



Ultra-Pure Lentivirus Purification Protocol

Cat. No. LV998

Materials provided:

- LentiBind Buffer A (320ml)
- LentiBind Buffer B (128ml)
- Lenti-Elution buffer (32ml)
- LentiBuffer (40ml)

Other Required Materials:

- 15ml sterile centrifuge tube (Sarstedt)
- Low protein binding, 0.45µM filter. (Millipore, Cat#SLHVR13SL)
- 100kDa cut-off buffer exchange column (Sartorius, Cat# VS2041)
- Centrifuge
- Orbital/rotating shaker

Protocol

1. Harvest lentiviral supernatant from culture. Centrifuge the collected supernatant at 4000 xg for 10 minutes at 4°C to pellet the cell debris. Filter the supernatant through a 0.45µm syringe filter.
2. For every **100ml of viral supernatant** to be purified, add **20ml of LentiBind Buffer A** and **8.0ml of LentiBind Buffer B**. Mix well and shake the mixture on a rotating/orbital shaker at 200-300rpm at 4°C for a minimum of 3 hours (ideally overnight).
3. Centrifuge at 10000 xg at 4°C for 30 minutes to collect the viral particles.
4. Discard supernatant completely without disturbing the pellet, and spin briefly again if necessary to remove any residual liquid.
5. Add **2.0ml Lenti-Elution buffer** to resuspend the viral pellet completely with a transfer pipette and transfer the viral suspension to a 15ml sterile centrifuge tube. Shake the tubes at 4°C for 30min to elute viral particles.
6. After incubation, spin at 10,000xg for 20 min to separate viral particles from the binding matrix.



7. Transfer the supernatant (viral particles) to 100kDa cut-off buffer exchange column.
8. Add **2.5ml LentiBuffer** and spin the column at 2000xg at 4°C to buffer exchange until the final volume is 200µl-1.0ml depending on viral titre desired. To speed up the process, use 1ml transfer pipette to re-suspend the supernatant every 30 minutes.
9. Final lentivirus preparations can either be used immediately for cell transduction or aliquoted into smaller volumes (10-20µl) for long-term storage at -75°C. The Ultra-Pure Lentivirus Purification Kit (LV998) can be stored at room temperature.