

Adenovirus Protocol

ADFECTION

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 infection 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

- a. Add 4 μ l of 1×10^{12} pt/mL, or the amount of viral particles necessary to infect at a certain MOI, to 1 mL of serum free MEM. Vortex lightly.
- b. Add 25 μ l of 1M CaCl₂. Vortex lightly.
- c. Incubate at room temperature for 20 min.
- d. Remove media from cells.
- e. Add 1 mL of MEM containing the Ad-CaPi precipitant to each dish.
- f. Incubate cells with the viral precipitant for 30 min at 37°C.
- g. Wash cells with fresh 2% FBS/1% P/S MEM or cell specific media.
- h. Add fresh 2% FBS/1% P/S MEM or cell specific media.
- i. 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>).

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