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Adenoviral Vectors: Storage and Transduction Instructions

Vector Handling

Recombinant adenoviral vectors, though replication deficient, transduce mammalian cells and should be handled with BSL2 standards.

Storage

- Upon receiving, the vector should be stored at -80°C.
- Vectors are provided in 1 ml, 500 µl, 100 µl, and 25 µl aliquots. After the first freeze/thaw cycle dispense the vector in 25 µl aliquots or larger, depending on the amount to be used in your experiments. Use 0.5ml tubes.
- Aliquots must be stored at –80°C.
- The vector titer starts to drop after the third freeze/thaw cycle.

Procedure

1. Thaw the vector on ice, and keep it on ice during the duration of the experiment.
2. Adenovirus (Ad5) transduction is cell type dependant. Some cell types exhibit low transduction efficiency, while others transduce very readily.
3. When designing adenovirus transduction experiments, it is recommend to use a reporter vector such as an Ad5 expressing eGFP to determine optimal transduction conditions.
4. Start transducing the cells at an MOI* between 10 and 100 if the cells are readily transducible. With some cell lines a higher MOI might be needed. Look for the highest transduction efficiency with minimal cell death. With some cell lines, high transduction levels cannot be achieved. If this is the case, it is recommended you try the "Adenovirus Adfection Protocol" (http://www.medicine.uiowa.edu/vectorcore/ under Protocols).
5. Use the minimum concentration of FBS that the cells can withstand when performing the transductions. For example, A549 cells are maintained using media containing 10% FBS. Transductions are performed using media containing 2% FBS.
6. Use the minimum amount of media necessary to cover the surface of the plate. For transductions performed in 6-well plates, use 1 ml of media per well.
7. To perform the transduction, combine vector and media, and then add the mixture to the cells. Remove media from wells 4-8 hours post transduction and replace it with complete media.
8. Look for expression at 24h, 48h, 72h and 96h, post transduction.

**MOI means Multiplicity of Infection. MOI equals number of viral particles per cell. In other words, and MOI of 1 means infecting with 1 viral genome (vg) per cell.*

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