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Lentiviral Production

In 2 collections

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We use this protocol and it's working

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Abstract

Protocol for producing lentivirus

Day -1: Preparing HEK Cells

1 Grow HEK cells in Dulbecco's Modified Eagle Medium (DMEM) with 10% bovine calf serum at 37°C in a 5% CO_2 incubator. Use 25 mL for a 15cm plate

2 Passage ~10^7 HEK cells per 15cm dish coated in 0.1% gelatin

Day 0: Transfecting HEK Cells

3 Change media on HEK cells, just prior to the following steps

4 Combine 14.7 ug pMDLg/pRRE (Addgene #12251), 5.7 ug pRSV.Rev (Addgene #12253), and 7.9 ug of pMD2.G (Addgene #12259) per 15 cm plate

5 Add 22.5 ug of transfer vector per 15 cm plate

6 Add 1 mL of 278 uM $CaCl_2$ and mix thoroughly

7 Add 1mL of 2x BBS solution (280 mM NaCl, 50mM BES, 1.5mM Na_2HPO_4; pH = 6.95) dropwise. Invert to mix.

8 Incubate for 1 minute at room temperature

9 Add dropwise to HEK cells, distributing throughout the plate. Shake the plate on the bench in an X-shape to distribute the plasmid mixture thoroughly

Day 1-3: Collecting Virus

10 Day 1: After 24 hours, change the media

11 Day 2: Collect conditioned media after another 24 hours

12 Day 3: Collect conditioned media after another 24 hours. Combine with the media collected on day 2

Day 3-4: Concentrating Virus

13 Centrifuge media at 2000xg for 10 minutes to pellet debris

14 Filter media through a 0.22um PES filter

15 Add PEG8000 to a final concentration of 5%

16 Add NaCl to a final concentration of 0.15M

17 Invert multiple times to mix well and incubate over night at 4°C

18 Centrifuge at 3000xg for 20 min to pellet viral particles

19 Discard the supernatant

20 Resuspend the pellet in DMEM/F12 (or media of your choice) to 1% of the original supernatant volume

21 Store 50uL aliquots at -80°C