800-586-6038
info@cellomicstech.com
www.cellomicstech.com

Protocol: Transducing Target Cells with Lentivirus

Materials

- Lentivirus stock
- Target cells (adherent or suspension)
- Appropriate cell culture medium
- Polybrene (optional for enhancing transduction efficiency)
- Phosphate-buffered saline (PBS)
- Trypsin-EDTA (for adherent cells)
- Cell culture plates or flasks

Equipment

- Incubator set to appropriate conditions (37°C, 5% CO_2)
- Centrifuge
- Hemocytometer or cell counter
- Inverted microscope
- Pipettes and sterile tips
- Biosafety cabinet

Protocol Steps

Though replication-deficient, recombinant lentivirus transduces mammalian cells and should be handled with BSL-2 standards. The following protocol is a general method for transducing adherent cells in a six-well plate. Use it as a starting point for determining the optimal transduction conditions for your target cells.

1. **Plate target cells:** Seed cells in 2ml complete growth medium, 12–18 hr before transduction.

   Note: *Use heat-inactivated FBS for transduction.*

2. **Prepare transduction medium:** Add polybrene to 2 ml of complete growth medium to the desired concentration. The optimum final concentration of polybrene may be determined empirically but generally falls within a range of 2–12 μg/ml.

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Note: *Polybrene is a polycation that reduces charge repulsion between the virus and the cellular membrane. Excessive exposure to polybrene (>24 hr) can be toxic to cells.*

3. **Prepare lentivirus with transduction medium:** Thaw aliquots of your lentiviral stocks on ice. Spin down briefly in a microfuge to bring the liquid to the bottom of the tube. You may see a residue in lentivirus stock in the stock. It is expected; do not worry. Mix the lentiviral vector gently by pipetting up and down. Transfer the proper volume of the lentiviral stocks into the prepared virus transduction medium to obtain the desired MOI; the total volume of the virus represents no more than 1/3 the final volume of the prepared virus transduction medium.
   Note: *Each freeze-thaw cycle will decrease titer.*

4. **Replace the cell medium with prepared lentivirus in the transduction medium:** Remove the plate(s) of target cells from the cell culture incubator and aspirate the culture medium. Add the prepared transduction medium with the virus to the cells. Incubate the plate(s) at 37°C for 8–24 hr in a CO_2 incubator. If you are concerned about exposure to the polybrene, limit the transduction to 6–8 hr.

   (Optional) *Centrifugation of the cultures at 1,200 x g for 60–90 min at 32°C or room temperature will significantly increase infection efficiency.*

5. **Refresh the cell culture medium**: Remove and discard the virus-containing transduction medium and replace it with a fresh growth medium. Continue to incubate the cells for 24–48 hr to allow the expressed protein to accumulate in the target cells. Harvest the cells for analysis or proceed with selection using the appropriate antibiotic.