

### Lentiviral particle assembly using Lenti2go Lentiviral Kit (10 cm dish)

1. One day (18-24 hours) prior to co-transfection, plate  $4 \times 10^6$  HEK293T cells in a 10 cm dish, and grow at 37°C to reach about 80% confluence.
2. Remove medium from the dish and rinse with serum free DMEM medium, then add 9 ml of warm, serum-free medium.
3. Prepare transfection solution: Set up two tubes (A) and (B). (A) is for DNA dilution, and (B) is for dilution of Transfectin.
4. In (A), add 10 µg of LentiPlus Master Mix, add 10 µg of Lenti-viral vector of your choice, and then add serum-free DMEM medium to make a final volume of 500 µl, Mix well.
5. In (B), add 45 µl of Transfectin, and then add 455 µl of serum-free DMEM medium to make a final volume of 500 µl. Mix well.
6. Combine (A) and (B) into a total volume of 1 ml. Mix DNA and Transfectin by vortexing, and incubate at room temperature for 15 min.
7. Add the 1 ml (A) + (B) mixture to the dish drop-wise. Mix gently by slowly rotating the dish. Incubate at 37°C for 6 hours.
8. Carefully remove the supernatant with a pipette (not to remove cells). Add 10 ml of warm DMEM with serum. Culture cells for 48 hours
9. Harvesting virus at 48 hours. Filter through a 0.45 µm filter to remove cellular debris. Aliquot and store the virus at -80°C.