UNIVERSITY OF IOWA
CARVER COLLEGE
OF MEDICINE

University of Iowa Health Care

Viral Vector Core Facility

500 Newton Road, 221 Eckstein Medical Research Building
Iowa City, Iowa 52242
319-335-6726 ***Tel***
319-335-6895 ***Fax***
www.medicine.uiowa.edu/vectorcore

Lentiviral Vectors: Storage and Transduction Instructions

Vector Handling

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

Storage

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

Reagents

- Polybrene (Hexadimethrine Bromide) Sigma Cat# H9268-5G
- Stock solution: (2000x)
  8mg/ml in H2O
  Filter sterilize
  Dispense in 1 ml aliquots
  Store at -20^{o}C

Procedure

1. Thaw the vector on ice and keep it on ice during the duration of the experiment.
2. Lentiviral transduction is cell type dependant. Some cell types exhibit low transduction efficiency while others transduce very readily.
3. When designing lentiviral transduction experiments, it is recommend to use a reporter vector such a lentiviral vector expressing eGFP to determine optimal transduction conditions.
4. Start transducting the cells at an MOI* between 0.1 and 10 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.
5. Use the minimum concentration of FBS that the cells can withstand when performing the transductions. 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 transduction, combine vector, media, and 1x Polybrene (4ug/ml of media), and add the mixture to the cells. Remove media from wells 4-8 hours post transduction and replace it with complete media with 10% FBS.
8. Look for expression at 24h, 48h, 72h and 96h, post infection.

**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.*