Adeno Associated Virus (AAV) Purification ViraKit™

Protocol

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR HUMAN CLINICAL PROCEDURES.
Please read through the entire kit instructions before performing virus infection and purification.

Catalog No. 003061 provides a single AAV purification application.
Catalog No. 003063 provides three single AAV purification applications.

Each kit contains the following components:		
	Catalog No. 003061	**Catalog No. 003063**
Components	**Quantity**	**Quantity**
Purification Filter Assembly[1]	1	3
Clarification Filter, bottle top	1	3
Glass Fiber Prefilter Disc	1	3
Dilution Buffer 1	40 ml	120 ml
Wash Buffer 2	30 ml	90 ml
Elution Buffer 3	10 ml	10 ml

1. Introduction – We make it easy

VIRAPUR provides an easy and quick membrane based method for laboratory scale purification of up to 10^{12} Virus Particles (AAV), Serotypes 2 and 5.

2. Overview and Precautions

a. In order to perform animal studies and some *in vitro* studies with AAV, it is usually necessary to purify the virus away from cellular contaminants and the expressed recombinant transgene. Purification results in concentrated virus in simple storage buffer that can be used for experimental studies. Traditionally, AAV has been purified by a cesium gradient ultracentrifugation process, but this cumbersome procedure involving large expensive equipment is lengthy. In addition, if you need large amounts of virus, the cesium procedure can be difficult to scale up.

b. The **AAV Purification ViraKit™** has assembled the technology, reagents and devices needed to perform AAV purifications from as little as one infected roller bottle or a maximum of six 15 cm dishes to get up to 10^{12} Viral Particles of purified AAV. This kit will make it as easy to purify AAV as it now is to purify plasmid DNA by Kit! Less time to purify and more time for experiments!

c. PRECAUTIONS: This kit permits the quick purification of AAV, an infectious agent which according to the National Institutes of Health (NIH) guidelines must be handled under Biosafety level 2 safety precautions (see: http://oba.od.nih.gov/rdna/nih_guidelines_oba.html). Although AAV is 99%+ replication incompetent, research viruses may over express their transgene, the transgene may be toxic or be involved in cellular regulation. All these unknowns indicate that a conservative safety approach should be followed and Biosafety level 2 practices should be used when appropriate. Wear hand, eye, face and body personal protection devices when manipulating AAV within a Class II Biosafety cabinet. Dispose of infected liquid and solid wastes according to NIH guidelines. Provider of kit takes no responsibility for improper use of kit.

d. STORAGE: The **AAV Purification ViraKit™** should be stored at room temperature between 10°C – 25°C.

3. User Provided Equipment and Supplies

- Table top centrifuge capable of spinning 100-200 ml at 2500 RPM
- Biosafety Cabinet for AAV manipulation
- Centrifuge tubes or bottles
- Sterile PBS (50 ml)
- One 20 ml syringe per preparation
- Three 3-5 ml syringes per preparation
- 37°C Water Bath

- Vacuum source
- Microcentrifuge tubes
- Microcentrifuge capable of 14,500 RCF if desired
- Microfuge capable of 14,500 RCF if desired.
- TurboNuclease or DNase

4. Summary of the Purification Procedure

a. AAV can be purified easily and quickly. First the user should release much of the virus from the transfected cells by multiple cycles of freezing and thawing. The cellular debris is removed by centrifugation leaving the viable virus particles in the supernatant. Supernatant is treated with DNase. The supernatant is further purified by passing it through a 0.45 micron filter. After adding **Dilution Buffer 1**, the virus solution is slowly passed over a treated filter which adsorbs the virus particles, allowing much of the cellular debris to pass through the filter. **Wash Buffer 2** is passed over the column to remove any bound debris, and the virus is eluted off the filter with **Elution Buffer 3**.

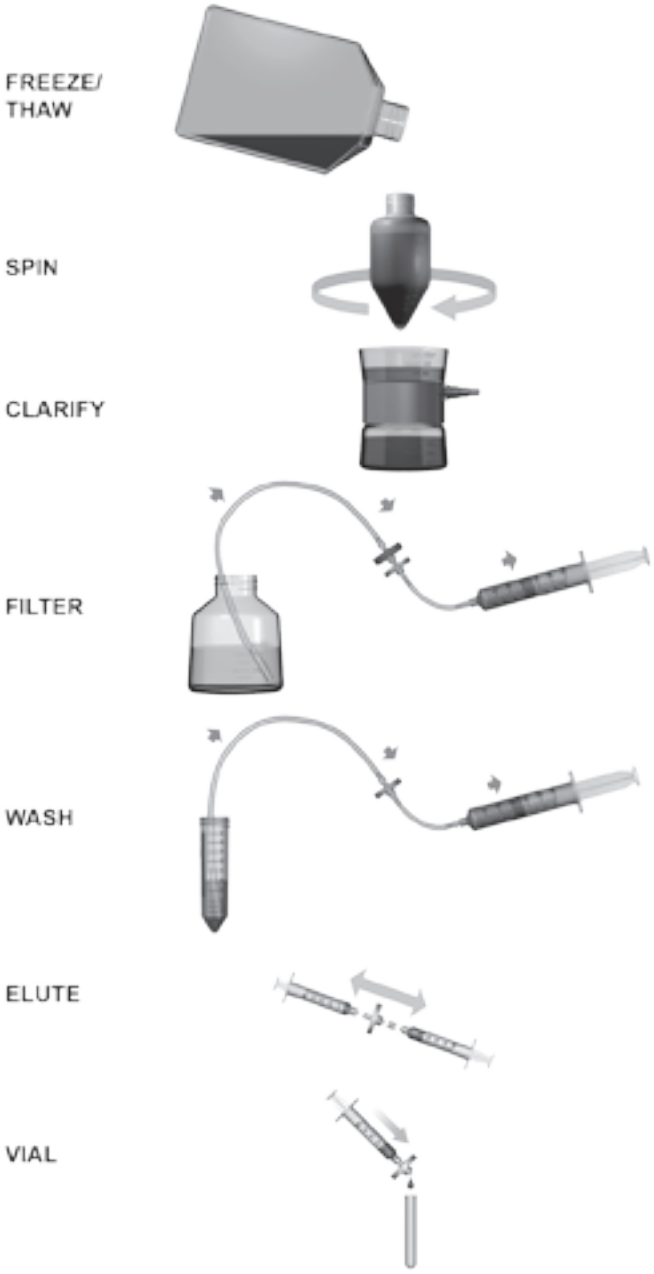

Figure 1

5. Initial Growth of cells in tissue culture vessels

a. HEK293 cells or their variants can be grown in tissue culture treated flasks. For the production of AAV, cells should be at a relatively early passage level. They should be kept on a regular passage program. Cells should not remain confluent for more than a few days. Cell that have remained confluent and unpassed for a more than several days can be passed at least one time at a low seeding density to reset the cells into an active growing state.

b. Cells should be seeded into the tissue culture flask at approximately 4 x 10^4 cells per cm^2 . The kit is designed to purify AAV from the equivalent of six 15 centimeter dishes for a total cell culture surface area of approximately 1050 cm^2. Recommended media: DMEM, high glucose with 4 mM glutamine and 10% Fetal Calf Serum. This media can be purchased through any reputable media vendor such as Life Sciences, JRH, Mediatech, or Irvine Scientific. The cell monolayer will become nearly confluent within approximately 2 to 4 days. You will transfect the cultures according to your own protocol with multiple plasmids. After transfection, harvest the cultures within 2 to 5 days by gently shaking or pipetting the cells off of the culture dish.

6. Harvest and Centrifugation of Infected Cell Lysate

a. At harvest, pool all the cell lysate and media into one capped vessel and freeze and thaw at least three times. Glass bottles are not suggested for this step as they may break. After the third thaw, pour the entire cell lysate into a centrifuge tube or bottle. Spin at 2500 to 2800 rpm for 30 minutes in a table top centrifuge. Collect the supernatant into another clean bottle; discard the pellet. The solution should be free of observable debris. If any flecks of debris are still observed, spin the supernatant again.

7. Recommended DNase Treatment pre bottle-top filter

a. Before using the bottle top filter unit to clarify the crude virus solution *and before* running the virus over the purification filter, you can remove much of the contaminating DNA by adding DNase to the virus supernatant, (1 µl for each 10 ml of your virus supernatant). Many laboratory grades of Bovine Pancreatic DNase will work, but one that is proven low in endotoxin may be preferred. As an optional item, you may obtain TurboNuclease[2] directly from VIRAPUR in a handy 50µl aliquot and the correct 25 units per µl concentration for this kit, Cat # 003005.

8. Prepare the Bottle Top Filter Unit

a. Unwrap the bottle top filter and place it in the biosafety hood. Carefully place a single **Glass Fiber Prefilter Disc** (provided) on top of the membrane in the top funnel of the filter unit. Attach the bottle top filter to a vacuum source and pre-wet the filters with approximately 5 ml of PBS or media. This pre-wetting adheres the prefilter to the 0.45 micron bottle top filter unit.

9. Clarify Infected Cell Lysate

a. Carefully pour the spun virus supernatant into the top of the filter unit, being careful not to flow the fluid stream under the prefilter or dislodge it. Filter all of the spun virus supernatant into the receiver bottle.

b. If it becomes full, disconnect the vacuum source from the filter unit, unscrew the top filter from the bottle and pour the filtrate into a clean new bottle that you have prepared. Reattach the screw-top filter to the bottle and finish filtering the virus supernatant.

10. Dilute Infected Cell Lysate (Use Dilution Buffer 1)

a. Measure the volume of the filtered supernatant.

b. Determine the volume of **Dilution Buffer 1** to be added to the filtered supernatant by using a ratio of 1 part **Dilution Buffer 1** for every 9 parts filtered supernatant. (For example: If you have 180 ml of filtered supernatant, add 20 ml of **Dilution Buffer 1.**) If necessary, pour the filtered supernatant into a new clean bottle that has a volume large enough for the diluted material.

c. Add the calculated volume of **Dilution Buffer 1** to the filtered supernatant. Mix gently but thoroughly. You are now ready to use the virus purification filter assembly.

11. Purification Procedure (Use Purification Filter Assembly)

Attach syringe to Purification Filter Assembly

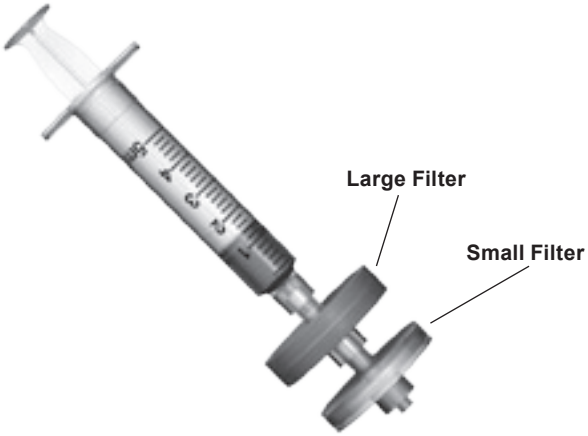

Figure 2

a. Attach the **Purification Filter Assembly** to a 3-5 ml syringe filled with sterile PBS (user provided) per **Figure 2**. Wet the interior of the **Purification Filter Assembly** by passing 3-5 ml of sterile PBS through the filters. Detach the syringe and prepare to attach the tubing assembly to the **Purification Filter Assembly**, and a 20-60 ml syringe per **Figure 3**.

Using purification filters to filter virus supernatant

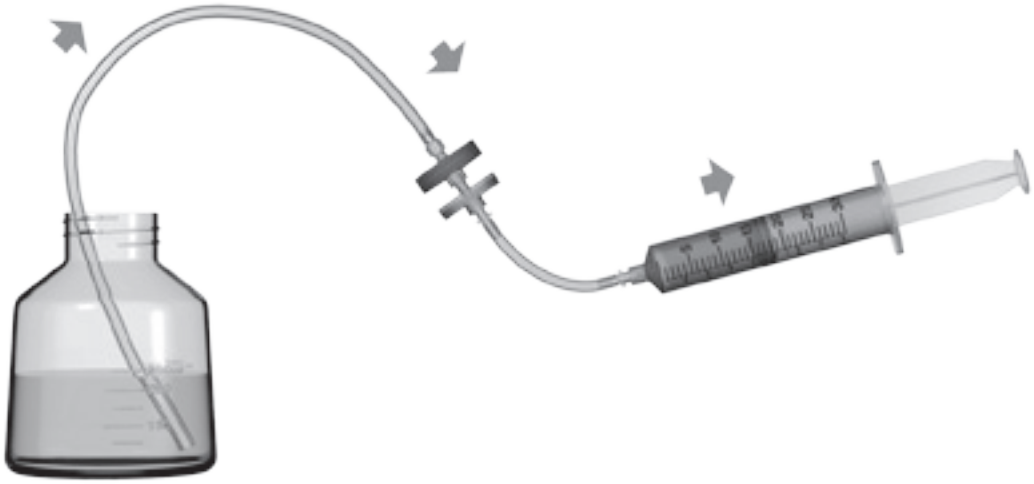

Figure 3

b. Pull the diluted virus supernatant through the filter assembly at a rate of about 20 ml per minute.
c. After the first syringe is full of media flow-through, pinch the two inch section of tubing on the syringe side of the filter to stop the fluid flow and remove the syringe.
d. Empty the syringe into a waste bottle large enough to accept the full volume of the diluted supernatant.
e. Reattach the syringe to the tubing and continue to pull the remaining virus supernatant through the purification filter at a rate of about 20 ml per minute, discarding each syringe-full until the entire volume has been filtered.
f. Disassemble the purification filter assembly and discard the LARGE filter.

12. Wash Procedure (Use Wash Buffer 2)

a. Reassemble the tubing assembly, SMALL filter, and syringe per **Figure 4**

b. Aliquot 30 ml of **Wash Buffer 2** into a sterile vessel (e.g. 50 ml conical tube).

c. Place the end of the tubing into **Wash Buffer 2** and pull the entire volume through the SMALL purification filter at a rate of about 20 ml per minute.

d. Empty the syringe into a waste bottle and discard syringe.

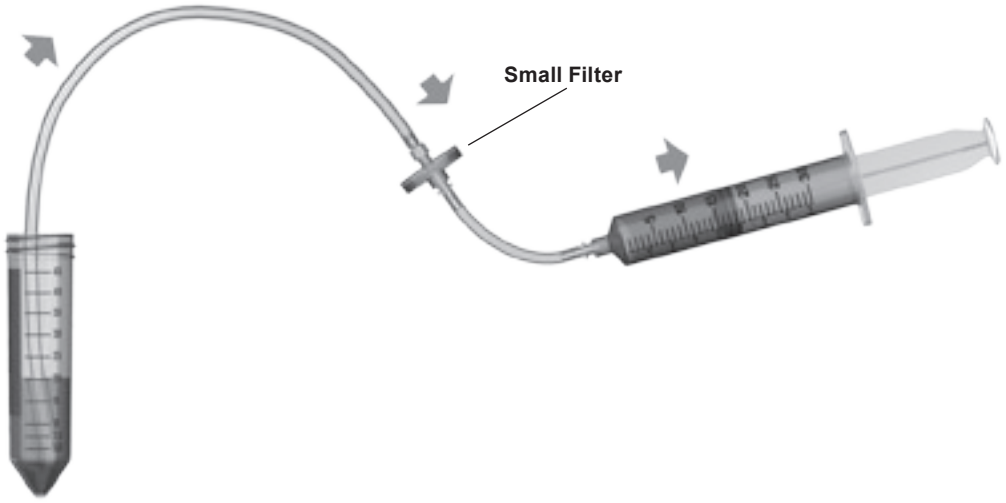

Figure 4

13. Elute Virus from the Filter (Use Elution Buffer 3)

a. Remove the tubing from both sides of the SMALL purification filter, and discard tubing.

b. Obtain two new 3 - 5 ml syringes. Draw approximately 1.5 ml of **Elution Buffer 3** into one syringe. Only 1.5 ml is sufficient to elute all bound virus, however, you may use more if you desire your virus to be more diluted.

c. Attach the two syringes to the SMALL filter using the female luer adaptor provided as shown in **Figure 5**.

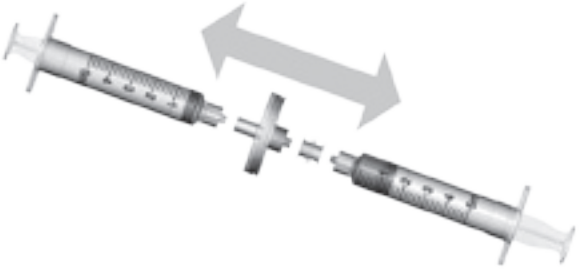

Figure 5

Figure 6

d. Pass the elution buffer slowly from the first syringe through the filter into the second syringe. Slowly pass the elution buffer back to the first syringe. Push a little air through the filter to collect all elution into one syringe, and discard the other empty syringe and filter.

Your virus is now in the syringe and in a moderate salt solution, **Figure 6**. It can be aliquoted and stored at 4º C. For long-term storage, 15% glycerol can be added and aliquots stored at -20º C.

14.

DISCLAIMER

VIRAPUR disclaims any and all responsibility for any injury or damage which may be caused by the failure of the buyer or any other person to use these products in accordance with the conditions outlined herein or in accordance with NIH guidelines for Biosafety Level 2 infectious agents and recombinant DNA material.

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1. Purification filter. Sartobine® is a registered trademark of Sartorius. The membrane adsorber technology is covered under U.S. Patent No. US005618418A.

2. TurboNuclease is manufactured by Accelagen, Inc. and is provided especially for VIRAPUR's ViraKits™ in aliquots of 50µl at 25 units per µl concentration, Catalog No. 003005.