

Adenovirus Adfection Protocol

Objective

Certain cells lack the Coxsackie-Adenovirus receptor (CAR). The absence of CAR or a low number of CAR receptor sites will yield no or low transduction efficiency. To overcome this problem, adenovirus particles can be introduced into the cell regardless of the number of CAR receptor sites by endocytosis of an Ad-Calcium Phosphate (CaPi) precipitation complex.

Reagents

- Serum - free Minimum Essential Medium (MEM)
- MEM with 2% Fetal Bovine Serum (FBS) and 1% Pen-Strep (P/S) or cell specific media
- 1M CaCl₂

Procedure

- Add 4 µl of 1x10¹² pt/mL (or 1x10¹⁰ pfu/mL), or the amount of viral particles necessary to transduce at a certain MOI, to 1 mL of serum free MEM. Vortex lightly.
- Add 25 µl of 1M CaCl₂. Vortex lightly.
- Incubate at room temperature for 20 min.
- Remove media from cells.
- Add 1 mL of MEM containing the Ad-CaPi precipitant to each dish.
- Incubate cells with the viral precipitant for 30 min at 37°C.
- Wash cells with fresh 2% FBS/1% P/S MEM or cell specific media.
- Add fresh 2% FBS/1% P/S MEM or cell specific media.
- Incubate in conditions appropriate to cell line being used.

Reference

Fasbender et al. J Clin Invest 102(1):184-193, 1998. <http://www.jci.org/cgi/content/full/102/1/184>.