

## Lentivirus and mMLV Protocol

### Storage Instructions and Infection Protocol

#### Vector Handling

Recombinant retroviruses and lentiviruses though replication deficient, they remain infectious and should be handled as such.

#### Storage

1. Upon receiving, the virus should be stored at  $-80^{\circ}\text{C}$ .
2. Vectors are provided in 100  $\mu\text{L}$  aliquots. After the first freeze/thaw cycle dispense the vectors in 25  $\mu\text{L}$  aliquots or larger, depending on the amount to be used in your experiments. Use 0.5 mL tubes.
3. Aliquots must be stored at  $-80^{\circ}\text{C}$ .
4. The vector titer starts to drop after the third freeze/thaw cycle.

#### Infection Protocol

##### Reagents:

Polybrene (Hexadimethrine Bromide)  
Sigma Cat# H9268-5G

Stock solution: (2000x)  
8mg/ml in  $\text{H}_2\text{O}$   
Filter sterilize  
Dispense in 1 ml aliquots  
Store at  $-20^{\circ}\text{C}$

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. Lentivirus infection is cell type dependant. Some cell types exhibit low infection efficiency while others infect very readily.
3. When designing lentivirus infection experiments, it is recommend the use of a reporter virus such a lentivirus expressing eGFP to determine optimal infection conditions.

**GENE TRANSFER VECTOR CORE**

- I. Start infecting the cell at an MOI\* between 0.1 and 10 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.
  - 4.
  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, and 1x Polybrene (4ug/ml of media), and add the mixture to the cells. Remove media from wells 4-8 hours post infection and replace it with complete media.
  8. Look for expression at 24h, 48h, 72h and 96h, post infection.

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

MOI = number of infectious viral particles per cell. In other words, an MOI of 1 means using 1 TU (transducing unit) per cell.

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