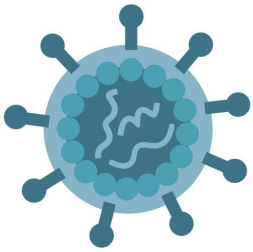
Oct 21, 2019 Version 2

Virus Concentration and Infection V.2



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Protocol status: In development
We are still developing and optimizing this protocol

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Materials

MATERIALS

Poly-L-Lysine

Lenti-X Concentrator (Takarabio)

Troubleshooting

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 ug/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 µl/cm2 to the RetroNectin-coated plate. (Approx 1.5 mL)

13 Place the plate in a centrifuge pre-warmed to 32℃ and centrifuge for 2 hours at 32℃ 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 × 105 cells/ml.

16 Do not allow the plate to dry. Immediately add target cells at a density of 0.5 - 2.5 × 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.
1500 RPM for 10 minutes

18 Incubate in a 37℃, 5% CO2 incubator for 2 - 3 days.