Fast-Trap™

Adeno Associated Virus (AAV) Purification and Concentration Kit

User Guide

- For research use only.
- Not for use in diagnostic or human clinical procedures.

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VIRAPUR
Virus Purification Experts

This kit has been developed in conjunction with Virapur®, LLC.

Contents

Introduction

The Fast-Trap Adeno Associated Virus (AAV) Purification and Concentration Kit contains the reagents, filtration devices, and concentration devices necessary to purify the virus away from cellular contaminants and most expressed recombinant transgenes. It yields concentrated virus in the exchange buffer of choice, suitable for *in vitro* and animal studies. The kit provides a quick, easy, membrane-based method for the laboratory scale purification of AAV serotype 2 up to 1050 cm^2 total cell culture surface area.

The purification kit protocol calls for collecting the virus from the media and transfected cells using multiple freeze-thaw cycles. Most of the large cellular debris is removed by centrifugation, leaving the viable virus particles in the supernatant. The supernatant is further clarified by passing it through a Stericup®-HV 0.45 µm filter. After adding a dilution buffer, the virus solution is slowly passed through a series of filters that bind contaminants and adsorb the virus particles. Smaller cellular debris passes through the filters. Following a wash to remove any bound debris, the virus is eluted off the filter with an elution buffer. Finally, the virus can be concentrated and exchanged into the desired buffer using an Amicon® Ultra centrifugal filter unit.

Kit Components

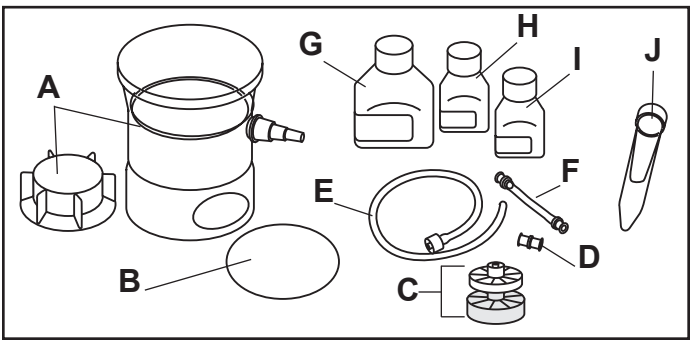
Millipore cat. no. FTAA 000 03 contains material for three single-use AAV purifications/concentrations with the following components:

3 – Purification filter assemblies, tubing sets, and luer adapters
3 – Stericup filtration systems
3 – Prefilter discs
1 – Dilution buffer, 120 mL
1 – Wash buffer, 90 mL
1 – Elution buffer, 30 mL
3 – Amicon Ultra-4 filter units, 50 kDa NMWL

Storing the Kit

The Fast-Trap AAV Purification and Concentration Kit should be stored at room temperature.

Parts and Functions of the Fast-Trap Kit



Letter	Part	Function
A	Stericup filtration system with cap	Clarifies virus sample
B	Prefilter disc	Protects membrane from fouling
C	Purification filter assembly	Big filter (yellow) — removes contaminants Small filter (clear) — captures virus
D	Female luer adapter	Connects syringes directly to small filter
E	Long tubing	Connects to big filter; draws from Stericup receiver bottle
F	Short tubing	Connects to small filter; allows small filter to be attached to syringe
G	Dilution buffer	Improves binding when added to clarified solution
H	Wash buffer	Washes non-bound or weakly bound components from the purification filter
I	Elution buffer	Removes bound virus from purification filter
J	Amicon Ultra filter unit	Concentrates virus sample and/or exchanges buffer

Additional Materials/Equipment

- Crude virus sample to be purified – up to 1050 cm^2 of transfected cell culture
- Dry ice/ethanol bath for freeze-thaw cycles
- Water bath, 37 °C
- Two 250 mL bottles, cleaned and sterilized
- One 20–60 mL syringe per preparation
- Three 3–5 mL syringes per preparation
- Sterile phosphate buffered saline (PBS), 50 mL
- Benzonase® nuclease or DNase

  NOTE: A grade of recombinant DNase/RNase such as Benzonase nuclease (EMD Biosciences cat. no. 71205), 1.0 µL enzyme per 10 mL of solution is recommended. Many laboratory grades of Bovine Pancreatic DNase may also be used, but one that is proven low in endotoxin may be preferred (for example, Sigma® cat. no. D4513, Type II-s from Bovine Pancreas).

- Vacuum source regulated to 15–23" Hg (for example house vacuum or chemical duty pump, Millipore cat. no. WP61 115 60)

  NOTE: House vacuum sources typically have a range of 20–23" Hg and can fluctuate.

  Use a Millex®-FA_{50} filter (or equivalent) and a vacuum flask to protect the vacuum source from contamination.

Additional Materials/Equipment, continued

- Equipment/Buffers for concentration and buffer exchange
  - Centrifuge with safety-covered, swinging bucket (preferred) or fixed angle rotor, capable of handling 17 mm × 124 mm 15 mL conical-bottom tubes at 1,500 × g
  - Exchange buffer of choice
  - Pipettor with 200 µL barrier tip (for concentrate sample recovery)
  - Microcentrifuge tube (for final concentrate sample)

Usage Guidelines

▲ WARNING: This kit permits the quick purification of AAV, an infectious agent which should be handled under BSL2 safety precautions. Wear hand, eye, face and body personal protective equipment (PPE) when using this kit. Provider of kit takes no responsibility for improper use of kit.

▲ WARNING: All virus purification steps (except water bath incubation and centrifugation) should be conducted in a biosafety cabinet.

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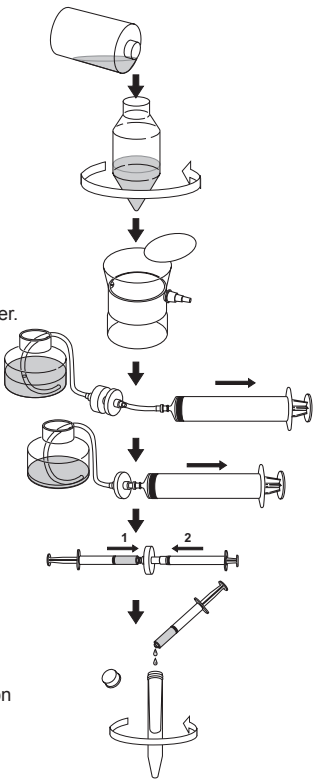
■ Variation in the amount of AAV purified with this kit may be due in part to transfection efficiency and cell conditions.

Overview of Procedure

1. Grow, seed, and transfect cells.
2. Harvest cells and media, and prepare cell lysate.
3. Add nuclease and filter through Stericup device to clarify viral supernatant.
4. Dilute viral supernatant with dilution buffer.
5. Pass viral supernatant through purification filter assembly.
6. Remove big filter and wash remaining small filter with wash buffer.
7. Elute virus with elution buffer.
8. Transfer eluted virus and exchange buffer to Amicon Ultra filter unit and centrifuge.
9. If desired, add exchange buffer to Amicon Ultra filter unit and centrifuge.
10. Recover virus sample from filter unit.



Virus Purification Protocol

This section breaks down the virus purification process into multiple procedures. The steps of each procedure should be followed carefully in order to ensure the best results for the Fast-Trap AAV Purification and Concentration Kit.

NOTE: The kit is designed to purify AAV from a cell culture surface area of approximately 1050 cm^2.

CAUTION: All virus purification steps (except incubation and centrifugation) should be conducted in a biosafety cabinet.

Cell Growth

HEK293 cells can be grown in tissue culture treated vessels. Use cells from an early passage level and keep them in a regular passage program to ensure optimal AAV production. If cells are confluent and have not been passaged for several days, seed cells sparsely at least once to bring them back into an active growing state.

1. Seed cells into the tissue culture flask at approximately 4×10^4 cells per cm^2.
2. Feed cells with recommended media (for example, DMEM, high glucose with 4 mM glutamine, Millipore cat. no. SLM-121-B, and 10% Fetal Calf Serum, Millipore cat. no. 1040-90, with antibiotics and supplements if required).
3. Culture cells until 70 to 80% confluency is achieved (approximately 2 to 4 days).
4. Transfect the cultures with multiple plasmids according to desired protocol.

Harvesting

1. After 2 to 3 days, collect the cells and media in capped vessels and freeze and thaw at least three times, using a dry ice/ethanol bath. After the third thaw, transfer approximately 100 mL of cell lysate into a centrifuge tube or bottle.

   NOTE: Glass bottles are not recommended for this step as they may break.

2. Centrifuge at 2500 to 2800 × g for 30 minutes. Collect the supernatant into another clean bottle; discard the pellet. The solution should be free of observable debris. If any traces of debris are observed, centrifuge the supernatant again.

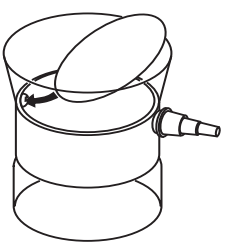
Clarification

▲ **WARNING:** Wear eye protection whenever using plastic vessels under partial vacuum.

1. It is recommended that the contaminating DNA be removed by adding Benzonase nuclease (1 µL for each 10 mL of crude virus), or the equivalent DNase (100 Kunitz units for each 10 mL of crude virus).

2. Cap container and gently invert the solution to mix thoroughly. Incubate at 37 °C for 30 minutes.

3. Unwrap the Stericup system and place it in the biosafety hood. Carefully install a single glass fiber prefilter disc under the tab in the top of the membrane funnel.



Clarification, continued

4. Attach the Stericup system to a vacuum source and pre-wet the filters with approximately 3 mL of sterile PBS or media to adhere the prefilter disc to the Stericup filter.

5. Carefully pour the viral supernatant into the membrane funnel, taking care not to dislodge the prefilter. Filter all of the viral supernatant into the receiver bottle.

   NOTE: For more information about using the Stericup system, please go to www.millipore.com and search for ***Stericup user guide*** in the ***Technical Library***.

6. Disconnect Stericup system from vacuum source. Remove and cap receiver bottle, and discard Stericup funnel.

Dilution

1. Measure the volume of the clarified crude virus.

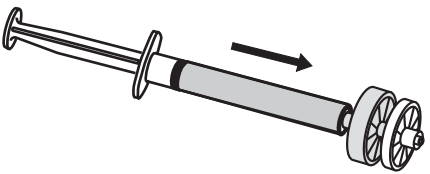
2. Determine the volume of dilution buffer to be added.

   Use 1 mL of dilution buffer for every 9 mL of clarified virus solution in the receiver bottle.

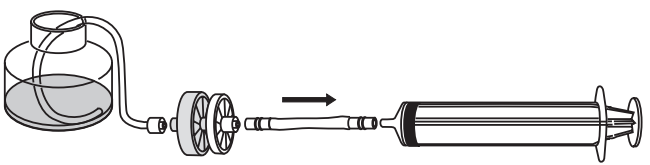
3. Add the calculated volume of dilution buffer to the filtered supernatant. Cap and mix gently but thoroughly.

Purification

1. Attach the purification filter assembly to a 3–5 mL syringe filled with sterile PBS. Wet the membranes by passing about 5 mL of sterile PBS through the filter assembly.



2. Detach the syringe.
3. Attach the purification filter assembly to the tubing assembly and a 20–60 mL syringe as shown below.



4. Pull the diluted viral supernatant through the purification filter assembly at a rate of about 20 mL per minute.
5. When the 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.
6. Empty the syringe into a waste bottle whose volume is at least equal to the volume of the diluted supernatant.

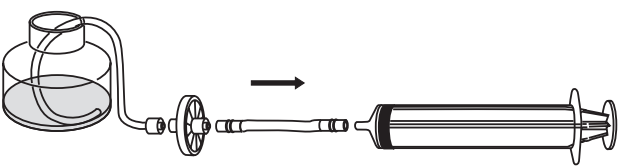
Purification, continued

7. Reattach the syringe to the tubing, release the pinch and continue to pull the remaining viral supernatant through the purification filter assembly at a rate of about 20 mL per minute. Discard each syringe-full of media flow-through until the entire volume has been filtered.

8. Disassemble the purification filter assembly and discard the large filter.

Washing

1. Reassemble the tubing assembly, small filter, and 20–60 mL syringe as shown in figure below.

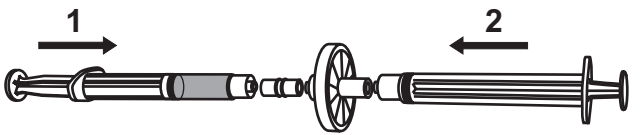


2. Aliquot 30 mL of wash buffer provided in the kit into a clean vessel (e.g., a Stericup receiver bottle or a 50 mL conical tube).

3. Place the end of the tubing into the wash buffer and pull the entire volume of the wash buffer through the small purification filter at a rate of about 20 mL per minute.

Elution

1. Remove the tubing from both sides of the small purification filter.
2. Obtain two new 3–5 mL syringes. Draw approximately 1.5 mL of elution buffer into one syringe.
3. Attach the two syringes to the small filter using the provided female luer adaptor as shown below.



4. 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. Pass a little air through the filter to collect all elution into one syringe.

Buffer Exchange and Concentration

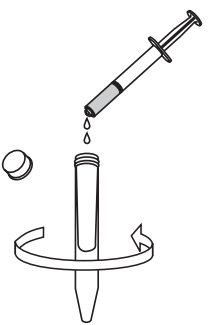
The Amicon Ultra-4 filter unit (50 kDa NMWL) may be used to further concentrate the virus. It may also be used for a complete buffer exchange into the buffer of choice. For more details on Amicon Ultra filter units, please go to www.millipore.com and search for ***Amicon Ultra user guide*** in the ***Technical Library***.

1. (Optional) Pre-rinsing: The Amicon Ultra filter unit contains trace amounts of glycerine. If this material interferes with analysis, pre-rinse the filter unit with 4 mL of exchange buffer or deionized water.

   CAUTION: Do not allow the membrane in Amicon Ultra filter unit to dry out once wet. If the filter unit is not being used immediately after pre-rinsing, leave fluid on the membrane until the filter unit is used.

2. Add the eluted virus sample to the Amicon Ultra filter unit. Bring sample volume up to a total of 4 mL with exchange buffer and cap unit.

   NOTE: If using a 23 degree fixed angle rotor, maximum volume should not exceed 3.5 mL.



3. Centrifuge at 1,500 × g for approximately 5 to 10 minutes. Check concentrate volume at 5 minutes. Centrifuge until 200–250 µL remain.

   N**OTE:** Do not allow the volume to be reduced below 200 µL, as this may cause the virus to aggregate. With higher titers, it may be necessary to increase the minimum volume.

Buffer Exchange and Concentration, continued

4. Discard filtrate in centrifuge tube appropriately and place filter unit back into centrifuge tube.

5. Bring sample volume up to a total of 4 mL (3.5 mL for 23 degree fixed angle rotor) with exchange buffer and cap filter unit.

6. Centrifuge at 1,500 × g for 5 minutes. Check the concentrate volume in the filter unit. Repeat centrifugation as required to reach desired volume. **Do not** allow the volume to be reduced below 200 µL, as this may cause the virus to aggregate. With higher titers, it may be necessary to increase the minimum volume.

7. To recover the final concentrated virus sample, insert a 200 µL pipettor into the bottom of the filter unit and withdraw the sample in several aliquots. Transfer the final concentrated virus sample to a microcentrifuge tube.

   NOTE: For optimal recovery, remove concentrated virus sample immediately after centrifugation.

8. Discard the filtrate, centrifuge tube, and filter unit appropriately.

Troubleshooting

Problem	Suggestions
Clarification step	
Membrane fouled/ clogged	Make sure prefilter is in place above the Stericup filter. Filter through another Stericup system. Re-centrifuge to remove large cellular debris.
Purification step	
Low recovery	Check flow rate (should be 20 mL/min.) Make sure big filter has been discarded and elution is being performed with small filter. Flush sample back and forth between two syringes to increase elution efficiency.
Buffer Exchange and Concentration step	
Low recovery	Do not over concentrate; virus may aggregate in volumes below 200 µL. With higher titers, it may be necessary to increase the minimum volume.

Technical Assistance

For more information, contact the Millipore office nearest you. In the U.S., call **1-800-MILLIPORE** (1-800-645-5476). Outside the U.S., see your Millipore catalogue for the phone number of the office nearest you or go to our web site at www.millipore.com/offices for up-to-date worldwide contact information. You can also visit the tech service page on our web site at www.millipore.com/techservice.

Product Ordering Information

This section lists catalogue numbers for the Fast-Trap AAV Purification and Concentration Kit. See “Technical Assistance” for information about contacting Millipore. You can also purchase Millipore products on-line at www.millipore.com/products.

Kits

Product	Catalogue No.	Qty/Pk
Fast-Trap AAV Purification and Concentration Kit: filtration devices and reagents for 3 samples	FTAA 000 03	3

Kit Components

Product	Catalogue No.	Qty/Pk
Stericup-HV filtration system, 150 mL	SCHV U01 RE	12
Prefilter	AP20 075 00	100
Amicon Ultra-4 filter units, 50 kDa NMWL	UFC8 050 08	8

Product Ordering Information, continued

Accessories

Product	Catalogue No.	Qty/Pk
DMEM with Glucose and L-Glutamine	SLM-121-B	500 mL
Fetal Calf Serum	1040-90	500 mL
Benzonase Nuclease	EMD Biosciences cat. no. 71205	25 KU
Bovine Pancreatic DNase Type II-s	Sigma cat. no. D4513	11 mg
Chemical duty pump	WP61 115 60 WP61 220 50	1 1
Millex®-FG_{50} filter unit	SLFG 050 10	10
Vacuum flask for vacuum trap, 2 L	XX16 047 05	1

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