Capital Biosciences
innovative solutions for life sciences

DATASHEET

Lenti-2 (Combo) Packing Mix

Cat. VSL-0020

Our lentiviral vectors are 3rd-generation and are compatible with packaging mixes that support the production of 3rd-generation vectors. Our optimized Lenti-2 (Combo) Mix supplies all the structural and replication proteins in-trans that are required for high-titer lentivirus production in packaging cells. Titers can be obtained up to 10^7IU/ml.

The number of lentiviral genes necessary for packaging, replication and transduction is limited to three (Gag/Pol/Rev), and their expression is derived from different plasmids, which all lack packaging signals. These plasmids share no significant homology to the expression vector, preventing the generation of replication-competent virus.

The following materials and reagents are required but not provided:

- Lentivirus vector
- 293T packaging cells
- Lentifectin
- Dulbecco's Modified Eagle's Medium (Invitrogen Cat: 11995)
- Fetal bovine serum (FBS) *Note*: serum need not be heat-inactivated.
- 200 mM L-Glutamine (Sigma Cat. No. G7513)
- Solution of 10,000 units/ml Penicillin G sodium and 10,000 µg/ml Streptomycin sulfate (Sigma Cat. No. P0781)
- Complete Medium: DMEM supplemented with 100 units/ml penicillin G sodium, 100 µg/ml streptomycin and 10% fetal bovine serum (FBS)
- • G418 *Note*: Make a 10 mg/ml active stock solution by dissolving 1g of powder in approximately 70ml of complete medium without supplements. Filter sterilize and store at 4°C.
- Puromycin
- Polybrene (Hexadimethrine Bromide; Sigma Cat. No. H9268)
- Trypsin-EDTA (Trypsin; Sigma Cat. No. T3924)
- Dulbecco's phosphate buffered saline (DPBS; VWR Cat. No. 82020-066)
- Tissue culture plates and flasks

9700 Great Seneca Hwy, Suite 247
Rockville, MD 20850
Tel: 800-475-2812; Fax: 240-363-0739

Transfection Procedure:

1. One day before transfection (Day 1), plate 293T cells in a 10cm tissue culture plate so that they will be 90-95% confluent on the day of transfection (i.e. 5x106 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 10µg of Lenti-Combo Mix and 10µg of pLenti expression plasmid DNA in 1.0ml of medium without serum. Mix gently.
    b. In a separate sterile 15ml tube, dilute 80µl 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.
    g. 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% CO_2 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.

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 determining the titer of your lentivirus stock.

If you plan to use your lentiviral construct for in vivo applications, we recommend filtering your viral supernatant through a sterile, 0.45ml low protein binding filter after the low-speed centrifugation step to remove any remaining cellular debris. The viral supernatant can be concentrated using the protocols discussed in the following section if higher titer virus is required.

For research use only

9700 Great Seneca Hwy, Suite 247
Rockville, MD 20850
Tel: 800-475-2812; Fax: 240-363-0739