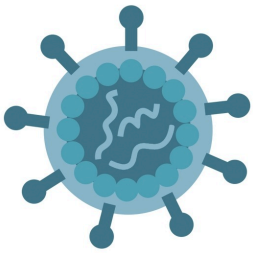
Oct 20, 2019 Version 3

Virus Concentration and Infection V.3



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Protocol status: Working
We use this protocol and it's working

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Materials

MATERIALS

Poly-L-Lysine

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 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.
2000 g for 20 minutes
2000 x g 00:20:00

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