

Infection of Cultured Cells with AAV vector

1. INTRODUCTION

This protocol is designed to test infection of cultured HEK 293 cells with AAV vectors. HEK 293 cells are used to serve as positive control for AAV infectivity. Other cell lines may be used with appropriate modifications of this protocol.

2. MATERIAL

AAV2-CMV-lacZ (Cat. No. 0304) or AAV2-CMV-GFP (Cat. No. 0303) at 1.0e12 GC/ml (or AAV vectors expressing other detectable marker genes)

HEK 293 cells

Growth media (DMEM/10% FBS + supplements)

37°C CO₂ incubator

Biosafety cabinet

3. METHOD

3.1 1 day before infection, seed HEK 293 cells in 12-well plates at a density of 2e5 cells/well in 1 ml.

3.2 Next day, trypsinize 2 wells and get the cell count. Take note on the number of cells per well.

3.3 Dilute AAV vector with HEK 293 growth media. Adjust the final AAV concentration as below:

$$\text{AAV concentration (GC/ml)} = \frac{\text{Number of cells/well} * 10,000}{0.1\text{ml}}$$

3.4 Prepare 500µl of above diluted AAV vector (dilution 1).

3.5 Perform serial 3-fold dilution by pipetting 150µl of high concentration AAV to 300µl media. Make 7 more concentrations (dilution 2 to 8).

3.6 AAV infection (3 options)

Remove all media from 12-well plate. Add 100µl/well of each diluted AAV per well. Add plain growth media to 2 wells as negative control. Incubate cells in 37°C/CO₂ incubator for 90mins with occasional rocking. At the end of 90min incubation, add 900µl/well of growth media and keep the cells in culture for 48 hours.

3.6.1 Direct infection

Add only diluted AAV to cells for infection.

3.6.2 Co-infection with Adenovirus

Together with diluted AAV, co-infect with wild-type adenovirus type 5 at MOI of 1 (20 particles/cell).

3.6.3 Treatment with etoposide

After incubation with AAV, add 900 μ l of media containing 33uM of etoposide (final concentration will be 30uM).

3.7 Perform appropriate assays to determine positive cells.

4. RESULTS

Here HEK 293 cells are infected at doses from 10,000 GC/cell to 3 GC/cell. For 293 cells, >50% cells are positive for the marker gene when infected at 1000 GC/cell (dilution 3). Both wt-adenovirus and etoposide will stimulate AAV genome replication thus enhancing the percentage of positive cells. >50% positive cells occur at or below 100 GC/cell (dilution 5).

5. NOTE

For questions about this protocol, please contact Applied Viromics, LLC at (510) 266-2646 or email info@appliedviromics.com.