THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

4×Lentivirus Concentrator Solution

Dissolve 80g PEG-8000, 14.0g NaCl in 80ml MillQ water and 20ml of 10×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 0.2μM. The concentrations of PEG-8000 and NaCl in the stock solution are 40% (W/V) and 1.2M, respectively. Store the solution at 4 ^{o}C.

Virus concentration protocol [*See* Note 1]:

1. Collect supernatant from 10-cm culture dish into a 15 ml tube;
2. Spin down at 800×g for 10 min at room temperature or through a sterile 0.45μm syringe filter (e.g. Millipore # SLHV033RS);
3. Carefully transfer the supernatant into a new 50 ml tube;
4. Add 1 volume of concentrator to 3 volumes of supernatant; [*See* Note 2]
5. Mix well by shaking for 60sec then incubate with constant rocking at around 60 RPM for at least 4 hours at 4 ^{o}C; [*See* Note 3]
6. Spin down at 1600× g for 60 min at 4 ^{o}C;
7. Carefully remove supernatant without disturbing the pellet; [*See* Note 4]
8. Thoroughly resuspend the viral pellet into PBS or desired medium (no serum & antibiotics) with 1/10~1/20 of the original volume by gently pipetting up and down; [*See* Note 5]
9. Aliquot and store at -80 ^{o}C until use;

Note:

1. *The PEG purification step leads to little loss in the infectious virus. However, up to 90% of the input infectious virus can be recovered after PEG precipitation.*

2. *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 4 ^{o}C without significant loss in titers.*

   Serum in cell culture supernatant may enhance virus precipitation; if you collect the virus in serum-free medium or low-serum medium, or the sample is free of turbidity/small particulates after addition of PEG-8000 concentrator, the addition of sterilized BSA (3% final concentration) may enhance virus precipitation.

3. *Longer incubation e.g. overnight will enhance recovery;*

   PEG will be pelleted with the virus. There may also be other proteins/particulates co-precipitated. It may be necessary to perform a virus purification step depending on downstream application. Residual PEG present in the concentrated virus does not seem to cause any toxicity in culture cells and PEG-purified virus generally is more stable during storage than crude vector preparations;

4. *The pellet size is not necessarily well correlated with the virus yield. Actually, the pellet you see after centrifugation is not virus only, and most of it is the serum proteins that get spun down from the media. There may also be some genomic DNA from disrupted cells, which does not go into solution, and PEG. To remove the serum protein, thoroughly re-suspend the "pellet" in DMEM without serum or PBS by letting it sit for 10 minutes, then gently pipetting up and down 20-30 times to avoid bubbles, which may inactivate virus. Then transfer this into a microfuge tube and spin in a microfuge full speed for 3 minutes to pellet the protein debris. Aliquot the supernatant and store in a -80C freezer.*

5. *If no pellet can be visualized, pipette up and down in the area (and just beyond) where the pellet would form.*

References

Lo HL, Yee JK. Production of Vesicular Stomatitis Virus G Glycoprotein (VSV-G) Pseudotyped Retroviral Vectors. In *Current Protocols in Human Genetics*, 2007 John Wiley & Sons, Inc. pp12.7.1-12.7.11;

Lentiviral Vector Preparation Application Note. Beckman Coulter. DS-18017A; https://www.beckmancoulter.com/wsrportal/bibliography?docname=DS-18017.pdf

Kutner RH, Zhang XY, Reiser J. Production, concentration and titration of pseudotyped HIV-1-based lentivirus vectors. Nature protocols 2009, 4: 495-905.

Protocol-Qualitative detection of norovirus and hepatitis A virus in soft fruit. http://www.crlcefas.org/content/softprotocol.pdf

FAQ: Retrovirus Transduction from Cell Biolabs, Inc. http://www.cellbiolabs.com/faq/viral-expression-faq/retroviral-transduction

Winter VomitingLab: protocols/PEG precipitation, http://openwetware.org/wiki/WinterVomitingLab:Protocols/PEG_precipitation

Sanyal D, Kudesia G, Corbitt G. Comparison of ultracentrifugation and polyethylene glycol precipitation for concentration of hepatitis B virus (HBV) DNA for molecular hybridization tests and the relationship of HBV-DNA to HBe antigen and anti-HBe status. J Med Microbiol 1991, 35: 291-293.

Colombet J, Sime-Ngando T. Use of PEG, polyethylene glycol, to characterize the diversity of environmental viruses. http://www.formatex.info/microscopy5/book/316-322.pdf

Marino MP, Luce MJ, Reiser J. Small- to large-scale production of lentivirus vectors In Lentivirus gene engineering protocols (edited by Federico M) 2003, Humana Press Inc. pp43-56