

Lentivirus Packaging Protocol

293T Cells Transfection

Protocol described below can yield 10ml of virus at a titer of at least 1×10^5 transducing units (TU)/ml. The amount of lentivirus produced under these recommended conditions is generally sufficient to transduce at least 1×10^6 cells at a multiplicity of infection (MOI) of 1. For example, 10 wells of cells plated at 1×10^5 cells/well in 6-well plates could each be transduced with 1ml of a 1×10^5 TU/ml virus stock to achieve an MOI of 1.

Transfection Procedure

1. One day before transfection (Day 1), plate 293T cells in a 10cm tissue culture plate so that they are 90-95% confluent on the day of transfection (i.e. 5×10^6 cells in 10ml of growth medium containing serum). As a general rule, one 15cm culture dish at 95% confluence can be subcultured into 5x 10cm dishes; whereas one 10cm dishes at 95% confluence can be subcultured into 2x 10cm dishes.
2. On the day of transfection (Day 2), set up the transfection mix as follows:
 - a. In a sterile 15ml culture tube, dilute 15 μ g of Lenti-Combo Mix and 10 μ g of pLenti expression plasmid DNA in 1.0ml of medium without serum. Mix gently.
 - b. In a separate sterile 15ml tube, dilute 80 μ l of Lentifectin (mix gently before use) in 1.0ml of medium without serum. Mix gently and incubate for 5 minutes at room temperature.
 - c. After the 5 minute incubation, combine the diluted DNA with the diluted Lentifectin. Mix gently.
 - d. Incubate for 20 minutes at room temperature to allow the Lentifectin/DNA complexes to form.
 - e. Add 4.5ml serum-free medium to the complexes followed by gentle mixing.
 - f. Remove the medium from the cells, and then add Lentifectin/DNA complexes carefully to culture dishes without dislodging cells. Incubate the cells for 5-8 hours at 37°C in a humidified 5% CO₂ incubator.

Note: 293T cells are poorly adhesive to most culture dishes. It is always recommended to add or change medium against the wall of culture dishes to avoid dislodging cells.
 - h. Add 0.65ml serum to each transfected culture dish and return the dishes to incubator. Incubate overnight.
3. The following day (Day 3), remove the medium containing the Lentifectin/DNA complexes and replace with 10ml complete culture medium. Incubate at 37°C in a humidified 5% CO₂ incubator.

Note: Expression of the VSVG glycoprotein can cause 293T cells to fuse, resulting in the appearance of large, multinucleated cells known as syncytia. This morphological change is normal and does not affect production of the lentivirus.

4. Harvest virus-containing supernatants 48-72 hours post-transfection (Day 4-5) by collecting medium into to a 15ml sterile, capped, conical tube.
Caution: Remember that you are now working with infectious virus at this stage. Follow the recommended guidelines for working with BL-2 organisms (see page 3 for more information).
5. Centrifuge supernatants at 3000rpm for 15 minutes at +4°C to pellet debris.
Optional: Filter the viral supernatant through 0.45µm PVDF syringe filter (Millipore, Cat. No. SLHVR25LS).
6. Aliquot viral supernatants into cryovials in 1.0ml portions and store viral stocks at -80°C.
Proceed to measuring the titer of your lentivirus stock. If you plan to use your lentivirus construct for in vivo applications, we recommend filtering your viral supernatant through a sterile, 0.45µm low protein binding filter after the low-speed centrifugation step to remove any remaining cellular debris. The viral supernatant can be concentrated using LentiClean kits also available from CBI.