

Lentivirus Production

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Materials

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

Medium: DMEM with 10% FBS with P/S for culturing 293FT cells

DMEM with 20% FBS with P/S for making virus

PEI (Sigma, #408727-100ML)

PEI Stock Conc: 1 μ g/ μ L

Opti-MEM

Plasmids:

psPAX2 (Addgene Catalog # 12260)

pMD2.G (Addgene Catalog # 12259)

Lenti viral plasmid: *e.g. plenti-AFP or plenti-Large T* 12 μ g/10 cm dish

0.45 μ m PES filter for filtering viral supernatant

PEG8000 for concentration of virus (Promega, #V3011)

Tranfection Steps (10cm dish):

1. Prepare DNA mixture

Opti-MEM 0.5 ml

psPAX2 9.2 μ g

pMD2.G 2.8 μ g

plenti-AFP 12 μ g

Mix and leave at RT for 5 min

2. Prepare PEI mixture

Opti-MEM 0.5 ml

PEI 33 μ l

Mix and leave at RT for 5 min

3. Mix DNA mixture with PEI mixture at *RT for 30 min*

4. Digest and count 293FT cells.

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

Cell number: Count ~ 8.5 to 9×10^6 cells to a 15ml tube, add plasmid and PEI mixture into the cell suspension, add additional DMEM with 20% FBS to a final volume of 12ml. Transfer the 12ml cell suspension into a 10 cm dish evenly.

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 $0.45 \mu\text{m}$ PES filter. Concentrate and aliquot the viral supernatant to store at -80°C .

Concentrate viral supernatant

Mix PEG8000 and viral supernatant at 1:3 ratio (e.g. 3ml PEG with 9ml viral supernatant).

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

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

Remove the supernatant thoroughly and re-suspend the viral pellet in PBS.

e.g. 10cm dish pellet can be suspended in 150 μl PBS for 3 mice immunization.

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