MILLIPORE

Fast-Trap®

Adenovirus Purification and Concentration Kit

User Guide

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

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Contents

Introduction

The Fast-Trap Adenovirus Purification and Concentration Kit contains the reagents, filtration devices, and concentration devices necessary to purify the virus away from cellular contaminants and the expressed recombinant transgene. 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 ≥ 3 mL of adenovirus serotype 5, up to a capacity of approximately $1x10^{13}$ total adenovirus particles.

The purification kit protocol calls for releasing the virus from the infected cells using multiple freeze-thaw cycles, then collecting the crude adenovirus. 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 Steriflip®-HV 0.45 µm filter. After adding a binding buffer, the virus solution is passed over a treated filter that adsorbs the virus particles, allowing the smaller cellular debris and media to pass through the filter. Following a wash to remove any weakly bound proteins, 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

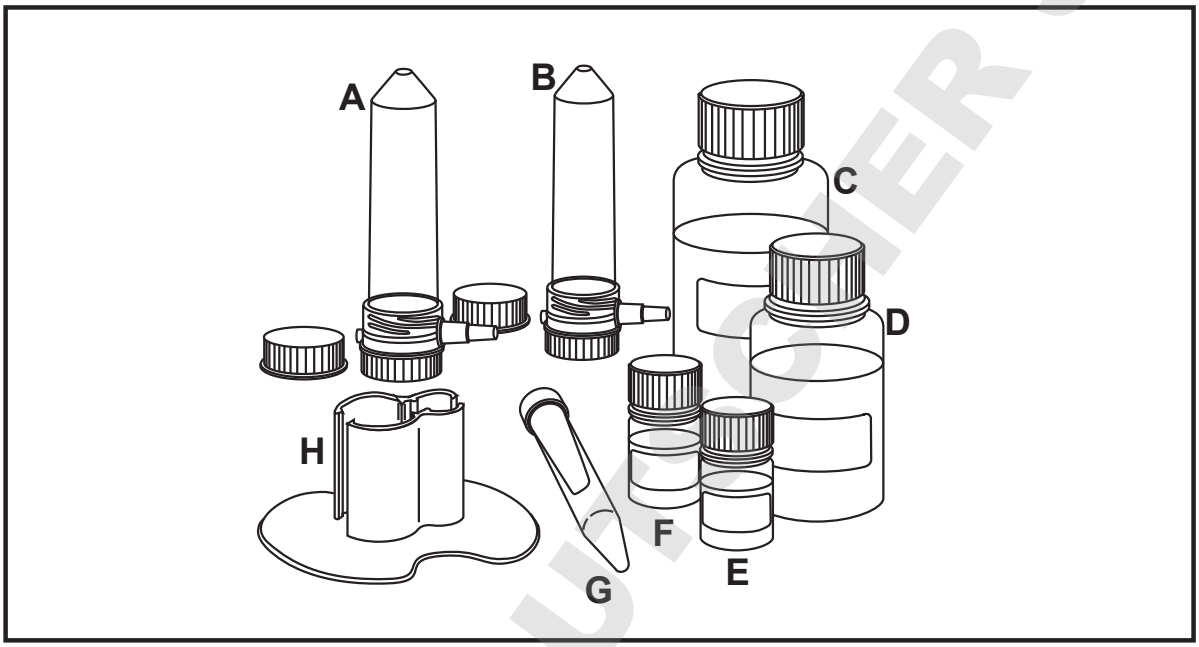
Millipore cat. no. FTAV 000 03 contains material for three single-use adenovirus purification/concentrations with the following components:

3 – Fast-Trap virus filter units
3 – Steriflip-HV filter units
1 – 10x Binding buffer, 20 mL
1 – Equilibration buffer, 100 mL
1 – Wash buffer, 200 mL
1 – Elution buffer, 21 mL
3 – Amicon Ultra-4 filter units, 50,000 NMWL
1 – Test tube stand
1 – User Guide
1 – Overview of Procedure card
1 – Material Safety Data Sheet

Storing the Kit

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

Parts and Functions of the Fast-Trap Kit



Letter	Part	Function
A	Steriflip-HV filter unit with cap	Clarifies virus sample
B	Fast-Trap virus filter unit with cap	Captures/elutes purified virus
C	Wash buffer	Removes weakly bound proteins
D	Equilibration buffer	Treats Fast-Trap virus filter unit
E	10x Binding buffer	Improves binding of virus sample
F	Elution buffer	Elutes purified virus from Fast-Trap virus filter unit
G	Amicon Ultra filter unit	Concentrates virus sample and/or exchanges buffer
H	Test tube stand	Holds Steriflip-HV filter unit and Fast-Trap virus filter unit

Additional Materials/Equipment

- Crude virus sample to be purified
- Dry ice/ethanol bath for freeze-thaw cycles
- 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 Aldrich® cat. no. D4513, Type II-s from Bovine Pancreas).
- Assorted pipettors, disposable pipettes, and aerosol barrier pipette tips
- Water bath, 37 °C
- 50 mL polypropylene tubes with double-lead threads (leakage may occur if using single-lead tube). Examples of appropriate tube types are shown in the following table:

Tube	Catalogue Number
BD Falcon™	352070, 352098
Fisherbrand®	05-539-6, 05-539-7, 05-539-8, 05-539-9
Greiner®	210261, 210270, 227261, 227270
Iwaki®	2341-050
Nunc™	334959, 334940
Perfector Scientific	2650

Additional Materials/Equipment, continued

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

  If a water aspirator is being used as the vacuum source, use a check-valve or in-line catch flask to prevent the system from accidentally drawing water into the receiving bottle.

- (Optional) Steriflip filter unit funnel top (Millipore cat. no. SC50 FL0 25). For more information about using the Steriflip funnel top, please refer to the Steriflip Filter Unit User Guide which can be found at www.millipore.com (search on **Steriflip User Guide**).

- 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 quick purification of adenovirus, 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. Millipore Corporation takes no responsibility for improper use of this kit.

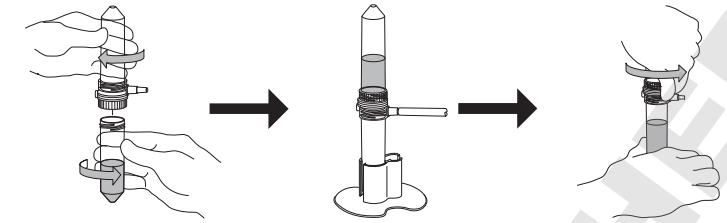
▲ **WARNING:** All virus purification steps (except water bath incubation and centrifugation) should be conducted in a biosafety cabinet.

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

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

Overview of Procedure

1. After collecting crude adenovirus, clarify by filtering through Steriflip-HV filter unit.



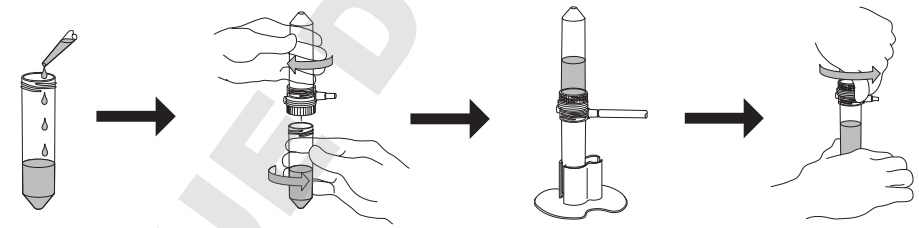
Connect Steriflip-HV filter to tube of crude adenovirus

Flip and apply vacuum

Disconnect and remove

2. Dilute crude adenovirus with 10x Binding buffer.

Using the Fast-Trap virus filter unit, follow the sequence below for Equilibration (step 3), Binding (step 4), Washing (step 5), and Elution (step 6).



Add appropriate solution to tube

Connect Fast-Trap virus filter unit to tube

Flip and apply vacuum

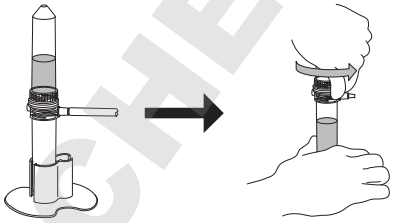
Disconnect and remove

3. Apply Equilibration buffer to Fast-Trap virus filter unit and filter.
4. Apply diluted crude adenovirus from step 2 to Fast-Trap virus filter unit and filter.
5. Apply Wash buffer to Fast-Trap virus filter unit and filter.
6. Apply Elution buffer to Fast-Trap virus filter unit and filter approximately 5 drops through.

Overview of Procedure, continued

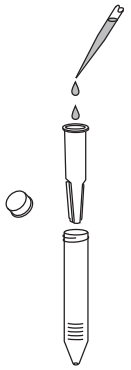
7. Incubate for 5 minutes.

8. Filter remaining Elution buffer to elute virus.



9. (Optional) Repeat steps 6 through 8.

10. Transfer eluted virus and exchange buffer to Amicon Ultra filter unit and centrifuge.
11. Add exchange buffer to Amicon Ultra filter unit and centrifuge.
12. 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 Adenovirus Purification and Concentration Kit.

NOTE: This kit is intended for use with volumes of 3 mL or greater.

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 adenovirus 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 x 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 90% confluency is achieved.
4. Infect the cultures with adenovirus according to desired protocol and incubate until cells round up and detach from the cell culture surface.

Harvesting

NOTE: Harvesting too early (cells remain attached and have not achieved optimal adenovirus production) or too late (cells break open and release virus into supernatant) may result in decreased viral titer.

1. Gently shake or pipet cells off the culture flask.
2. Pool the cells and media into 50 mL centrifuge tubes or capped vessels. Centrifuge at 500 to 1,500 × g for 15 minutes and resuspend pellet in fresh media. Freeze and thaw three times using a dry ice/ethanol bath. After the third thaw, transfer the cell lysate into a centrifuge tube or bottle.
3. Centrifuge at 2,500 × g for 15 to 30 minutes. Collect the supernatant into clean 50 mL polypropylene centrifuge tubes; discard the pellet(s). The solution should be free of observable debris. If any traces of debris are observed, centrifuge the cell lysate again.

Clarification

CAUTION: To ensure proper filtration, be sure to keep the Steriflip-HV filter unit in an upright position while filtering.

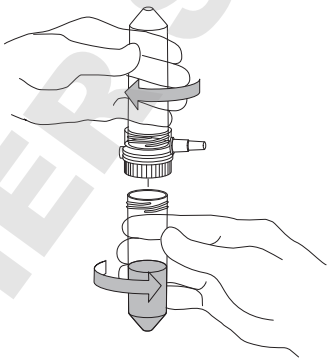
▲ **WARNING:** Wear eye protection whenever using plastic vessels under partial vacuum.

1. It is recommended that the contaminating DNA be removed by adding Benzonase nuclease (1 µL for each 10 mL of crude adenovirus), or the equivalent DNase (100 Kunitz units for each 10 mL of crude adenovirus).
2. Cap container and gently invert the solution to mix thoroughly. Incubate at 37 °C for 30 minutes.
3. In the biosafety hood, unwrap the Steriflip-HV filter unit using aseptic technique.

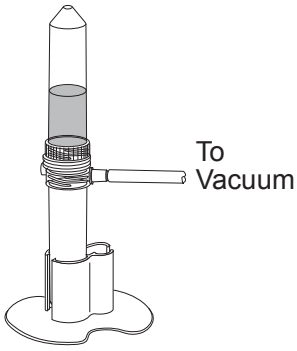
Clarification, continued

4. Unscrew the cap of Benzonase nuclease (or DNase) treated virus sample tube. **Connect** the Steriflip-HV filter unit to the top, open end of the sample tube. **Secure** it tightly.

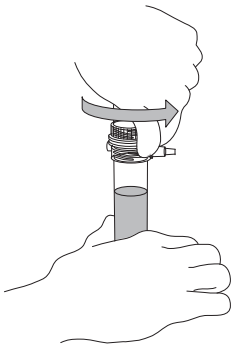
   NOTE: For more information about using the Steriflip filter unit, please refer to the Steriflip Filter Unit User Guide which can be found at www.millipore.com (search on **Steriflip User Guide**).



5. **Flip** the assembly over so that the sample tube is above the Steriflip-HV filter unit. Place the complete assembly in the Test tube stand.
6. Attach vacuum source to the vacuum port on the side of the Steriflip-HV filter unit. The universal tubing adapter on the Steriflip-HV unit fits most vacuum hoses.



7. Turn on the vacuum source and draw the solution through the Steriflip-HV filter unit into the empty sample tube at the base.
8. Disconnect the vacuum source when filtration ends. Remove the Steriflip-HV filter unit, along with the empty sample tube, and dispose of appropriately. Using aseptic technique, cap and save the tube containing the filtered sample, and reserve for processing in the **DILUTION** procedure that follows.



Dilution

1. Measure the volume of the clarified crude adenovirus.
2. Determine the volume of 10x Binding buffer to be added.

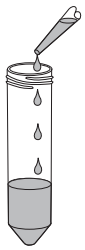
   Use 1 mL of 10x Binding buffer for every 9 mL of clarified virus in the collection tube.
3. Add the calculated volume of 10x Binding buffer to the clarified adenovirus. Cap and mix gently but thoroughly.
4. The diluted, clarified virus sample is now ready to use in the **VIRUS BINDING** procedure.

Equilibration

CAUTION: To ensure proper filtration, be sure to keep the Fast-Trap Virus filter unit in an upright position while filtering.

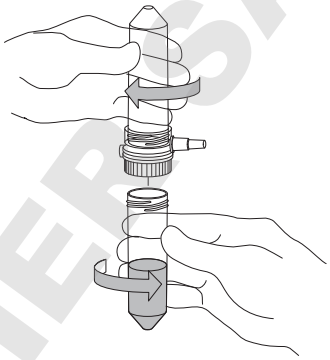
▲ **WARNING:** Wear eye protection whenever using plastic vessels under partial vacuum.

1. Prepare a 50 mL polypropylene tube with 25 mL of Equilibration buffer, and place it in the Test tube stand.

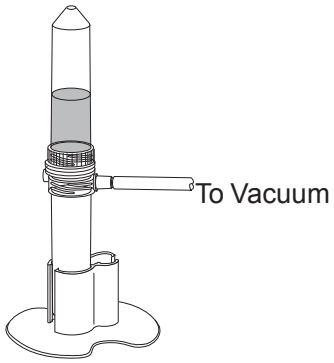


Equilibration, continued

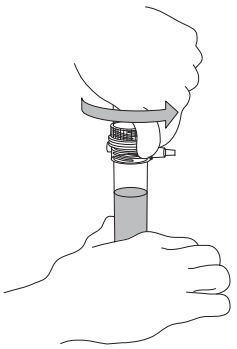
2. Remove the Fast-Trap virus filter unit from packaging and **connect** by screwing it to the top of the 50 mL tube containing Equilibration buffer. **Secure** tightly.



3. **Flip** the assembly over so that the sample tube is above the Fast-Trap virus filter unit. Place the complete assembly in the Test tube stand.
4. Attach vacuum source to the vacuum port on the side of the filter unit.
5. Turn on the vacuum source and draw the Equilibration buffer through the Fast-Trap virus filter unit into the empty sample tube at the base.
6. Disconnect the vacuum source when filtration ends.



7. Remove the tube containing the filtered Equilibration buffer and discard appropriately. Using aseptic technique, attach a new 50 mL tube to the Fast-Trap virus filter unit. Remove empty Equilibration buffer tube and discard tube appropriately. Place assembly in Test tube stand.
8. The Fast-Trap virus filter unit is ready for the **VIRUS BINDING** procedure that follows.

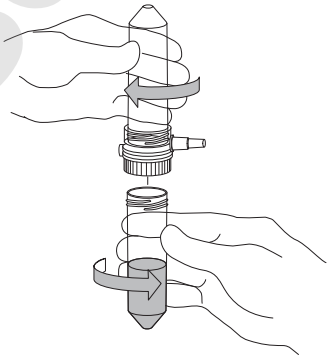


Virus Binding

CAUTION: To ensure proper filtration, be sure to keep the Fast-Trap virus filter unit in an upright position while filtering.

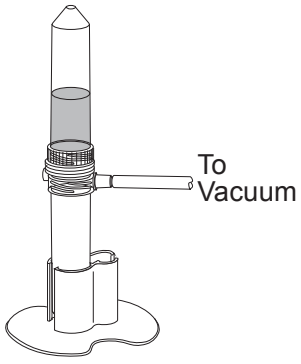
▲ **WARNING:** Wear eye protection whenever using plastic vessels under partial vacuum.

1. Place the 50 mL tube of virus diluted with 10x Binding buffer (as prepared in the **DILUTION** procedure) in the Test tube stand.



2. **Connect** and **secure** the Fast-Trap virus filter unit to the top of the 50 mL sample tube containing diluted virus.

3. **Flip** the assembly over so that the sample tube is above the Fast-Trap virus filter unit. Place the complete assembly in the Test tube stand.



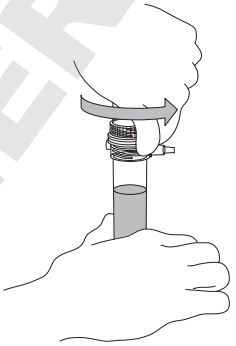
4. Attach vacuum source to the vacuum port on the side of the filter unit.

5. Turn on the vacuum source and draw the diluted virus sample through the Fast-Trap virus filter unit into the empty sample tube at the base.

   NOTE: As adenovirus binds to the Fast-Trap virus filter unit, a decrease in flow rate may be observed.

Virus Binding, continued

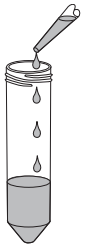
6. Disconnect the vacuum source when filtration ends.
7. Remove the tube containing the filtered adenovirus and discard the filtrate appropriately. Using aseptic technique, attach a new 50 mL tube to the Fast-Trap virus filter unit. Remove the empty crude adenovirus tube and discard appropriately. Place assembly in Test tube stand.



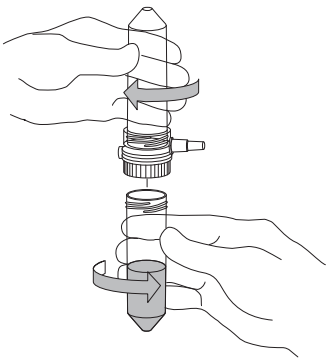
8. The assembly is ready for the **WASHING** procedure that follows.

Washing

1. Prepare a 50 mL polypropylene tube with 20 mL of Wash buffer and place it in the Test tube stand.

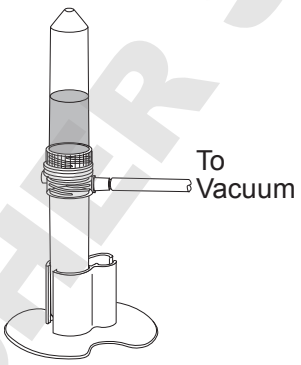


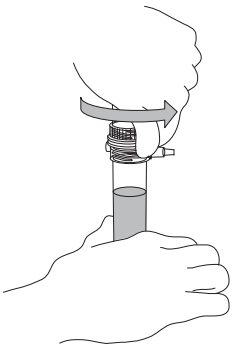
2. **Attach** and **secure** the Fast-Trap virus filter unit to the top of the 50 mL sample tube containing Wash buffer.



Washing, continued

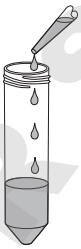
3. **Flip** the assembly over so that the Wash buffer tube is above the Fast-Trap virus filter unit. Place the complete assembly in the Test tube stand.
4. Attach vacuum source to the vacuum port on the side of the filter unit.
5. Turn on the vacuum source and draw the Wash buffer through the Fast-Trap virus filter unit into the empty sample tube at the base.
6. Disconnect the vacuum source when filtration ends.
7. Remove the tube containing the filtered Wash buffer and discard the tube appropriately. Using aseptic technique, attach a new 50 mL tube to the Fast-Trap virus filter unit. Remove empty Wash buffer tube and discard tube appropriately. Place assembly in Test tube stand.
8. The assembly is ready for the **ELUTION** procedure that follows.



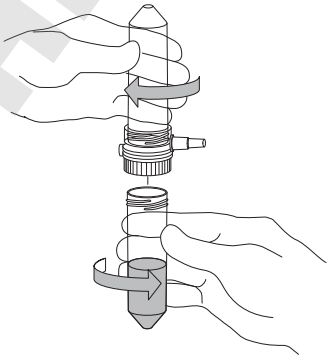


Elution

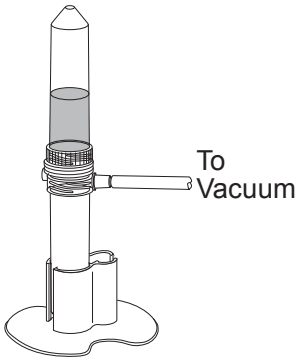
1. Prepare a 50 mL polypropylene tube with 3 mL of Elution buffer, and place it in the Test tube stand.



2. **Attach** and **secure** the Fast-Trap virus filter unit to the top of the 50 mL sample tube containing the Elution buffer.

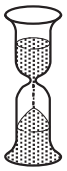


3. **Flip** the assembly over so that the Elution buffer tube is above the Fast-Trap virus filter unit. Place the complete assembly in the Test tube stand.
4. Attach vacuum source to the vacuum port on the side of the filter unit.
5. Turn on the vacuum source and **draw approximately 5 drops** of the Elution buffer through the filter unit into the empty sample tube at the base to ensure adequate buffer contact with the entire filter surface.
6. Disconnect and turn off the vacuum source.

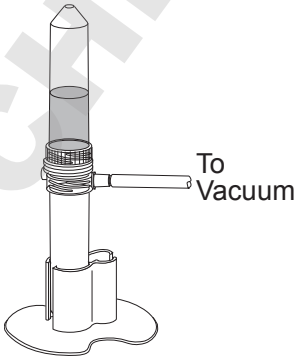


Elution, continued

7. Incubate for 5 minutes.

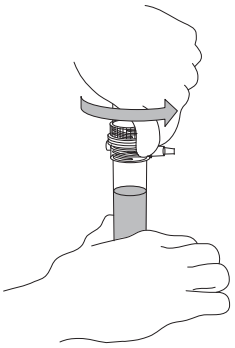


8. Re-connect vacuum source, turn vacuum on and continue until filtration is complete. Disconnect the vacuum source when filtration ends.



9. Unscrew the Fast-Trap virus filter unit and the tube containing the eluted virus sample. Cap and save the eluted virus sample for the **BUFFER EXCHANGE and CONCENTRATION** procedure that follows.

   NOTE: The eluted virus sample may appear slightly pink due to phenol red in the media.



10. (Optional) Repeat steps 1–9 above for a second, 3 mL elution and save eluted virus sample for **BUFFER EXCHANGE and CONCENTRATION.**
11. Discard the Fast-Trap virus filter unit and empty 50 mL tube appropriately.

Buffer Exchange and Concentration

The Amicon Ultra-4 filter unit (50,000 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 information on using Amicon Ultra filter units, please refer to the Amicon Ultra-4 User Guide which can be found at www.millipore.com (search on **Amicon Ultra 4 User Guide**).

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.

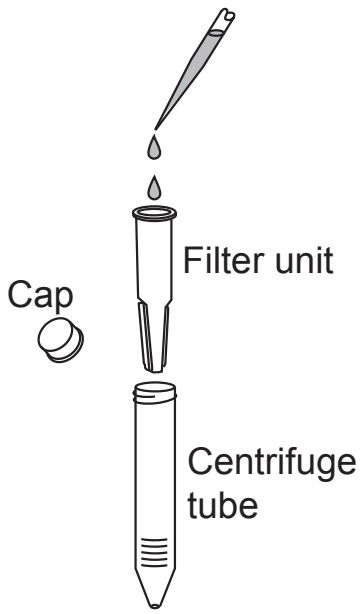
   CAUTION: Do not allow the membrane in Amicon Ultra filter unit to dry out once wet. If the filter unit is not being used immediately after pre-rinsing, leave fluid on the membrane until the filter unit is used.

2. Add approximately 3 mL of 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 500–600 µL remain.

   N**OTE:** Do not allow the volume to be reduced below 500 µL, as this may cause the virus to aggregate.



Buffer Exchange and Concentration, continued

4. Discard filtrate in centrifuge tube appropriately and place filter unit back into centrifuge tube. If there is no second elution, proceed to step 6.
5. **(Optional)** If a second elution was performed, uncap filter unit and add second eluted virus sample to the concentrated sample in the filter unit. Bring sample volume up to a total of 4 mL (3.5 mL for 23 degree fixed angle rotor) with exchange buffer and cap unit. Centrifuge again as in step 3.
6. 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.
7. 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 500 µL, as this may cause the virus to aggregate.
8. 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 adenovirus sample to a microcentrifuge tube.

   NOTE: For optimal recovery, remove concentrated virus sample immediately after centrifugation.
9. Discard the filtrate, centrifuge tube, and filter unit appropriately.

Troubleshooting

Problem	Suggestions
Clarification step	
Membrane fouled/ clogged	Filter through another Steriflip-HV filter unit
	Re-centrifuge to remove large cellular debris from supernatant
Purification step	
Slow flow/no flow	Flow rate is expected to decrease when loading high virus titer
	Do not exceed device capacity of approximately 1 x 10^{13} total virus particles
	Use vacuum pressure > 15" Hg
Low recovery	Filter approximately 5 drops of Elution Buffer through assembly to completely saturate the filter
	Perform second elution
	Use 10x Binding buffer prior to loading virus onto Fast-Trap virus filter unit
	Do not exceed device capacity of approximately 1 x 10^{13} total virus particles
Buffer Exchange and Concentration step	
Low recovery	Do not over concentrate; virus may aggregate in volumes < 500 µL

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 Adenovirus Purification and Concentration Kit. See "Technical Assistance" for more information about contacting Millipore. You can also purchase Millipore products on-line at www.millipore.com.

Kits

Product	Catalogue No.	Qty/Pk
Fast-Trap Adenovirus Purification and Concentration Kit: Filter units and reagents for 3 samples	FTAV 000 03	3/pk

Additional Components

Product	Catalogue No.	Qty/Pk
Steriflip-HV filter units, 50 mL	SE1M 003 M00	25/pk
Amicon Ultra-4 filter units, 50,000 NMWL	UFC8 050 08	8/pk

Product Ordering Information,
continued

Accessories

Product	Catalogue No.	Qty/Pk
Steriflip Funnel attachment, 50 mL	SC50 FL0 25	25/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 Aldrich cat. no. D4513	11 mg
Chemical duty pump	WP61 115 60 WP61 220 50	1/pk 1/pk
Millex® FG_{50} filter unit	SLFG 050 10	10/pk
Vacuum flask for vacuum trap, 2L	XX16 047 05	1/pk

Standard Warranty

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