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Virus Concentration and Infection V.3

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Works for me

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MATERIALS

NAME ▾

CATALOG # ▾

VENDOR ▾

Poly-L-Lysine

MATERIALS TEXT

Lenti-X Concentrator (TakaraBio)

Lentivirus Concentration

- 1 Harvest the lentivirus-containing supernatants. (Caution: supernatants contain live lentivirus.) Pool similar stocks, if desired. Filter through a 0.45 µm filter.
- 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.
- 3 Incubate mixture at 4°C for 30 minutes to overnight. ⚡ 4 °C
⌚ 00:30:00
- 4 Centrifuge sample at 1,500 x g for 45 minutes at 4°C. After centrifugation, an off-white pellet will be visible. ⚡ 1500 x g
⚡ 4 °C
- 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.

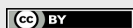
RetroNectin Plate Preparation

- 7 Prepare RetroNectin solution (30 µg/mL) by diluting RetroNectin powder (0.5 mg) into 16.6 mL of PBS

- 8 Dispense an appropriate volume of sterile RetroNectin solution into each well (1.5 mL) per 6 well dish.
- 9 Keep at room temperature for 30 minutes.
🔧 Room temperature
🕒 02:00:00
- 10 Remove the RetroNectin solution and then block with an appropriate volume of sterile 2% bovine serum albumin (BSA, Fraction V) in PBS (1.5 mL of a 6 well dish) Allow the plate to stand at room temperature for 30 minutes. 🔧 Room temperature 🕒 00:30:00
- 11 Remove the BSA solution, and wash the plate once with an appropriate volume of HBSS/Hepes or PBS. After removing the wash solution, the plate is ready for use.

Virus Infection

- 12 Add the retrovirus stock solution or diluted solution at 125 - 500 $\mu\text{L}/\text{cm}^2$ to the RetroNectin-coated plate. (Approx 1.5 mL)
- 13 Place the plate in a centrifuge pre-warmed to 32°C and centrifuge for 2 hours at 32°C at 1,000 - 2,000g to facilitate binding of virus particles with RetroNectin reagent.
🕒 02:00:00 🌡 32 °C 🌀 2000 x g
- 14 Discard the supernatant, but do not allow the plate to dry. Wash the plate with an appropriate volume of PBS or PBS containing 0.1 - 2% albumin (BSA or HSA).
- 15 Collect the target cells and count the number of living cells. Then suspend the cells in the growth medium at a concentration of 0.2 - 1 x 10⁵ cells/mL.
- 16 Do not allow the plate to dry. Immediately add target cells at a density of 0.5 - 2.5 x 10⁴ cells/cm².
 * 6 well dish has SA of 9.6 cm²
- 17 To promote contact between the target cells and viral particles, plates can be centrifuged after adding the cells.
 2000 g for 20 minutes
🌀 2000 x g 🕒 00:20:00
- 18 Incubate in a 37°C, 5% CO₂ incubator for 2 - 3 days.



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