

Lenti-X™ Concentrator Protocol-at-a-Glance (PT4421-2)



A. Summary

The Lenti-X Concentrator (Cat. Nos. 631231 & 631232) provides a fast and simple method for concentrating lentiviral stocks. Concentration is achieved by mixing a lentiviral supernatant with this concentration reagent, followed by a short incubation step and centrifugation in a standard centrifuge. The process is easily scaled up to accommodate larger supernatant volumes. No ultracentrifugation is required. The concentration procedure can be completed in as little as 1 hour, or for convenience, longer incubation times can be used. The Lenti-X Concentrator is designed for use with all lentiviral supernatants, including all of Clontech's Lenti-X vectors. Using this reagent increases vector titer (IFU/ml) by 1–2 logs in a short amount of time with minimal loss of material.

B. Protocol

Viral supernatant is collected from your virus-producing cell line and centrifuged to remove cells and debris. It is then mixed with the Lenti-X Concentrator and incubated for a short time at 4°C. The mixture is then centrifuged at low speed to obtain a high-titer virus-containing pellet which can then easily be resuspended and used for transduction of your intended target cells.

1. Harvest the lentivirus-containing supernatants. (Caution: supernatants contain live lentivirus.) Pool similar stocks, if desired. Centrifuge briefly (500 x g for 10 min) or filter through a 0.45 µm filter.

NOTE: If filtering, use only cellulose acetate or polyethersulfone (PES) (low protein binding) filters. Do not use nitrocellulose filters. Nitrocellulose binds surface proteins on the lentiviral envelope and destroys the virus.

2. Transfer clarified supernatant to a sterile container and combine 1 volume of Lenti-X Concentrator with 3 volumes of clarified supernatant. Mix by gentle inversion. Larger volumes may be accommodated through the use of larger (i.e., 250 ml or 500 ml) centrifuge tubes.

NOTE: For easy calculation of the amount of Lenti-X Concentrator to use, simply measure the amount of viral supernatant to be concentrated, divide by 3 and add the resulting amount of Lenti-X Concentrator to your viral supernatant.

3. Incubate mixture at 4°C for 30 minutes to overnight.

NOTE: We have tested incubation times as short as 15 minutes and up to 1 week at 4°C with minimal losses observed. Thorough cooling of the sample is essential, so larger volumes (>100 ml) may require longer incubation times.

4. Centrifuge sample at 1,500 x g for 45 minutes at 4°C. After centrifugation, an off-white pellet will be visible.
5. Carefully remove supernatant, taking care not to disturb the pellet. Residual supernatant can be removed with either a pipette tip or by brief centrifugation at 1,500 x g.
6. **Gently** resuspend the pellet in 1/10 to 1/100th of the original volume using complete DMEM, PBS, or TNE. The pellet can be somewhat sticky at first, but will go into suspension quickly.
7. Immediately titrate sample or store at –70°C in single-use aliquots.

NOTE: For fast determination, the Lenti-X qRT-PCR Titration Kit (Cat. No. 632165) directly quantifies the viral genomes in your virus stock, which is much faster and often more useful than antibiotic selection. Since it exploits conserved regions contained in most lentiviral vectors, all particles, regardless of expression features (promoters, cDNAs, etc.), can be quantified.

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