

Concentrate Lentivirus with PEG8000 precipitation

-The Han Lab

1. Prepare 4x PEG8000/NaCl solution:

Dissolve 80g PEG-8000, 14.0g NaCl in 80ml MillQ water and 20ml of 10x PBS (pH7.4), Mix with gentle stirring, heating gently if necessary, until the solids are dissolved then adjust pH to 7.0~7.2 and the final volume to 200ml. Sterilize by filtering through 0.2 μ m. The concentrations of PEG-8000 and NaCl in the stock solution are 40% (W/V) and 1.2M, respectively. Store the solution at 4C.

2. Collect supernatant from 10-cm culture dishes;

3. Spin down at 2000xg for 10min at room temperature or through a sterile 0.45 μ m filter

4. Add 1 volume of PEG8000/NaCl solution into 3 volumes of virus media;

5. Mix well by shaking for 60 sec then incubate with constant rocking at around 60 RPM for at least 4 hours at 4C;

6. Spin down at 1600xg for 60min at 4C;

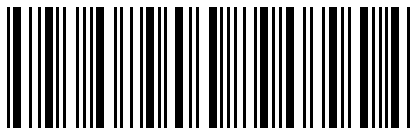
7. Carefully remove supernatant without disturbing the pellet;

8. Thoroughly resuspend the viral pellet into PBS or DMEM (no serum & antibiotics) with 1/10 to 1/20 of the original volume by gently pipetting up and down;

9. Aliquot and store at -80C until use.

Note: The final concentrations for PEG-8000 and NaCl are 10% (w/v) and 0.3M, respectively; Virus is quite stable in PEG solution and can be kept overnight at 4C without significant loss in titers.

(The end)



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