

Lenti Starter kit

Components:

1. pPACKH1-Plamid Packaging Mix (40µl)
2. PEG-it (5 ml)
3. TransDux (200µl at 200x)

What you will need:

1. Your Lentivector construct (3rd generation preferable)
2. HEK 293TN or FT cells and culture media
3. Tabletop low speed centrifuge (ex. Beckman GS-6R)

Protocol

Pseudovirus Production

Day 1

1. Plate 3×10^6 293TN cells in a fresh 10-cm plate in 10 ml of antibiotic free DMEM medium (DMEM+FBS+Glu).

Day 2

2. The cells should be 50 to 70% confluent.
3. Transfect cells with plasmid containing gene of interest (2µg) and **pPACKH1-plasmid mix (10µg=20µl)**. Use Lipofectamine+Plus transfection reagent from Invitrogen (Follow manufacturer's protocol).

Day 4

4. Harvest viral supernate (culture media) and add **PEG-it** at a final volume of 1:5. Example: 2.5 ml of PEG-it should be added to 10 ml of viral supernate, invert 10 times to mix well. Keep everything cold from this point onwards. Store viral Sup+ PEG-it at 4°C overnight to 3 days.

Day 5

5. Harvest PEG-it precipitated virus by centrifuging at 4°C at 1500xg for 30 min. Aspirate off the supernate and resuspend the milky-white pellet in a small volume (30 to 50µl) of cold sterile PBS or cold DMEM.
6. Freeze virus at -80°C.

Transduction of Target Cells

TransDux protocol

Day 1

1. Plate 50,000 cells per well in a 24 well plate in culture medium.

Day 2

2. Cells should be between 50 to 70% confluent.
3. Aspirate medium from cells.
4. **Add TransDux to complete medium to a final concentration of 1X (Example; add 60µl of 200x TransDux to 12 ml of medium – transfer 0.5ml of this mixture per 24 well)**
5. Add virus to each well at different MOIs or different volumes.

Day 5

6. 72 hours post transduction, the viral genome will be integrated into the host cell genome.
7. Look at the cells for reporter expression if the viral construct has a reporter like GFP and/or begin appropriate antibiotic selection to establish stable cell line.

OPTIONAL – Virus Titering

1. Aspirate off medium. Wash each well with PBS (at this point the plate can be frozen at -80°C).
2. Add 100µl of Lysis Buffer (SBI's Ultra Rapid Titer Kit) to each well.
3. Titer virus according to protocol given in the Ultra Rapid Titer Kit (SBI cat# LV960A-1).

