

Adeno-associated Virus Protocol

Storage Instructions and Infection Protocol

Vector Handling

Recombinant adeno-associated viruses though replication deficient, they remain infectious and should be handled as such.

Storage

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

In-vitro infection Protocol

Reagents:

Hoechst 33342

Trihydrochlorine, trihydrate (16.2mM).

Molecular Probes Cat# H-3570

2.5 μM Hoechst: add 1.54 μl of 16.2 mM Hoechst to 10mL of media.

D-MEM with 2% Fetal Bovine Serum (FBS) and 1% Pen-Strep (P/S)

D-MEM with 10% Fetal Bovine Serum (FBS) and 1% Pen-Strep (P/S)

1. Thaw the vector on ice, and keep it on ice during the duration of the experiment.
2. Adeno-associated virus (AAV) infection is cell type dependant. Some cell types exhibit low infection efficiency, while others infect very readily.
3. When designing adeno-associated infection experiments, it is recommend the use different serotypes of a reporter virus such an AAV expressing eGFP to determine optimal serotype for infection of your tissue or cell culture.

GENE TRANSFER VECTOR CORE

4. Start infecting the cell at an MOI* between 1×10^4 and 1×10^6 vg per cells if the cells are readily infectible. With some cell lines a higher MOI might be needed. Look for the highest infection with minimal cell death. With some cell lines high infectivity levels cannot be achieved.
5. Use the minimum concentration of FBS that the cells can withstand when performing the infections. For example, HT1080 cells are maintained using media containing 10% FBS. Infections are performed using media containing 2% FBS.
6. Use the minimum amount of media necessary to cover the surface of the plate. For example, infections performed in 6-well plates, 1 ml of media per well is used.
7. To perform the infections, combine virus, media with Hoechst. Add the mixture to the cells. Remove media from wells 4-8 hours post infection and replace it with complete media with Hoechst.
8. Look for expression at 24h, 48h, 72h and 96h, post infection.

Note: *Hoechst can be toxic to certain cell and cause cell death. Check the effects of Hoechst in your cells prior to using it in your experiment.*

* **MOI** means **M**ultiplicity **O**f **I**nfection.

MOI = number of viral particles per cell. In other words, an MOI of 1 means infecting with 1 viral genome (vg) per cell.

***In-vivo* infections**

Vectors are provided in high salt. The virus needs to be dialyzed prior to injections. For dialysis instructions refer to “**AAV Dialysis Protocol**” (www.uiowa.edu/~gene under Protocols).

--- o 0 o ---