

GFP expressed lentiviral titering

I. Materials

1. 96-well cell culture treated plates
2. 15 mL conical vials
3. 5 ml Polypropylene Test Tube
4. Polybrene (Hexadimethrine bromide; Sigma #H9268) or Protamine sulfate (Sigma #P4020)
5. 1x Dulbecco's Phosphate Buffered Saline (PBS)
6. Trypsin-EDTA
7. Human and mouse cell line and appropriate growth media. (For example, HEK 293T cell and DMEM culture medium containing 10% heat inactivated fetal calf serum and 1 X Pen/Strep.)

II. Instructions

A. Optimization of lentiviral infection

1. (DAY 1) Plate 2×10^5 293T cells per well in a 6-well plate and incubate at 37 °C, 5% CO₂ for 18-20 hours
2. (Day 2) Prepare 15ml of DMEM culture medium containing polybrene (final concentration 8 ug/ml).
3. Thaw lentivirus stock at room temperature and store the virus stock on ice. Mix by gently tapping the tube several times with finger.
4. Prepare 2 mL 10-fold serial dilutions ranging from 10^{-1} to 10^{-4} in 15 ml conical vials. Mix gently by inverting the tubes 10 times.
5. Add 1 ml DMEM culture medium containing polybrene to one well as a mock control. Then add 1ml of each of diluted virus to the remaining wells of the plate. Incubate at 37 °C for 18-20 hours.
6. (Day 3) Remove the medium containing virus from well and replace with 2 ml of DMEM culture medium (without polybrene).
7. (Day 4 and forward) Replace medium every 2-3 days until GFP expression. Trypsinize cells, inactivate with culture media, spin and resuspend in cold PBS for FACS analysis.
8. FACS analyzes for GFP expression and record the percentage of cells that are GFP positive. Use a well that has between 1% and 20% of cells expressing GFP to determine titer.

Formula for virus titer calculation: $\text{titer} = \{(F \times Cn) / V\} \times DF$

F: The frequency of GFP-positive cells determined by flow cytometry;

Cn: The total number of target cells infected.

V: The volume of the inoculum.

DF: The virus dilution factor.

Example:

Dilution	0	10^{-1}	10^{-2}	10^{-3}	10^{-4}
DF	0	10	100	1000	10000
F	0 (0%)	0.12 (12%)	0.01 (1%)	0.003 (0.2%)	0.0004 (0.05%)
Cn	2×10^5	2×10^5	2×10^5	2×10^5	2×10^5
V (ml)	1	1	1	1	1
Titer (TU/ml)	0	2.4×10^5	2×10^5	6×10^5	8×10^5

Thus, the titer of the lentiviral stock is 2.2×10^5 TU/ml (*i.e.* .average of 2.4×10^5 and 2×10^5)