

Infecting cells with an adenovirus Ad5-strain

Grow required cells (eg 293, HeLa, MRC5) in appropriate medium (see TC-3) For P100 grow cells to 75%-80% confluency. For immunofluorescence purposes cells should not exceed 30-40% confluency at the time of fixation/ infection.

1. Remove medium and add approximately $5 - 6 \times 10^8$ pfu/cell per dish. Virus can be diluted with PBS, if necessary. Tilt dishes once or twice to spread the virus evenly.
2. For virus adsorption, incubate cells at 37°C for at least 30 min (at most up to 1hr) in tissue culture incubator "for virus cells only". Tilt once more during adsorption period.
3. Then make up to 10 ml of final volume with α -MEM containing 2% HS and then incubate dishes for time wanted in virus tissue culture incubator.

Note:

A. To infect 293 N3S cells incubate for 18-24 hrs. The virus induces a cytopathic effect (CPE), which alters the cell morphology. When 80-85% of the cells have been infected (judged by morphological change) then you are ready to harvest the cells for further processing.

B. To infect 293 cells for growing up virus stocks incubate cells for 48 hrs.

C. To infect MRC5 cells incubate cells at least for 40 hrs to achieve early mT/Gp expression.