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Lentiviral Transduction Protocol

**The following protocol is based on a 24-well plate. If your experiments require a different size of culture plates, just scale up or down accordingly, based on the relative surface area.

Tissue Culture Vessel	Growth area, cm^2/well
96-well plate	0.35
24-well plate	2
12-well plate	4
6-well plate	9.5
35 mm plate	8
60 mm plate	20
100 mm plate	60

Day 1, Seed Cells:

1) Seed $0.5x10^5$ HEK293T cells or your specific cells in each well of the 24-well plate to 50% confluency upon transduction. Serum free medium is not required for the transduction. Use the optimal cell specific medium and conditions.

2) Incubate 18–20 hours at 37°C in a humidified 5% CO2 incubator.

   Note: While determining the plate density, please consider both the growth rates of cells and the length of time the cells will be growing before transduction.

Day 2, Transduction:

1) Calculate the amount of viral particles to be added, according to the desired multiplicity of infection (MOI).

   Multiplicity of Infection (MOI):
   Multiplicity of Infection (MOI) is the number of transducing lentiviral particles per cell

   To calculate:
   (Total number of cells per well) x (Desired MOI) = Total transducing units needed (TU)

   (Total TU needed)/(TU/mL) = Total mL of lentiviral particles to be added to each well

2) Thaw the lentiviral particles on ice. Gently spin down before opening. Keep them on ice. Mix gently before use.

3) Remove medium from the cell culture and add the appropriate amount of lentiviral particles, culture medium, and polybrene (final concentration is 8 µg/mL) to the total volume of 500 uL. Gently swirl the plate to mix.

Polybrene (Hexadimethrine bromide):

Polybrene is a small, positively charged molecule that binds to cell surfaces, neutralizes surface charge, increases binding between pseudoviral capsid and the cellular membrane. Polybrene (Hexadimethrine bromide) has been proved to greatly enhance transduction efficiency. Some cells, like primary neurons, are sensitive to polybrene. Do not add polybrene to these types of cells. If working with a cell type for the first time, a polybrene control only well should be used to determine cell sensitivity.

4) Incubate 18–20 hours at 37°C in a humidified 5% CO2 incubator. Overnight incubation may be avoided when toxicity of the lentiviral particles is a concern. Cells may be incubated for as little as 4 hours before changing the medium containing lentiviral particles.

Day 3, Change media: Remove the medium containing lentiviral particles from the wells and replace with 500 µL fresh pre-warmed complete culture medium.

Day 5, 72 hrs post transduction:

Harvest the cells, perform Western blot to detect gene expression with the tag antibody (DDK antibody, cat# TA50011 or mGFP antibody, cat# TA150122) or observe the GFP under a fluorescent microscope. Keep growing cells if you plan to isolate stable cell lines.

To generate stable cell lines (upon confirming that the WB or GFP is positive), you will need to split the transduced cells and seed them at very low density, such as 500-1000 cells per 10cm culture dish. Change medium in the presence of an antibiotic every 3-4 days until single colonies can be identified (generally, 10-12 days later). Pick a minimum of 5 single colonies and expand further.