VIRAPUR
Virus Purification Experts

Catalog Numbers **003058** **003059**

Adenovirus Mini Purification Virakit™

Adenovirus 5 and Recombinant Derivatives

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 003058 provides five Adenovirus purification applications.
Catalog 003059 provides twenty-four Adenovirus purification applications.

Each kit contains the following components:		
	Catalog No. 003058	**Catalog No. 003059**
Components	**Quantity**	**Quantity**
Purification Filters, Spin[1]	5	24
Loading Buffer A	2.5 ml	11 ml
Wash Buffer B	7.0 ml	30 ml
Elution Buffer C	2.5 ml	11 ml
Freeze Buffer D	500 µl	3 ml
One purification application can purify virus from infected cells harvested from a volume as small as one well of a 96-well plate up to a volume as large as one half of a 10 cm culture dish.		

1. Introduction – We make it easy

Virapur's Adenovirus Mini Purification ViraKit™ provides you with an easy, quick membrane based method to obtain at least 10^{10} virus particles of pure adenovirus from small volume samples for quick analysis of new viral constructs, or continuous monitoring of large scale bioprocesses, or small pilot experiments. Each spin filter in this kit can process a cell pellet from one well of a six-well dish.

Our **Adenovirus Standard Purification ViraKit™** (cat# 003051 & 003054) has provided laboratory scale purification of up to 10^{12} adenovirus particles since we introduced it to the research community in 2000.

2. Overview and Precautions

a. Virapur's Adenovirus purification kit is designed to purify and concentrate your recombinant virus derived from Serotype 5. In order to perform animal studies and some in vitro studies with adenovirus, whether recombinant or wild type, 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. Cesium chloride density gradients have been the time-tested way to purify adenovirus, but this cumbersome and tricky procedure involving large expensive equipment can take days.

b. Adenovirus purification using filter technology is effective and highly comparable to purification by cesium chloride gradient centrifugation. Our Adenovirus Purification ViraKit™ utilizes ion exchange chromatography technology, identical to procedures used to purify clinical grade adenovirus for human trials. Virus obtained through our method is appropriate for in vitro studies and is of high titer and high purity. The ViraKit™ surpasses previous methods of adenovirus purification (cesium chloride density gradient ultracentrifugation), saving the user time, money and generating an end product of equal quality. An additional buffer exchange into user selected buffer can be performed using a centrifugal filter device.

c. Our Adeno ViraKit™ now purifies virus from the infected cell pellet, increasing the efficiency and speed of the virus extraction and purification procedure. The Adeno Purification ViraKit™ Mini will purify up to 1 x 10^{11} viral particles (VP) and 1 x 10^{10} infectious units (IU) from the number of infected cells indicated.

d. The amount of adenovirus purified with this kit depends on how well your virus grows. Viruses may have reduced yield if the foreign transgene effects cell machinery and the efficiency of virus packaging. Virus isolates that produce less than 1 x 10^{10} particles per ml in the unpurified total cell and supernatant extracts may give lesser yields in the Adenovirus Purification ViraKit™.

e. PRECAUTIONS: This kit permits the quick purification of Adenovirus, an infectious agent which according to the National Institutes of Health (NIH) guidelines must be handled under Biosafety Level 2 safety precautions (see: http://www4.od.nih.gov/oba/rac/guidelines_02/NIH_Guidelines_Apr_02.htm). Although adenovirus recombinants are 99%+ replication incompetent, the transgene may be toxic or be involved in cellular regulation. All these unknowns indicate that a conservative safety approach should be followed and Biosafey Level 2 practices should be used when appropriate. Wear hand, eye, face and body personal protection devices when manipulating adenovirus 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.

f. STORAGE: The **Adenovirus Mini Purification ViraKit™** should be stored at room temperature.

3. User Provided Equipment and Supplies

- Table top centrifuge capable of spinning microcentrifuge tubes at 2200 x g and 1250 x g
- Class II Biosafety Cabinet for Adenovirus manipulation
- Sterile microcentrifuge tubes

4. Summary of the Purification Procedure

a. Virus can be purified easily and quickly. Two to four days after infection of 293 cells with your virus, the cells display cytopathic effect. Infected cells are harvested from the container and centrifuged away from the tissue culture media. After several rounds of freeze/thaw, lysed infected cells are pelleted in the centrifuge. The pellet is discarded, and the clarified lysate is passed over the virus purification filter where adenovirus particles are preferentially adsorbed to the filter. The filter is washed with buffer and virus is eluted off the filter with a small volume of elution buffer. You can accomplish this procedure within 30 minutes and be ready to take your virus into experiments!

b. Virus can be further purified and exchanged into the freeze buffer for storage to –70°C.

PLEASE READ ENTIRE PROTOCOL BEFORE INITIATING CULTURES AND USING KIT

5. Determine amount of adenovirus stock to infect your cells

a. Each virus stock varies slightly in infectious titer.

i. Preparations recently derived from a plaque may be at a titer of 10^6 to 10^7 infectious units (IU) per ml.

ii. Routinely passaged adenovirus stocks may be at titer of 10^8 to 10^9 IU per ml

iii. Purified stocks could be as high as 10^{10} IU per ml.

b. Aim to infect 293 cultures with a multiplicity of 3 IU per cell. Do not over-infect your cultures with too much virus because the cells will not produce high virus titers. One approximation is to use about 100 µl of crude infected virus stock (a.ii., above) to infect a 10 cm dish.

6. Initial Growth of (Human Embryonic Kidney) 293 Cells in Tissue Culture Vessels

a. 293 cells or their variants are grown in tissue culture treated flasks. For the production of adenovirus, cells should be at a relatively early passage level and be 95-100% confluent. They should be kept on a regular passage program. Cells should not remain confluent for more than a few days. Cells that have remained confluent and unpassaged for 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. Cells that have been confluent for more than 24 hours will give reduced virus yields if infected.

b. Cells should be seeded into the tissue culture flask at approximately 4 x 10^4 cells per cm^2. Recommended media: DMEM, high glucose with 4 mM glutamine, and 10% Fetal Calf Serum plus antibiotics if desired. The cell monolayer will become nearly confluent within approximately 2 days. When the monolayer is 90-95% confluent, it is time to infect with your adenovirus.

c. Add your sterile adenovirus to the culture to initiate the infection.

d. Replace the vessels back in the incubator for 2-4 days until cytopathic effect is complete. Cells will progress from Day 2 through Day 4 of infection from isolated round cells to clumps of floating cells. Cultures should be harvested when all of the cells are rounded and few are floating. This is usually at Day 3.

7. Harvest and Centrifugation of Infected Cell Lysate

a. At harvest, ensure that all the cells are detached from the culture vessel by shaking the vessel or pipetting the cells. Pool the cells and media into a sterile, capped tube. Spin at 1250 x g for 10-15 minutes in a microfuge. Discard all but 1.0 ml of the supernatant. Resuspend the pellet in the remaining 1.0 ml of supernatant and freeze/thaw the pellet for three consecutive cycles. A cycle consists of placing the pellet in a dry ice/ethanol bath or in the -70°C freezer for a complete freeze and then into the 37°C water bath until thawed.

b. After the final thaw, centrifuge the lysate at 2200 x g for 10 minutes to initially clarify the solution. Retain the supernatant which contains your virus.

c. Do not plan to load more virus than the harvest from the *equivalent of one half of a 10 cm dish*. Virus yields will be reduced if the filter is overloaded.

8. Purification Procedure

LOAD

a. Equilibrate the purification filter by adding 0.4 ml of **Loading Buffer A** to the purification filter and centrifuge at 2200 x g for 5 minutes. Discard flow through.

b. Load 0.4 ml of the clarified lysate containing your virus on the purification filter and centrifuge at 2200 x g for 5 minutes. Discard flow through.

c. Add remaining volume of clarified lysate to purification filter and repeat centrifugation (may take 2 – 3 more loads). Discard flow through.

WASH

d. Wash the purification filter by adding 0.4 ml of **Wash Buffer B** to the purification filter and centrifuge at 2200 x g for 5 minutes. Discard flow through. Repeat this wash step two times.

ELUTE

e. Elute the virus by first placing the **Purification Filter** into a sterile microcentrifuge tube.

f. Add 0.4 ml of **Elution Buffer C** to the purification filter and centrifuge at 2200 x g for 5 minutes. *This flow through contains the purified virus.*

g. Determine the adenoviral titer by one of the below options:

1. Perform an OD_{260} reading on a dilution of the eluate in 0.1% SDS. See www.virapur.com for this protocol. This technique will result in a total virus particle value per ml.
2. Perform a plaque assay as described at www.virapur.com

h. Purified adenovirus can be stored at this point. Add glycerol to 10% final concentration or add a volume of **Freeze Buffer D** equal to 25% of the volume of your eluted virus to achieve a sugar concentration which will be cryoprotective. Virus should be snap frozen in dry ice and stored at -70° C.

i. Purified adenovirus can now be used for in vitro experiments.

DISCLAIMER

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[1] Purification Filters, Spin. The Sartorius Membrane Adsorber technology is covered by US patent # US005618418A