Speedy Lentivirus Purification Protocol

Cat. No. LV999

Notes before starting:

The 293T cell line is widely used as the optimal cell line for lentivirus production. The health of 293T cells at the time of transfection is a critical factor for the success of lentivirus production. The use of "unhealthy" cells will negatively affect the transfection efficiency, resulting in lower titre lentiviral stocks. For optimal lentivirus production, follow the guidelines below to culture 293T cells before use in transfection:

- Ensure cell viability is greater than 90%.
- Do not allow cells to overgrow before passaging.
- Use cells that have been subcultured for less than 16 passages.
- Make sure 293T cells are free of mycoplasma contamination.

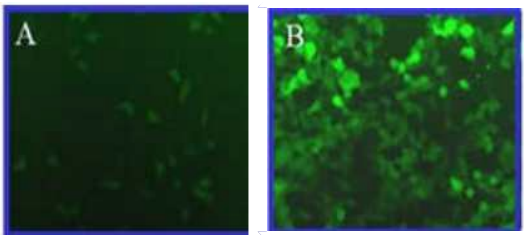

Fig.1. Lentiviral Infection Efficiency. 293 cells in a 12-well plate were infected with 1µl of unpurified viral supernatant (A) and 1µl of purified viral stock (B). Pictures were taken 48 hours after virus infection.

Protocol

1. Harvest lentiviral supernatant from culture. Centrifuge the collected supernatant at 2500 g for 10 minutes to pellet the cell debris and filter it through a 0.45 µm syringe filter.
2. For every 45 ml of the viral supernatant, add 5 ml of Lenti-binding solution and mix thoroughly by inversion.
3. Centrifuge at ≥ 5000g at 4°C for 10 minutes to collect the viral particles.
4. Decant the supernatant completely. Be careful not to disturb the pellet.
5. Add 0.45 ml ~ 4.5 ml of serum-containing medium, depending on the concentration of virus needed, to re-suspend the viral pellet completely.
6. Aliquot the re-suspended virus immediately and store at -70°C.

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