**用PEG8000沉淀浓缩慢病毒**

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 um. 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 10 min at room temperature or through a sterile 0.45 um 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 1600 xg for 60 min 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 -80 C 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)

Han实验室

1. 制备4x PEG8000/NaCl溶液:

将80g PEG-8000，14.0g NaCl溶于80ml MillQ水和20ml 10X PBS (pH7.4)中，轻轻搅拌混合，必要时轻轻加热，直到固体溶解，然后调整pH至7.0~7.2，最终体积为200ml。通过0.2 um过滤灭菌。原液中PEG-8000和NaCl的浓度分别为40% (W/V)和1.2 M。将溶液储存在4C。

2. 收集10cm培养皿上清液;

3. 在室温下以2000 xg旋转10分钟或通过无菌的0.45um过滤器

4. 将1体积PEG8000/NaCl溶液加入3体积病毒培养基中;

5. 摇60秒混合均匀，然后在4C下以大约60转/分钟的速度摇至少4小时孵育;

6. 转速为1600 xg，转速为60min，温度为4C;

7. 小心地去除上清液，不干扰颗粒;

8. 将病毒颗粒以原体积的1/10 ~ 1/20轻移至PBS或DMEM(不含血清和抗生素)中;

9. 分装后在-80℃保存直至使用。

注:PEG-8000和NaCl的最终浓度分别为10% (w/v)和0.3M;病毒在PEG溶液中相当稳定，在4C保存过夜，滴度无明显下降。

(结束)