

I. Introduction

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 down or up to accommodate supernatant volumes. No ultracentrifugation is required. The concentration procedure can be completed in as little as 30 mins (for small volumes), 1.5 hours (for standard volumes), or for convenience, longer incubation times can be used.

Lenti-X Concentrator is designed for use with all lentiviral supernatants, including all of Takara Bio'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.

II. Required Materials

This protocol applies to the following Takara Bio products:

- Lenti-X Concentrator (Cat. Nos. 631231 & 631232)

Additional materials required:

- Lentivirus stock
- 15 & 50 mL conical tubes
- 1.5 mL microcentrifuge tubes
- Filtered pipets and pipetting device
- Micropipettes and tips (with hydrophobic filters)
- 0.45 µm filter (cellulose acetate or polyethersulfone, do not use nitrocellulose)
- Complete cell culture medium (DMEM + 10% FBS) or 1X PBS containing Ca²⁺ and Mg²⁺
- Centrifuge
- Microcentrifuge
- Lenti-X™ CoStix™ Plus (Cat. Nos. 631280 or 631281) (Optional)

III. 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.

Section A is a standard protocol for > 1 mL up to 1.5 L volumes of virus. Section B is a scaled down protocol for a quick concentration of smaller volumes (≤ 1 mL) that are compatible with the Lenti-X Transduction Sponge (Cat. #631478).

A. Standard Protocol

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 with 1/3 volume of Lenti-X Concentrator. 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, ensuring not to disturb the pellet. Residual supernatant can be removed with either by pipetting or by brief centrifugation at 1,500 x g.
6. **Gently** resuspend the pellet with 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 the sample and use, or store at –70°C in single-use aliquots.

NOTE: For fast determination, Lenti-X GoStix Plus (Cat. No. 631280) provides a rapid, simple, and effective method for instantly quantifying lentivirus in packaging cell supernatants. The test involves applying 20 µl of supernatant to a GoStix Plus cassette and waiting 10 minutes for the appearance of test and control bands that indicate the presence of lentiviral p24. The results on the cassette can then be analyzed using a free smartphone app, which quantifies lentivirus titer by comparing the intensities of the test and control bands.

B. Small-Scale (vol. ≤ 1 mL) Protocol

1. Harvest the lentivirus-containing supernatants. Centrifuge briefly (500 x g for 10 min) to remove debris.
2. Transfer clarified supernatant (≤ 1 mL) to a 1.5 mL centrifuge tube and combine with 1/3 volume of Lenti-X Concentrator. Mix by gentle inversion.
3. Incubate mixture at 4°C for 15 min.

NOTE: For this shorter incubation time, it is important that the sample is at 4°C for the entire 15 minutes.

4. Centrifuge sample at 12,000 x g for 5 min at 4°C. After centrifugation, a small, off-white pellet will be visible.
5. Carefully remove the supernatant, ensuring not to disrupt the pellet. Any remaining supernatant can be removed by pipetting or by brief centrifugation at 1,500 x g.
6. Gently resuspend the pellet in 100 µl (1/10th original volume) using complete DMEM or PBS. The pellet can be somewhat sticky at first, but will go into suspension quickly.
7. Immediately titrate the sample and use, or store at –70°C.

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