

Storage and Transduction Instructions for AAV Vectors



Vector Handling

Recombinant adeno-associated virus vectors, though replication deficient, transduce mammalian cells and should be handled with BSL2 standards.

Storage Buffers Provided

Virus is provided ready for injection in biocompatible **F68/PBS Buffer**: 1XPBS adjusted to 180mM NaCl, pH 7.4 / 0.001% Poloxamer 188.

Storage

1. Upon receiving, the vector 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 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 Transduction Protocol

Reagents:

- *Hoechst 33342 Trihydrochlorine, trihydrate (16.2mM). Molecular Probes Cat# H-3570*
- 5 μM Hoechst: add 3 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)

Procedure:

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 transduction efficiency, while others transduce very readily.
3. When designing adeno-associated transduction experiments, it is recommended to use different serotypes of a reporter vector such as AAV expressing eGFP to determine optimal serotype for transduction of your tissue or cell culture.
4. Start transducing the cells at an MOI* between 1×10^4 and 1×10^6 vg per cell if the cells are readily transducible. With some cell lines a higher MOI might be needed. Look for the highest transduction with minimal cell death. With some cell lines, high transduction levels cannot be achieved.
5. Use the minimum concentration of FBS that the cells can withstand when performing the transduction. For example, HT1080 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 example, transductions are performed in 6-well plates, 1 ml of media per well is used.
7. To perform the transductions, combine vector and media with Hoechst. Add the mixture to the cells. Remove media from wells 4-8 hours post transduction and replace it with complete media with Hoechst.
8. Look for expression at 24h, 48h, 72h and 96h, post transduction.

Note: Hoechst can be toxic to certain cells 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. An MOI of 1 means infecting with 1 viral genome (vg) per cell.