Pre-Made Adenovirus Amplification

Protocol

Always amplify one adenovirus at a time and in different culture hoods and incubators. If only one set of equipment is available, amplify the viruses sequentially and use UV radiation for 30 minutes in-between each virus. Use separate trypsin and medium containers for each virus. Cross-contamination when working with two or more adenoviruses is more common than you think. Once it occurred, your results will be greatly compromised. After you have individual stocks for each adenovirus, you can then work with two or more adenoviruses in your targeting cells.

Furthermore, large-scale virus production and purification is necessary for *in vivo* injection and most *in vitro* gene transductions.

1. When you receive your recombinant adenovirus, make two to three aliquots and use one for amplification in 293 cells. Freeze the other aliquots in -70°C as a seed stock for future use.
2. Amplify your adenovirus in 293 cells by infecting the cells with 10μL of the adenovirus for a 60mm dish, or 200μL for a 100mm dish.
3. When more than 95% of 293 cells are detached from dishes, collect both cells and medium.
4. Freeze (-70°C freezer or dry ice / ethanol) and thaw (37°C water bath) the collection three times.
5. Pellet cell debris by centrifugation at 3,000 rpm at room temperature for 10 minutes.
6. Transfer supernatant into a new tube. Store at 4°C for short-term use (two to three weeks) or add glycerol (final concentration 10%) and freeze at -70°C (stable for one to two years).
7. Use the supernatant to infect your target cells. Subsequently analyze your gene expression by Western blotting, Q-PCR, or under microscope if your gene of interest is a reporter gene (i.e. β-gal or EGFP).
8. For any further questions, please contact us at info@abmGood.com and we will get back to you promptly.