Amplification Protocol

Pre-made Adenovirus Amplification

Pre-made adenovirus from **abm** is provided as a seed-stock only and will require further amplification for downstream *in vitro* transduction. Large-scale virus production and purification will be necessary for *in vivo* injections.

IMPORTANT INFORMATION
It is strongly recommended to always amplify one adenovirus seed- stock at a time and in different culture hoods and incubators if possible. Where only one set of equipment is available, amplify the viruses sequentially and use UV radiation for 30 minutes in-between working with each virus. As cross-contamination when working with two or more adenoviruses is a major risk, we also recommend using separate trypsin and medium containers for each virus.

Protocol

1. When you receive your recombinant adenovirus, make two to three aliquots and use one for amplification in 293 cells. Freeze the remaining aliquots in -70°C as a seed stock for future use.

2. Amplify your adenovirus in HEK 293 cells, plated at 60-70 % confluency. For a 60 mm dish, infect the cells with 70 µL of the adenovirus, for a 100 mm dish, infect the cells with 200 µL of virus.

3. When more than 95% of 293 cells are detached from the dishes, collect both the cells and medium into a large falcon tube.

4. Freeze (in a -70°C freezer or dry ice / ethanol) and thaw (in a 37°C water bath) the collection three times.

5. Pellet the cell debris by centrifugation at 3,000 rpm at room temperature for 10 minutes.

6. Transfer the supernatant into a fresh tube. Store at 4°C for short-term use (two to three weeks) or add glycerol to a final concentration of 10% and freeze at -70°C (stable for one to two years).

7. **Transduction Procedure:** If the virus is to be used in an *in vitro* transduction, double CsCl purification is not required as the viral supernatant will provide 100% gene transduction efficiency in most human cell lines. For *in vivo* studies purification is essential to remove defective particles, cell debris, and residual media components, since these contaminants can induce significant immune responses. In addition, CsCl purification will concentrate the virus to a level suitable for *in vivo* injections.

1. Prepare target cells in a 6-well plate or 10 cm at 70% confluency one day prior to transduction.

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2. Aspirate the culture medium and overlay with viral culture supernatant (1ml for a 6-well plate and 4-5 ml for 10 cm dishes) to cover the cells for one hour in an incubator.

3. Remove the media containing the virus and replace it with fresh complete media.

4. Gene transduction can be evaluated 48-72 hours after transduction by different assays, such as Western blot or qPCR. Alternatively, confirm reporter gene expression (i.e. ß-gal or EGFP, if applicable) under a microscope.

For any further queries, please contact our technical support team at technical@abmgood.com and we will get back to you promptly.