Protocol-at-a-Glance

Adeno-X™ Mega Purification Kit
Protocol-at-a-Glance

(PT4007-2)

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 (see other side for a graphic overview of the protocol)

1. When the cytopathic effect is complete, centrifuge the cells in a swinging-bucket rotor at 500 x g for 10 min.
2. Resuspend the pellet in 25 ml of fresh, serum-free medium.
3. Lyse the cells by freeze-thawing three times.
4. Centrifuge the lysate in a swinging-bucket rotor at 1,500 x g for 10 min. Discard the pellet.
5. Add 20 µl Benzonase Nuclease to the supernatant and incubate for 20 min at 37° C.
6. Add an equal volume of 1X Dilution Buffer to the lysate.
7. Clarify the lysate by filtering it through a Stericup Filter Unit. Wash the unit with 5–10 ml of Equilibration Buffer, then combine the wash with the clarified lysate.
8. Equilibrate the Purification Assembly with 10 ml 1X Equilibration Buffer.
9. Pass the diluted and clarified lysate (~50 ml) through the Purification Assembly to allow the virus to bind.

Notes:

a) This, and all subsequent steps, should be performed with a continuous-flow pump at a flow rate of **3 ml/min**.

b) The inside diameter (ID) of the 10 ml syringe is ~15 mm; that of the 60 ml syringe is ~27 mm.

10. Pass 30 ml of 1X Wash Buffer through the Purification Assembly.
11. Remove the Filter Stack from the Assembly.
12. To elute the adenovirus, attach the Filter Stack to a sterile 10 ml syringe containing 10 ml 1X Elution Buffer. Pass 3 ml of Elution Buffer through the Filter Stack into a sterile 15 ml conical tube.
13. Turn off the pump and wait 5 min.
14. Turn on the pump and allow the remaining elution buffer to pass through the Filter Stack. Use residual air in the syringe to push any remaining liquid through the filter.
15. Determine the adenoviral titer.

Notes:

- We recommend using the Adeno-X qPCR Titration Kit (Cat. No. 632252) for rapid determination of genome copies.
- We recommend using Adeno-X GoStix™ (Cat. No. 632270) for rapid detection of adenovirus hexon protein.
- We recommend using the Adeno-X Rapid Titer Kit (Cat. No. 632250) to determine IFU

16. Use immediately, or aliquot and store the adenovirus at –70°C.

Note:

For improved long-term stability, and proper tonicity for *in vivo* applications, we recommend a buffer exchange of the eluted adenovirus into 1X Formulation Buffer.

1X Formulation Buffer:
2.5% glycerol (w/v), 25 mM NaCl, and 20 mM Tris-HCl, pH 8.0
(GTS buffer; Hoganson, *et al.*, 2002)

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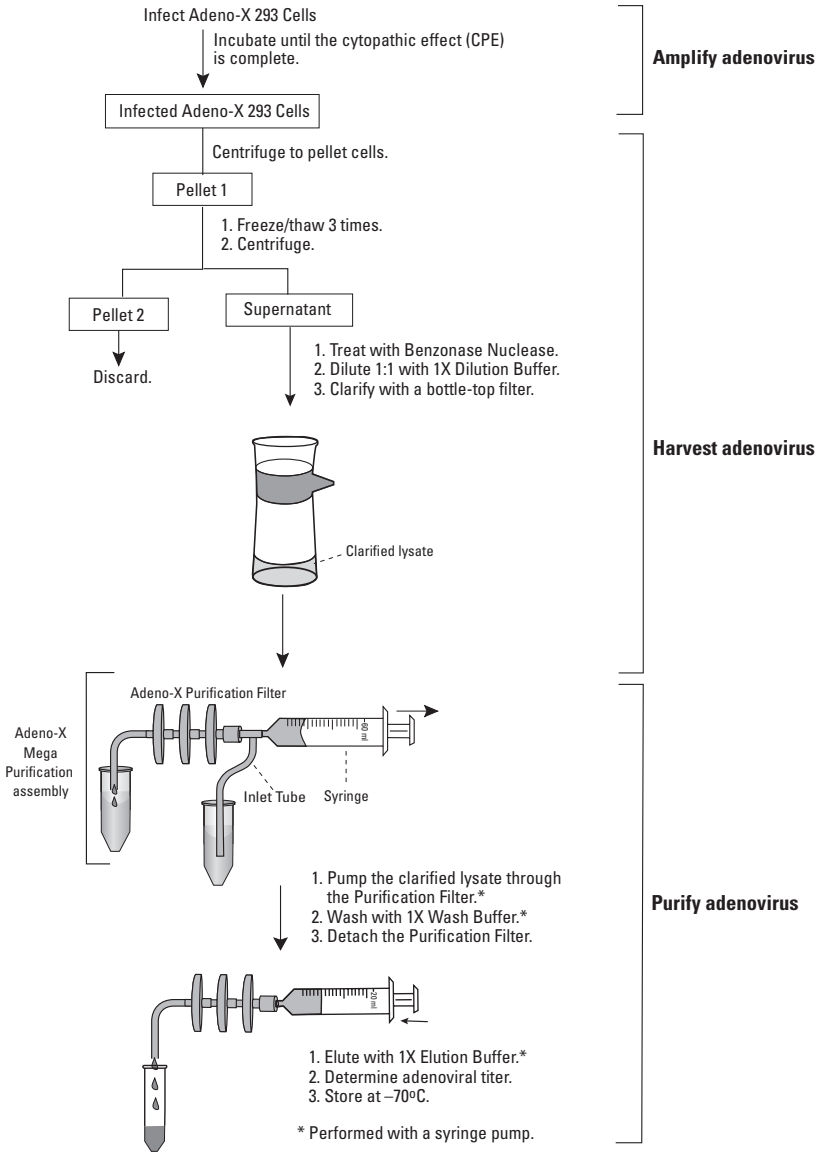

Figure 1. Overview of the Adeno-X Mega Purification Protocol.

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