Amplification and Purification of Adenovirus

Amplification of adenovirus and creation of crude stock

1) Culture 293 cells in 162 cm² flask with DMEM+10%FBS, and grow them to 80-90% confluence. You can also use confluent 293s to grow adenovirus.

2) Aspirate the medium to leave behind approximately 1ml of DMEM, and add 250-500ul of adenovirus (crude prep) or 50-150ul of purified virus. Rock the flask to ensure that the entire surface area is covered, then incubate the flask for 60 minutes, rocking the plate every 15-20 minutes ensure that the whole flask is covered.

Alternatively, aspirate all the medium, then add back 500ul of crude adenovirus with 1ml of DMEM, and rock for 1 hour. 1.5ml is sufficient to cover the entire bottom of the flask.

--If you are growing up adenovirus stock from an aliquot (such as one obtained from another lab), I would recommend growing a 10cm² dish worth of 293 cells and using only a small aliquot of the virus to create a “working stock” of virus that can be used to amplify lots more virus with the big (162cm²) flasks. This also preserves the original stock in case something goes wrong.

3) Add back 10-12 ml of DMEM+5% FBS (note the decreased amount of FBS), and culture the cells until all the cells detach (around 3-6 days). If it takes longer than one week to kill all the cells, your viral stock is too low, and needs to go through 1 or 2 more rounds of amplification before purification.

4) Collect the cells and medium in a 50 ml conical tube, and store at –80°C, or proceed immediately to next step.

5) Thaw viral prep at RT if frozen, then sonicate the cells for 10 minutes (alternatively, you can freeze/thaw 5 times). Spin the conical tubes at 3000rpm for 10 minutes to pellet the cellular debris. The supernatants are now your high titer stock that can now be purified, or used in cell culture experiments.

NOTE: Since there are ~10⁸ 293 cells in a 162 cm² flask, and adenovirus can grow up to 10,000 copies per cell, the theoretical final titer can be up to ~10¹⁰⁻¹¹ pfu/ml.

Purification for in vivo use

Buffers: 10mM HEPES (1.3g HEPES in 500ml)  
2.2M CsCl (38g CsCl in 100ml of 10mM HEPES)  
4.0M CsCl (67g CsCl in 100ml of 10mM HEPES)  
Saturated CsCl (67g CsCl in 50ml of 10mM HEPES)

All of the above should be autoclaved and pH adjusted to 7.4

PBS+ 10% glycerol

Equipment: SW28 tubes  
SW41 tubes  
SW28 and SW41 rotors and adapters  
50ml conical tubes, syringes, needles

1) Create a CsCl gradient in a SW28 tube as follows:
Make sure that you *gently* create each layer. The better your layers, the higher your yield. One trick is to use a BSA solution and gently rub the inside of the centrifuge tube. This will reduce the surface tension and allow the cesium chloride solution to cling to the walls.

2) Centrifuge at 25,000 rpm for 2 hrs to overnight at 4°C. After the spin, you should find the viral band between the 4M and 2.2M layer.

3) To retrieve the band, you can either aspirate the media from the top to reach the viral band, or you can insert a needle directly into the tube to retrieve the band. I recommend the latter. Use an 18 gauge needle attached to a 3ml syringe and poke the needle into the tube about 0.5 cm below the white viral band. Push the needle upwards and take out 2.5 ml of virus.

--You may see a thick band of white cell debris. This is not the virus! The viral band is always at the interface of the 4M and 2.2M gradient (the lowest white band). Try not to suck up cell debris if possible, but if you do, it’s OK at this stage, since we will re-purify the band in the next step.
4) Mix the 2.5 ml of virus with 2.5 ml of saturated CsCl, then add to a new SW41 tube. Layer on top of this 2 ml of 4 M CsCl followed by 4 ml of 2.2 M CsCl. Again, gentle layering results in higher viral yields. Use the mixed virus + sat cesium chloride to reduce the surface tension by pipetting this mixture down the inside of the tube.

5) Centrifuge at 35,000 rpm for 3 hours to overnight at 4°C. After the spin, you should find the viral band between in the middle of the 2.2 M layer.

6) Again, retrieve the band with either by pipetting off the top layers or by sucking out the virus with a needle. A 1 ml syringe and 21 gauge needle should be all you need to remove the viral band. Repeat as directed above to remove the viral band with a needle.

7) Transfer the virus solution into a dialysis cassette (Pierce# 66110, 3.5K MWCO), and dialyse in PBS + 10% glycerol for 1-2 hours.

8) Change the buffer to fresh PBS + 10% glycerol after the first dialysis and dialyze overnight.

-- You may also wish to dialyze into adenovirus buffer (see below) + 10% glycerol. This has been shown to keep adenoviruses more stable, but in hands it has been equivalent to PBS + 10% glycerol. If you are injecting into mice, the pH and salinity are very important.

9) Quantify virus before storing at –80°C indefinitely.

Quantifying viral amount:

Adenovirus Titering
1) Take a 5 ul aliquot of the dialysate as a blank and dilute 1:100 in 500 ul (TE/0.1% SDS). You can use 10 ul if you think the viral titer is low.
2) Dilute 5 ul of viral sample into TE/0.1% SDS and put into a UV spec. If you took 10 ul for your blank, also use 10 ul here.
3) Measure the OD_{260}. An OD_{260} = 1x10^{12} viral particles/ml, but remember that you diluted your sample 1:100.
Thus, if you have an $OD_{260}$ of 0.0534:

$$0.0534 \times (100) \times (10^{12}) = 5.34 \times 10^{12} \text{ viral particles/ml}$$

- Viral particles do not equal pfus. Typically, a pfu value is ~100 fold less than the viral particles. The only way to measure pfu is a plaque assay. Even commercially available kits are only estimations of pfu/ml.
- It is typical to make aliquots of $1 \times 10^{11}$ vp/ml aliquots at this stage, since freezing/thawing the purified virus decreases its biological potency.

- **REMEMBER:** It is easy to overlook, but when you make your aliquots, use the adenovirus storage buffer (below) for dilutions!

- **Good luck with the adenoviral work!**

**Buffer for Adenovirus:**

20mM Tris pH=8 (20ml in L from 1M stock) 25mM NaCl (5ml/L from 5M stock) + 5% glycerol (can also be 10%)

For 50ml conical tube=250ul NaCl, 1ml Tris, dilute with dH2O up to 50ml.