System Biosciences
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LentiStarter 2.0 kit

Components:

Reagent	Amount
pPACKH1 HIV lentiviral packaging plasmids	**100 µl**
PEG-It virus precipitation reagent	**20 ml**
PureFection Transfection Reagent	**120 µl**
TransDux virus transduction reagent	**50 µl**

There will be enough reagents provided in the Kit to produce high-quality pseudoviral particles from 2 x 150mm or 5 x 100mm plates of HEK293T/FT producer cells (cells not included).

What you will need:

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

Protocol

Pseudovirus Production (For 100mm plates)

Volumes for 150mm plates are denoted in red

Day 1

1. Plate 3x10^6 293T/FT cells (7-8 x10^6) in a fresh 100mm (150mm) plate in 10 ml (20 ml) of antibiotic-free DMEM medium (DMEM+FBS+Glu).

Day 2

1. The cells should be 50 to 70% confluent at day of transfection

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2. Add 0.8 ml (1.6 ml) of serum-free DMEM media into an Eppendorf tube. Add 2 µg (4 µg) of transfer plasmid and 20 µl (45 µl) pPACKH1-plasmid mix to the tube and mix by pipetting

3. Add 24 µl (55 µl) of PureFection reagent to the tube, and vortex for 10 sec.

4. Incubate mixture at room temperature for a minimum of 15 min.

5. Add mixture drop-wise to the dish, and swirl to disperse evenly throughout the plates.

6. Incubate plates in 37^{0}C tissue culture incubator overnight.

Day 3

1. Replace transfection media with fresh complete growth media w/antibiotics

Day 4 and 5

1. Collect the medium (which now contains pseudoviral particles) into 50-ml sterile, capped conical centrifuge tubes.

2. Centrifuge at 3000 x g for 15 minutes at room temperature to pellet cell debris.

3. Transfer the viral supernatant into new fresh tubes.

4. Add **PEG-it** at a final volume of 1:5. Example: 5 ml of PEG-it should be added to 20 ml of viral supernatant, invert 10 times to mix well. Keep everything cold from this point onwards. Store virus supernatant containing PEG-it at 4^{0}C overnight to 3 days.

Day 6

1. Harvest PEG-it precipitated virus by centrifuging at 4^{0}C at 1500 x *g* for 30 min. Aspirate off the supernatant and resuspend the milky-white pellet in a small volume (1/100 to 1/1000 of original volume) using cold sterile PBS or cold DMEM.

2. Freeze virus aliquots at -80^{0}C.

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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 25µl of 200x TransDux to 5 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^{0}C).
2. Add 100µl of Lysis Buffer (SBI's UltraRapid Global Titering Kit) to each well.
3. Titer virus according to protocol given in the UltraRapid Global Titering Kit (SBI Cat# LV961A-1).