Lentivirus Packaging Kit

Name: Lentivirus Packaging Kit

Cat. #: P904

Application: Kit for packaging Lentivirus. This product is for research use only.

Product Size: 10 reactions, 100mm dish per reaction

Product Description: This kit is for packaging 3rd generation Lentivirus.

To package Lentivirus, use the provided transfection reagent to transfect 293T cells with your lentiviral vector together and the provided packaging mix. The packaging plasmids will express the elements required for lentivirus production. Replication-incompetent, HIV-1-based lentivirus containing your gene of interest will be produced.

Shipping/Storage: Ship at **4°C,** store condition see below

Shelf Life: 6 months

Component:

Component	Cat. #	Amount	Storage
293T Transfection Reagent	P902	450 µl	4°C
Lentivirus Packaging Mix	P904P	200 µl	−20°C, avoid freeze-thaw cycles

Remark: The 3rd generation packaging system offers maximal biosafety as the lentiviral Rev gene is supplied as an independent vector from other structure genes, further eliminating the possibility of reverse recombination of vectors into a replication competent viral particle. The third generation lentiviral packaging mix will only support lentiviral expression vector with a chimeric 5' LTR in which the HIV promoter is replaced with CMV or RSV, thus making it TAT-independent.

Follow the recommended NIH BSL-2 guidelines for all materials containing Lentivirus.

Protocol (example of producing virus in **100mm petri dish)**

Day 1: Plating cells

1. 24 hours before transfection, coat 100-mm dishes with **6 ml 1x Gelatin** for 60 min.
2. Aspirate gelatin, and plate **~5 X 10^6** fast growing 293T cells per plate, in 10 ml medium.

Note: Before plating, pipet the 293T cell suspension vigorously to achieve single cells.

Plate the cells in evenly distribution manor.

Day 2: Transfection

Check to make sure the cells are 70-90% confluent.

1. **Change medium: 2 hours before** transfection, remove culture medium and add **10 ml fresh** complete culture medium (with 10% serum / without antibiotics)

2. In tube 1 add:
   2.5 µg Lentivirus vector (contains your gene of interest)
   20 µg Lentivirus packaging mix
   500 µl DMEM (serum-free, High Glucose)
   Pipet up and down to mix well

3. In tube 2 Add:
   40 µl 293T Transfection Reagent
   500 µl DMEM (serum-free, High Glucose)
   Gently pipet up and down to mix well

4. Incubate at room temperature (20–25°C) for 3 min.

5. Add tube 2 into tube 1, pipet up and down several times. **Vortex** for **10 seconds**.

6. Incubate for **15 minutes** at **room temperature**.

7. Add the incubated mixture **drop-wise** to the cells, and **gently rock / swirl** the plate to disperse evenly.

8. Return the cells to 37°C incubator with 5% CO_2.

Day 3: Add Titer-Up

Replace the supernatant with 10 ml fresh medium.

(Optional) Add **20 µl** of **Titer-Up** (500x, Cat. #: P906) to the medium, to enhance the virus titer.

Return the plates to the cell culture incubator.

Day 4: Collect virus

1. Collect virus supernatant twice at **48 and 72 hours** post transfection into a 50mL sterile FALCON tube. Centrifuge at **3,000rpm** for **15 minutes** at 4°C to remove cell debris. Filter the clear supernatant through **0.45 µm** syringe tip filter.

2. The filtered clear supernatant is virus soup. Use it immediately or aliquot into sterile 1.5-mL tubes and store at **-80°C**, for **up to 3 months.**

3. (Optional) To concentrate virus, add **5X Lentivirus Concentration Solution** (101Bio, Cat. #: P904C) to the viral supernatant (volume of Lentivirus Concentration Solution vs. volume of viral supernatant = 1:4) and mix thoroughly. Put the mixture to 4°C refrigerator overnight and spin the virus pellet down next day. Please refer our P904C user manual for details.

-- The end –