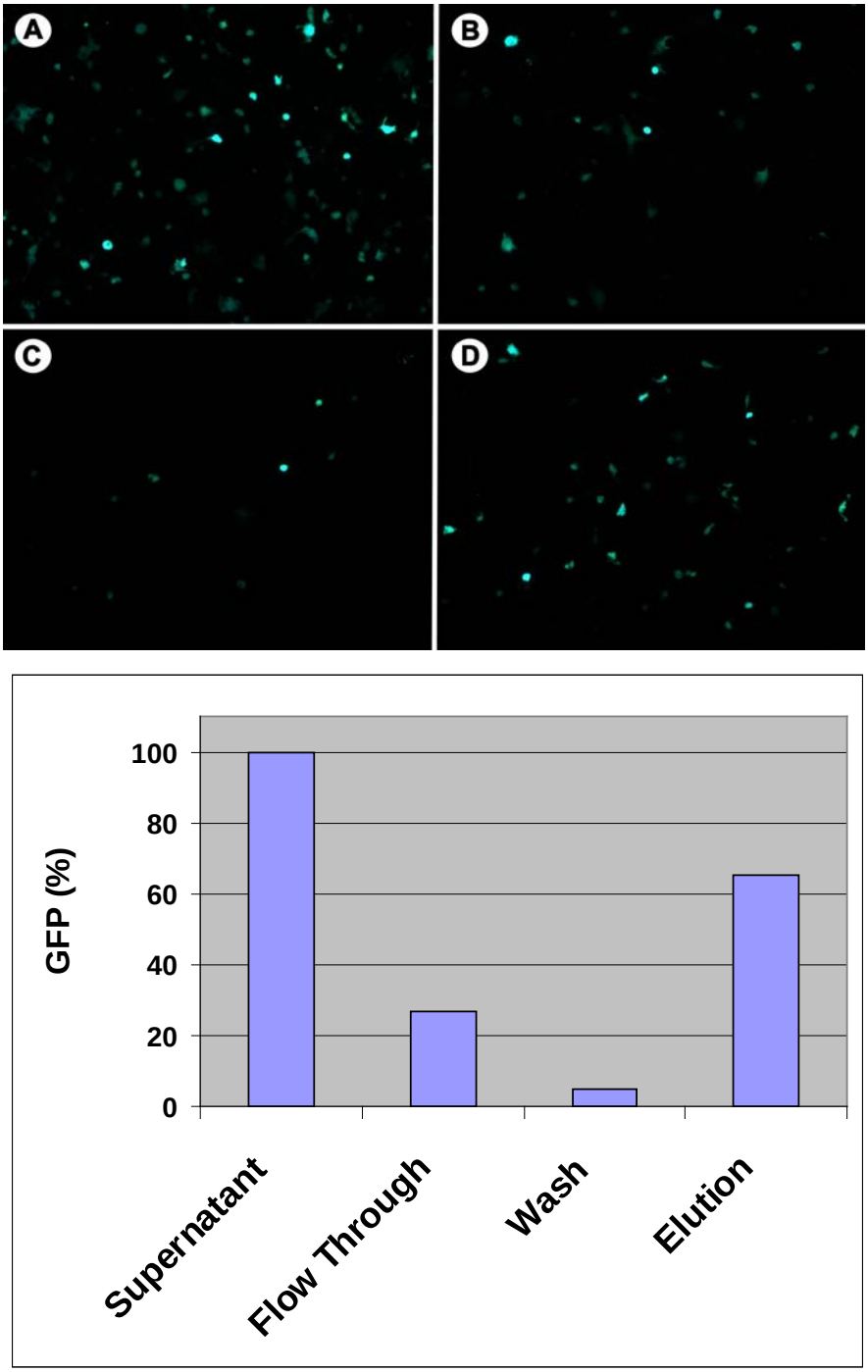


Figure 1: Purification of AAV2-GFP. AAV2-GFP was produced by a helper-free system in 293 cells. AAV supernatant was subjected to the purification steps. Samples from each fraction were used to infect 293 cells, GFP positive cells were scored by counting after three days. A: AAV Supernatant; B: Flow through;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

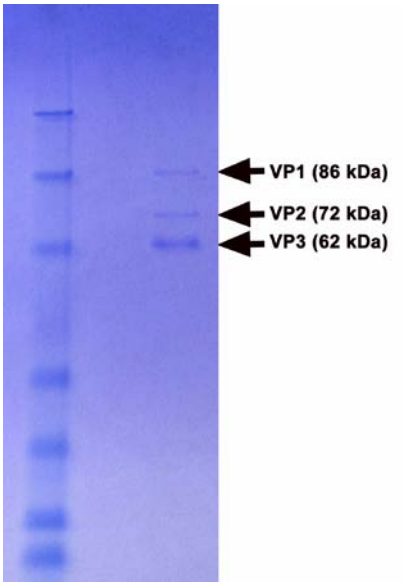


Figure 2: **Electrophoretic Profile of Purified AAV2-GFP.**

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Recent Product Citations

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