FenicsBIO

Lentivirus Transduction Protocol

The following protocol is a general protocol for transducing cells in a six-well plate. Use it as a starting point for determining the optimal transduction conditions for your target cells.

Materials

Polybrene (10 mg/ml): provided with FenicsBIO's premade and custom lentiviruses.

Media: DMEM with 10% FBS or optimized culture medium for cell type.

Note: Use heat inactivated FBS for transduction.

Cell Culture Plate: 6-well cell culture plate

Cells: Exponentially growing mammalian cells

Note: The lentiviral particles are classified as Biosafety level 2. Follow all BSL-2 guidelines for handling.

Method

Day 1

Add ~ 5x 10^5 cells in 2 ml complete cell culture medium to the numbers of wells needed in a 6-well plate. Incubate 18-20 hours in a cell culture incubator. For adherent cells, the target cells should be approximately 70% confluent when transduction.

Note: Growth rate of cells vary a lot. Adjust the numbers of cells plated to accommodate a confluency of 70% upon transduction.

Day 2

1. Prepare transduction medium: For each 6-well, add polybrene to 1 ml of complete growth medium to desired concentration. The optimum final concentration of polybrene may be determined empirically but generally falls within a range of 2–12 µg/ml.

Note: Polybrene is a polycation that reduces charge repulsion between the virus and the cellular membrane. Excessive exposure to polybrene (>24 hr) could be toxic to cells. Some cells, such as primary neurons, are sensitive the polybrene. Do not add polybrene to these types of cells.

2. Thaw aliquots of your lentiviral stocks on ice. Mix gently by pipetting up and down. Add proper volume of the lentiviral stocks into the prepared virus transduction medium to obtain the desired MOI (Multiplicity of Infection), the total volume of the virus should represent no more than 1/3 the final volume of prepared virus transduction medium.

   Note: When transducing the cell line with lentivirus for the first time, a range of MOI, such as 1, 2, 5, 10, 20 should be tested for optimal transduction efficiency. To calculate:
   (total number of cells per well) x (desired MOI) = total transducing units (TU) needed
   (total TU needed) / (lentivirus concentration TU/ml) = total ml of lentivirus to add to each well

3. Remove the plate(s) of target cells from the cell culture incubator. Aspirate culture medium. Add prepared transduction medium with lentivirus to the cells. (Optional) Centrifuge the cultures at 1,200 x g for 60–90 min at 32°C or room temperature (Centrifugation can significantly increase infection efficiency). Return the plate(s) to the cell culture incubator and incubate 18-20 hours.

   Note: If toxicity of the lentiviral particles or polybrene is a concern, replace the lentivirus containing medium with fresh growth medium 4-6 hours later.

Day 3

Remove and discard the lentivirus containing transduction medium and replace it with fresh growth medium. Continue to incubate the cells for 24–48 hours to allow the expressed protein to accumulate in the target cells. Harvest the cells for analysis or proceed with selection using the appropriate antibiotic.