

GFP-Adenovirus : Preparation of High Titer Viral Stocks

1. Plate 293 or 911 cells in T-175 flasks to be 90% confluent at time of infection. Usually, 6 T-175 flasks are sufficient to make a high titer stock.
2. Infect cells with virus supernatant at a multiplicity of infection (MOI) of 5 to 10 PFU per cell. Use about 80 – 120 μ l of the virus supernatant to be amplified. Dilute in 90 ml of complete medium and leave on cells for 90 min (15 ml per flask). Aspirate and add 45 ml fresh medium. When all cells have rounded up and about half of the cells are detached (usually at 3 to 4 days post infection), harvest the cells by hitting the flasks. Spin them down in 50 ml conical tubes at 5000 rpm and 4°C for 5 min. Remove the supernatant.
3. Resuspend all pellets in a total of 8.0 ml sterile PBS. Perform four cycles of freeze / thaw / vortex using a dry ice-ethanol bath and water at 37°C. Centrifuge lysate in a 50 ml conical tube at 5000 rpm and 4°C for 5 min.
4. Weigh 4.4 g of CsCl in a 50-ml conical tube, transfer the virus supernatant to the tube, and mix well by vortexing. Transfer the CsCl solution (should be about 10 ml, density of 1.35 g/ml) to a (polyallomer) tube for SW40 rotor. Overlay to the top of the tube with mineral oil. Prepare a balance tube. Spin the gradient in a SW40 rotor at 31500 rpm and 10°C for 18 to 24 hrs.
5. Collect virus fraction (about 0.5 to 1.0 ml) with a 3cc syringe and an 18G needle, by puncturing the tube just below the virus band and drawing the virus down into the needle. Mix with an equal volume 2x Storage Buffer (see below). Store virus stocks in aliquots at -20°C. (It sometimes happens that aliquots partly freeze. This however seems not to dramatically affect virus performance.)
6. Check viral titer by GFP (preferred), or by plaque assays, or by immunohistochemical staining, or simply read OD₂₆₀. To read OD₂₆₀, add 15 μ l virus to 15 μ l blank solution (Blank Solution = 1.35g/ml CsCl mixed with an equal volume 2x Storage Buffer) plus 100 μ l TE / 0.1% SDS; vortex 30 sec, centrifuge 5 min. measure A₂₆₀. One A₂₆₀ unit contains ~10¹² viral particles (particles:infectious particles = ~20:1).

2x Storage Buffer = 10 mM Tris, pH 8.0
100 mM NaCl
0.1% BSA
50% glycerol

Filter sterilise and store at 4°C