Purification of *Adenovirus* by Cesium Chloride Density Gradients

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[Abstract] *Adenovirus* are efficient gene delivery systems. The standard method for purification of adenoviral vectors is based on using a cesium chloride (CsCl) density gradient combined with ultracentrifugation. This method is suitable for small-scale purification and is less expensive than column chromatography or commercial purification kits.

Materials and Reagents

1. HEK293 cell
2. Glycerol
3. Liquid nitrogen
4. 70% ethanol
5. Distilled water
6. SDS
7. $CaCl_2$
8. $MgCl_2$
9. $CsCl_2$ (Thermo Fisher Scientific, catalog number: BP1595-500)
10. Tris-HCl (pH 8.0)
11. TE
12. EDTA
13. SDS
14. Sucrose
15. 5% deoxycholate (see Recipes)
16. Dialysis buffer (see Recipes)
17. Balance buffer (see Recipes)
18. Saturated $CsCl_2$ (see Recipes)

Equipment

1. Centrifuges
2. Ultracentrifuge

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3. Laminar flow hood
4. Syringe
5. Water bath
6. Slide-A-Lyzer 10 K dialysis cassette (Thermo Fisher Scientific, catalog number: 66203)
7. Spectrometer
8. Ti50.2 tube

Procedure

1. Thaw HEK293 cell suspension (with *adenovirus* in TE +5% glycerol 20.5 ml) from liquid nitrogen at 37 °C.
2. Add 1.23 ml 5% deoxycholate to 0.3% final concentration and vortex.
3. Incubate on ice for 30 min (vortex every 10 min). During incubation, prepare homoginizer: wash with 70% ethanol, then with distilled water, then sterilize under ultraviolet light.
4. Homoginize until cell suspension becomes free flowing (set to 25, three times, 2 min for homogenization and 3 min on ice each time).
   Wash homogenizer: 1x 1% SDS, 5x regular water, 3x sterilized dH_2O, dry up.
5. Incubate cell suspension on ice for 15 min (vortex each 5 min).
6. Spin 30 min at 9,000 *x g* to remove cell debris on 4 °C.
7. Load 11.9 ml of saturated $CaCl_2$ and 20.5 ml of the homogenate onto an ultracentrifuge tube (fit to Ti50.2 tube).
8. Place a cap on to the ultracentrifuge tube. Do not introduce any air bubbles and mix well.
9. Load the other balanced buffer tube, place cap and avoid bubbles. Weigh two ultracentrifuge tubes. If there is over 0.05 g difference in weight, adjust the balanced tube with TE or $CsCl_2$.
10. Ultracentrifuge 35,000 rpm for 20 h at 4 °C. After that, you will see a viral particle band.
11. In a laminar flow hood, carefully remove the tubes from the rotor, then open the cap.
12. Using a 1 ml syringe with a 23 G1 needle, puncture the side of tube below the viral particle band (cloudy white).
13. Aspirate the viral band (about 1 ml) carefully, avoid collecting other bands and impurities. If the viral band is over 1 ml, use another 1 ml syringe with 23 G1 needle and puncture at different position of tube to collect virus.
14. Transfer virus directly to a Slide-A-Lyzer 10 K dialysis cassette that should be hydrated by immersing into dialysis buffer for 30 min.
15. Insert the tip of the needle at a top corner of the cassette, and inject virus slowly.
16. Transfer the virus cassette into an autoclaved 1 L baker containing 1,000 ml dialysis buffer with the lowest speed of stir bar spin.

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17. Dialyze at 4 °C for 2 h then change fresh buffer and dialyze overnight. On the second day, change 1,000 ml fresh buffer and dialyze for another 4 h.
18. Carefully collect the virus by sucking with syringe to a clean sterilized tube, measure OD_{260} with 1:50 dilution.
19. Calculate virus concentration (VP/ml): OD_{260} x 50 x 10^{10}. Aliquot, store at -80 °C.

Recipes

1. TE
   10 mM Tris-HCl (pH 8.0)
   1 mM EDTA
2. Saturated $CsCl_2$
   Add $CsCl_2$ 25 g to TE, and stir, repeat the addition until $CsCl_2$ doesn't dissolve anymore. Transfer the solution to 37 °C water bath, add more $CsCl_2$. It takes about 100 g $CsCl_2$ to saturate 50 ml buffer. Autoclave and store at RT.
3. 5% deoxycholate
   5% deoxycholate in water, filter sterilized.
4. 1% SDS (for washing the probe), filter sterilized.
5. Balance buffer (10 ml)
   6.4 ml TE and 3.6 ml of saturated $CsCl_2$
6. Dializing buffer (4 L)
   10 mM Tris (pH 8.0)
   2 mM $MgCl_2$
   5% sucrose

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