Protocol-at-a-Glance

Retro-X™ Concentrator Protocol-at-a-Glance

(PT5063-2)

A. Summary

The Retro-X Concentrator (Cat. Nos. 631455 & 631456) provides a fast and simple method for concentrating retroviral stocks. Concentration is achieved by mixing a retroviral supernatant with this concentration reagent, followed by an incubation step and centrifugation in a standard centrifuge. The process is easily scaled up to accomodate larger supernatant volumes. No ultracentrifugation is required. The Retro-X Concentrator is designed for use with all retroviral supernatants, including all of Clontech's Retro-X vectors. Using this reagent increases vector titer (IFU/ml) by 1–2 logs in a reasonable 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 Retro-X Concentrator and incubated overnight 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 retrovirus-containing supernatants. (Caution: supernatants contain live retrovirus.) 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 retroviral envelope and destroys the virus.

2. Transfer clarified supernatant to a sterile container and combine 1 volume of Retro-X Concentrator with 3 volumes of clarified supernatant. Mix by gentle inversion. Larger volumes may be accomodated through the use of larger (i.e., 250 ml or 500 ml) centrifuge tubes. It is recommended to start with at least 10 ml or more of viral supernantant if the viral titer is expected or known to be low (~10^5 IFU/ml).

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

3. Incubate mixture overnight at 4°C.
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 Retro-X qRT-PCR Titration Kit (Cat. No. 631451) directly quantitfies 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 retroviral vectors, all particles, regardless of expression features (promoters, cDNAs, etc.), can be quantified.

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