

Lentivirus Production

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Materials

Packaging cell line: 293FT better to use early passaged cells

Medium: DMEM with 10% FBS with or without P/S for culturing 293FT cells

DMEM with 20% FBS **without P/S** for making virus

Lipofectamine2000 30 μ l/10 cm dish, 5 μ l /well of 6-well plate

Opti-MEM

Transfection plasmids stored at -20°C, common plasmid box:

psPAX2, 2nd generation lentiviral packaging plasmid. (Addgene Catalog # 12260)

pMD2.G, VSV-G envelope expressing plasmid. (Addgene Catalog # 12259)

Lenti viral plasmid: *e.g. pLent-EGFP-puro* 11 to 12 μ g/10 cm dish

0.45 μ m PES filter for filtering viral supernatant

PEG8000 for concentration of virus (stored at cell culture room 4°C)

Prepare 293FT cells, split cells 2 to 3 times a week, cells should remain healthy.

Tranfection Steps:

Prepare plasmids mixture **0.5 - 1 ml/10 dish**

0.25 or 0.5 ml Opti-MEM

30 μ l Lipofectamine2000

Mix and leave at *RT for 5 min*

0.25 ml or 0.5 ml Opti-MEM

psPAX2 9.2 μ g

pMD2.G 2.8 μ g

pLent-EGFP 11 to 12 μ g

Mix DNA with Lipofactamine2000 at *RT for 30 min*

Passage 293FT cells.

Count cell number and suspend cells in DMEM with 20% FBS.

Cell number ~ 7.5×10^5 /ml, 12 ml /10 cm dish, ~ 8.5 to 9×10^6 /10 cm dish.

Add plasmid mixture into 12 ml cell suspension and place into a 10 cm dish evenly.

Also can place cells in the dish first then add the plasmid mixture dropwise evenly. Incubate cells no medium change.

Harvest virus at 48 or 72 hours post transfection.

Centrifuge the medium with virus at ~500 g for 3 minutes and filter the viral supernatant through a 0.45 μ m PES filter. Viral supernatant can be stored at 4°C for less than 1 week. It is better to concentrate and aliquot the viral supernatant to store at -80°C.

Concentrate viral supernatant

Mix PEG8000 and viral supernatant at 1:4 ratio (3 ml PEG in 12 ml viral sup.).

Mix and shaking gently on ice or at 4°C for 5 to 6 h.

Spin down at 1600 g for 60 min at 4°C.

Remove the supernatant and re-suspend the viral pellet in PBS, 1/20 original volume. e.g. 2ml in a 2 ml tube, the pellet can be suspended in 100 μ l PBS.

Aliquot the concentrated virus if need and store at -80°C.