

# ADENOVIRAL AMPLIFICATION AND PURIFICATION - ADENOPURE®

## **Material required:**

1. Puresyn's Adenopure® Purification Kit (Cat #70100)
2. DMEM, 10% FBS, 1% Pen/Strep (TC media)
3. DMEM, 2% FBS, 1% Pen/Strep (Infection media)

## **Viral Stocks** (any of these can be used):

1. Glycerol/BSA stock.
2. Elution Buffer viral stock or 3% Sucrose/PBS stock at  $1.0 \times 10^{12}$  pt/ML.
3. Viral Lysate in media (should be freeze/thawed 3X).

## **Cell Preparation**

For each virus to be amplified, prepare 5 (150 mm) plates at 80% confluency.

## **Infection**

1. Warm infection media (DMEM 2% FBS) in 37°C water bath.
2. Aliquot infection media into Pyrex autoclaved bottles. Allow for 20 ml of media per plate.  
5 plates - 100 ml infection media.

3. Infect the media:

*For glycerol or sucrose stock:* Look up the pfu to calculate  $\mu\text{L}/\text{plate}$

$$\frac{2.5 \times 10^8}{\text{pfu/ml}} \times \frac{\text{dilution}}{1000} = \mu\text{l per plate}$$

factor

*For 5 (150mm) plate viral lysates:*

Spin down the lysate at 2500 rpm or 600 g for 5 minutes.

Use 250  $\mu\text{l}$  total of the supernatant = 50  $\mu\text{l}$  per plate.

*For 1 (60mm) transfection plate viral lysates:*

Spin down the lysate at 2500 rpm or 600 g for 5 minutes.

Use 4 ml of cell/media lysate.

4. Aspirate the existing media from the 293 cells on the 80% confluent 150 mm plates.
5. Touching the pipette tip to the edge of the dish, slowly plate 20 ml of infected media to each plate of cells.

6. Label the top plate of each of the stacks with the virus name, the date of infection, the time of infection, and your initials. Then mark the sides of the plates with a slash to indicate the plates have been infected. Also fill out the top section of the virus worksheet with the infection information.
7. Incubate the plates at 37°C overnight until 100% cpe is observed. This should be after approximately 48-96 hours of infection/incubation.

## **Harvest**

1. Check plates to be harvested for 100% cpe.
2. Label 50 ml conical tubes  
5 plates - need 2 tubes
3. Harvest cells (using a 25 ml pipette) by pipetting media up and down on the plate to wash the infected cells from the plate into the media.
4. Add cell media to 50 ml conical tubes, dividing it evenly among tubes.
5. Store at -20°C.
6. Record the time of harvest on the Virus Worksheet.

## **Purification**

1. Freeze/Thaw cell/media lysate three times using a 37°C water bath and a dry ice/ethanol bath.
2. Centrifuge the sample at 3500 rpm or 1179 g for about 5 minutes at room temperature.
3. Filter the supernatant through a 0.2 µm filter unit (low protein binding filter such as Millipore Durapore with the PVDF membrane).
4. Add 50 µl of 25 U/µl of Benzonase® and mix gently. Incubate at 37°C for 30 minutes.
5. Calculate amount of 10X Dilution Buffer needed per total amount of supernatant using the following formula:

$$\text{Volume of filtered cell lysate} / 9 = \text{Volume of 10X Dilution Buffer required.}$$

Accurate sample volume is critical.

6. Add 10X Dilution Buffer to supernatant.
7. Attach the Adenopump® to a ring stand.
8. Aliquot into a 50 ml conical tube amount of Equilibration-Wash Buffer needed. To avoid cross-contamination do not place feed tube directly into buffer stock bottle.
9. Equilibrate the Adenovirus Binding Module by passing 30ml of Equilibration-Wash Buffer through the Module. Leave a small amount of buffer in the syringe to avoid passing air through the Virus-Binding Module.
10. Load the diluted-filtered lysate onto the Virus-Binding Module.

11. Pass the entire sample over the Virus-Binding Module. A slow flow rate is necessary. Use a flow rate of 10 ml/minute or lower.
12. Aliquot into a 50 ml conical tube 50 ml of the Equilibration-Wash Buffer.
13. Wash the adenovirus bound to the Virus-Binding Module by passing 50 ml of Equilibration-Wash Buffer through the Module. Leave a small amount of buffer in the syringe to avoid passing air through the pump.
14. Fill a 10 cc syringe with 3 ml of Elution Buffer and 7 ml of air.
15. Detach the Virus Binding Module from the one-way dual check valve T-fitting of the Adenopump<sup>®</sup>. Re-attach the module to the 10 cc syringe with Elution Buffer.
16. Collect the material in the next step as one fraction. It contains the purified virus.
17. Pass 15 drops of Elution Buffer through the Virus-Binding Module. Incubate the Module for 5 minutes at room temperature. After the incubation period, pass the remainder of the Elution Buffer. Push the air through the module to expel all remaining Elution Buffer.
18. Approximately 4.5 ml final elution volume is generally obtained.
19. Dialyze to your storage or formulation buffer of interest.
20. Calculate the concentration.
21. If the virus is kept in the Elution Buffer, store the virus at 4°C for short-term storage up to one week. Store at -20°C for long-term storage.

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