Virongy Biosciences
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Infectin Sample Protocols

Overview – Infectin™ is a viral infection enhancer designed to facilitate viral penetration of the cortical actin barrier, thereby greatly enhancing productive viral infection. Infectin™ can be used to facilitate the infection of a variety of host cells by different viruses and viral vectors. Below is an example procedure for enhancing lentiviral infection of suspension CEM-SS T Cells with Infectin™. We recommend using this procedure as a starting point and testing multiple doses to determine the optimal concentration for your cells.

Suspension Cell Protocol – Lentiviral infection enhancement of suspension CEM-SS T-cells

1) Count cells and determine viability.
   Note: Cell viability should be ≥ 80%.
2) Pellet cells by centrifugation at 300 x *g* for 5 minutes.
3) Resuspend cells in complete media at a concentration of ~2 x 10^6 cells/mL.
4) Add 100µL of cell suspension (~2 x 10^5 cells) per infection.
5) Pre-treat cells by adding 10 µL of Infectin™ (10X) so that Infectin™ concentration is approximately 1X.
6) Mix and incubate for 10-30 minutes at 37°C in a CO^2 incubator.
7) Thaw viral particles on ice.
8) Add the desired volume of virus for infection to a separate microcentrifuge tube.
9) Add the volume of Infectin™ (10X) equal to 10% of the volume of the virus to the microcentrifuge tube containing the virus.
10) Mix gently.
11) Add the virus/Infectin™ mixture to the cells and incubate the infection for 2 hours at 37°C in a CO^2 incubator.
12) Add 1 mL of fresh complete media.
13) Centrifugation at 300 x *g* for 5 minutes.
14) Remove supernatant
15) Add 1 mL of fresh complete media.
16) Culture the infected cells for 2-3 days.
17) Quantify viral infection.

Table 1: Scaleup recommendations for viral infection using Infectin™

Infection Cell Number	Cell Volume	Infectin™ Buffer (10X)	Final Volume
2 x 10^5	100 µL	10 µL	1 mL
5 x 10^5	250 µL	25 µL	2.5 mL
1 x 10^6	500 µL	50 µL	5 mL
2 x 10^6	1 mL	100 µL	10 mL
5 x 10^6	2.5 mL	250 µL	25 mL
1 x 10^7	5 mL	500 µL	50 mL
5 x 10^7	25 mL	2.5 mL	250 mL
1 x 10^8	50 mL	5 mL	500 mL

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Adherent Cell Protocol – Lentiviral transduction enhancement of adherent HDFn cells

1) Count cells and determine viability.
   Note: Cell viability should be ≥ 80%.
2) Pellet cells by centrifugation at 300 x *g* for 5 minutes.
3) Resuspend cells in complete medium at a concentration of ~2 x 10^6 cells/mL.
4) Add 250µL of cell suspension (~5 x 10^5 cells) to a 6-well plate.
5) Add 1750µL of complete medium to each well.
6) Gently mix and incubate for 4-12 hours at 37°C in a CO^2 incubator.
7) Remove medium.
8) Wash cells with 2mL of fresh medium.
9) Remove 1.5mL of wash medium (0.5mL medium remaining).
10) Pre-treat cells by adding 50 µL of Infectin™ (10X) so that Infectin™ concentration is approximately 1X.
11) Mix and incubate for 10-30 minutes at 37°C in a CO^2 incubator.
12) Thaw viral particles on ice.
13) Add the desired volume of virus for infection to a separate microcentrifuge tube.
14) Add the volume of Infectin™ (10X) equal to 10% of the volume of the virus to the microcentrifuge tube containing the virus.
15) Mix gently.
16) Add the virus/Infectin™ mixture to the cells.
17) Mix and incubate the infection for 2 hours at 37°C in a CO^2 incubator.
18) Remove medium
19) Add 2 mL of fresh complete media to wash.
20) Remove wash medium
21) Add 2 mL of fresh complete media.
22) Culture the infected cells for 2-3 days.
23) Quantify viral infection.

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