

Adeno-X™ Virus Purification Kits Protocol-at-a-Glance

(PT3680-2)

Please read the *User Manual* before using this Protocol-at-a-Glance. This abbreviated protocol is provided for your convenience, but is not intended for first-time users.

Purification Protocol

1. Remove the Adeno-X™ Purification Assembly from the packaging. **Do not place the tubing in the virus-containing solution yet.**
2. Remove air from the Filter and Tubing Assembly.
 - a. Fill a 10 ml syringe with 5–10 ml of sterile PBS.
 - b. Attach the syringe to the filter and push the PBS through the assembly.
3. Place the Tubing Assembly in the virus-containing solution as shown in Figure 1.
4. Pull the virus-containing solution into the syringe and then push through the filter at ~20 ml/min (drops should be countable). Avoid drawing air into the system.
5. Transfer the inlet Tubing Assembly from the holding vessel to the 1X Wash Buffer.
6. Pull the wash buffer into the syringe through the tubing, and then push the entire volume of Wash Buffer through the filter at ~20 ml/min.
7. Remove the filter from the one-way valve.
8. Using a clean 5 ml Luer-Lok™ tip syringe, elute the adenovirus: Fill a syringe with 3 ml of 1X Elution Buffer; connect the syringe to the female port on the filter cartridge; then push 1 ml of Elution Buffer through the filter into a sterile 15 ml conical centrifuge tube. Incubate the filter at room temperature for 5 min, then push the remaining elution buffer through to collect the remaining adenovirus. Use air from the syringe to push any remaining virus from the filter.
9. [Optional] To concentrate the virus even further, or to exchange 1X Elution Buffer for 1X Formulation Buffer, transfer the eluate to a Millipore YM-50 Centrifugal Filter Unit. Follow the instructions provided with the unit. Do not allow the volume to be reduced below 0.5 ml, as this may cause the virus to aggregate. When the volume has been reduced to 1 ml, add 2–3 ml of 1X Formulation Buffer and spin again.
10. Determine adenoviral titer.

Note: We recommend using the Adeno-X™ Rapid Titer Kit (Cat. No. 631028).
11. Aliquot and store the adenovirus at -70°C .

Standard Purification Assembly

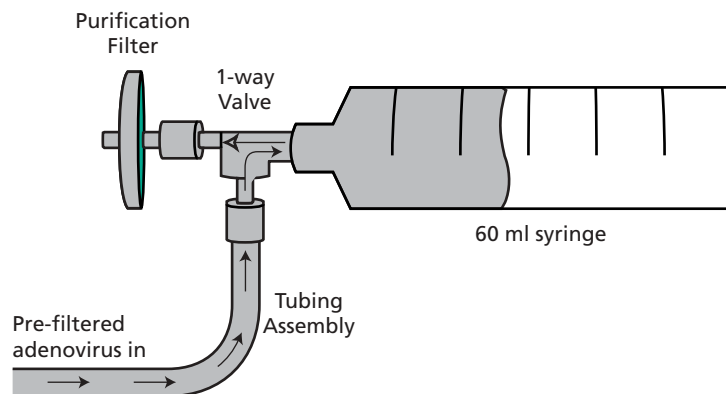


Figure 1. The Adeno-X™ Virus Syringe-Filter Assembly. The apparatus should be used under sterile conditions in a Biosafety Level 2 hood.

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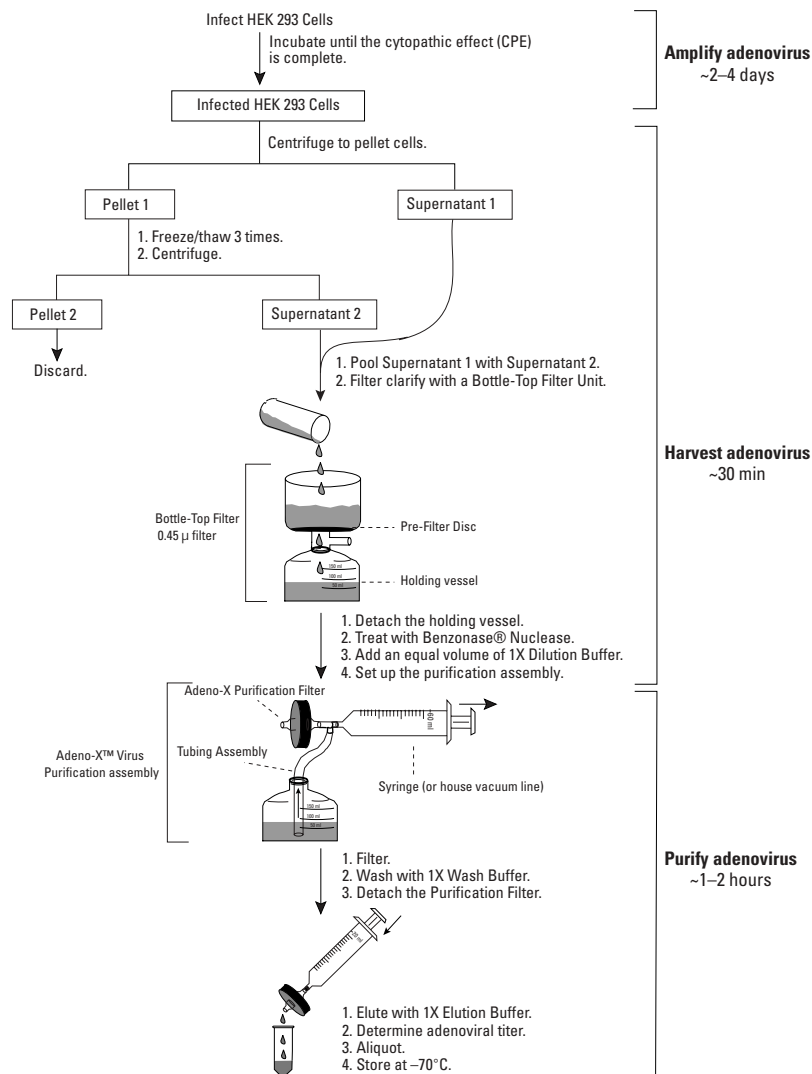


Figure 2. Overview of the Adeno-X™ Virus Purification Protocol. The illustrations shown correspond to the apparatus and procedures used in Standard-size Purification Kits.

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