User Manual of Lentivirus

Infection Protocol for Recombinant Lentivirus

--- Storage Instructions and Infection Protocol

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Introduction

Lentivirus, a genus within the *Retroviridae* family, possesses the unique ability to efficiently infect both dividing and non-dividing cells. Due to this property, lentiviral vectors are widely used as one of the most effective tools for gene delivery in mammalian cells, applicable to both *in vitro* and *in vivo* studies.

Important Guidelines for Transduction

- The optimal multiplicity of infection (MOI) is cell type–dependent. It is recommended to perform pilot transductions using reporter viruses (e.g., LV-EF1a-GFP or LV-CMV-GFP) across a range of MOIs (e.g., 0.5, 2, 8, 20, 40) to determine conditions that maximize transduction efficiency while minimizing cytotoxicity.
- The addition of hexadimethrine bromide (polybrene) at a final concentration of 3–8 μg/mL can enhance transduction efficiency by 2–10 fold in most cell types.
- For suspension cells, a spinoculation step is recommended to improve viral contact with cells and enhance transduction efficiency.
- Transduction efficiency should be evaluated approximately 72 hours post-infection if no antibiotic selection is applied.
- For difficult-to-transduce cells, antibiotic selection may be required to enrich for stably transduced populations prior to downstream applications.

In Vitro Transduction Protocol for Adherent Cells

Step 1. Preparation of Virus-Containing Medium

- Thaw lentiviral stock at room temperature.
- Dilute the virus in complete growth medium to achieve the desired MOI.

Calculation:

Required TU=MOI×number of target cells

Example:

To infect 5×10^4 cells at an MOI of 10:

Required TU = $10 \times 50{,}000 = 5 \times 10^5$ TU

If viral stock = 1×10^9 TU/mL → use 0.5 μL of virus.

Step 2. Transduction of Cells

- Remove the existing culture medium from cells.
- Add the virus-containing medium prepared above.
- Add polybrene if required (final concentration: 3–8 μg/mL).
- Incubate cells under standard culture conditions.
- Replace with fresh medium after 12 ~ 24 hours if necessary.
- Assess transduction efficiency after ~72 hours.

In Vitro Transduction Protocol for Suspension Cells

This protocol serves as a general guideline for a 12-well and 24-well plate format and can be scaled accordingly.

1. Materials and Reagents

Lentiviral stock: High-titer (purified concentrated preferred).

Transduction Medium: Growth medium supplemented with Polybrene (Hexadimethrine bromide) at a working concentration of 4 ~ 8 μg/mL.

Culture Vessel: 12-well or 24-well plates (flat bottom usually works best for spinoculation).

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Centrifuge: Equipped with a plate swing-out rotor.

2. Preparation of Cells (Day 0)

- **Count and Seed:** Harvest suspension cells in the log phase of growth. Seed cells at a density of $0.5x10^6$ to $1x10^6$ cells/mL in a total volume of 0.5 ~ 1.0 mL per well.
- **Add Polybrene:** Add Polybrene to the cell suspension.

  Note: Some sensitive cells (like primary neurons or certain hematopoietic stems) are sensitive to Polybrene. Perform a toxicity titration if the cell line is new to your lab.

3. Viral Transduction (Spinoculation)Calculate MOI:

- Determine the volume of virus needed based on your target **Multiplicity of Infection (MOI)**. For suspension cells, an MOI between 10 and 50 is often required.

  Volume of Virus (mL) = (Number of Cells x Desired MOI)/Viral Titer (TU/mL)

- **Inoculation:** Add the calculated volume of lentiviral stock to each well. Mix gently by pipetting.
- **Spinoculation:** Place the plate in a centrifuge and spin at **800–1000 x g for 90–120 minutes** at 32°C.

  Note: This physical force increases the frequency of contact between the viral particles and the cell membrane.
- **Post-Spin Incubation:** After centrifuging, do not remove the medium. Place the plate in a CO2 incubator at 37°C for 4 ~ 6 hours (or overnight, depending on cell sensitivity to the viral supernatant).

4. Media Exchange (Day 1)

- **Centrifuge Cells:** Transfer the cell suspension to a conical tube and centrifuge at 300 x g for 5 minutes.
- **Resuspend:** Aspirate the supernatant containing the Polybrene and residual viral particles.
- **Fresh Medium:** Resuspend the cell pellet in fresh, complete growth medium (without Polybrene) and transfer back to the culture vessel.

5. Analysis (Day 3 ~ 5)

- **Incubation:** Culture the cells for an additional 48 ~ 72 hours to allow for stable integration and protein expression.
- **Evaluation:**
  - **Fluorescence:** If using a GFP/RFP reporter, analyze via flow cytometry.
  - **Selection:** If using an antibiotic resistance marker (e.g., Puromycin), start the selection process 48 hours post-transduction.
  - **qPCR:** Perform genomic DNA extraction to determine the **VCN (Vector Copy Number)** if precise integration data is required.

Optimization Tips

- **VCN Control:** To avoid insertional mutagenesis, aim for a VCN of 1–2 copies per cell.
- **Cell State:** Ensure cells are highly viable (>95%) before starting; dead cells release DNA which can cause clumping and reduce transduction efficiency.

Storage and Handling

Lentiviral stocks are supplied in sterile PBS at the indicated titer. Viral particles can be stored at −80°C for up to 24 months without significant loss of activity.

Upon receipt, it is recommended to aliquot the virus and store at −80°C immediately.

Avoid repeated freeze–thaw cycles, as this will significantly reduce viral titer and transduction efficiency.

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