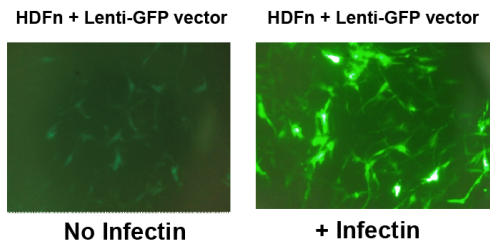


Turbo-Infectin™

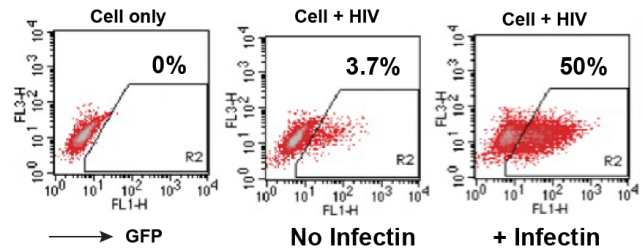
A Virongy proprietary cutting-edge technology

Turbo-Infectin™ is a viral infection enhancer designed to facilitate viral attachment to cells and viral penetration of the cortical actin barrier, which greatly enhance productive viral infection. **Turbo-Infectin™** can be used to facilitate the infection of a variety of host cells by different viruses and viral vectors. **Turbo-Infectin™** can enhance viral infection rates by 3 to 30 fold. Virongy developed **Turbo-Infectin™** based on **Infectin™** and its scientific theory that the actin cytoskeleton is a natural barrier for viral entry and post-entry intracellular migration (Yoder et al., *Cell*, 2008, 134:782). **Turbo-Infectin™** is formulated to combine **Infectin™** with additional technologies that facilitate virus attachment to target cells.

Effects of Infectin™ on Lentiviral Transduction of HDFn cells



Effects of Infectin™ on HIV infection of CD4 T cells



Important

- **Turbo-Infectin™** should be stored at 2-8°C, and is stable for 3 months. *Do not freeze and leave Turbo-Infectin™ at room temperature.*
- **Turbo-Infectin™** viral infection enhancer works with most cell lines to enhance viral infection. On average, Turbo-Infectin™ enhances productive viral infection by **3- to 30-fold***.
- **Turbo-Infectin™** is newly formulated into **Buffer A** and **Buffer B**, both as 10X concentrated.

*(*The degree of enhancement is affected by the types of viruses and cells. Enhancement is strongest for enveloped viruses entering cells via membrane fusion.)*

Applications

- ◆ Enhancing lenti- or retroviral transduction of target cells
- ◆ Enhancing infection rates of other enveloped viruses
- ◆ Facilitating recovery of infectious viruses from cell or tissue cultures
- ◆ Facilitating anti-viral drug screening efficiency

Infectin is intended for Research Use Only and is not for diagnostic or therapeutic purposes or uses in humans or animals.

Protocol

Example – Turbo-Infectin™ enhances lentiviral infection of suspension CEM-SS T cells:

(please see **Table 1** for scale-up recommendation)

- 1) Count cells to be infected, and pellet cells by centrifugation at 300 x g for 5 minutes.
Note: Cell viability should be ≥ 80%.
- 2) Resuspend cells in complete media at a concentration of $\sim 2 \times 10^6$ cells ml⁻¹.
- 3) Use 100 µl of cells ($\sim 2 \times 10^5$) per infection.
- 4) Pre-treat cells by adding 10 µl of **Buffer A (10X)** and 10 µl **Buffer B (10X)** so that Turbo-Infectin™ concentration is approximately **1X**. Mix and incubate for 10-30 minutes at 37°C in a Co2 incubator
- 5) Take out virus from freezer and thaw on ice. **Record the volume of virus used.**
- 6) To the virus solution, add **Buffer A (10X)** and **Buffer B (10X)** in an amount equal to 1/10 of the virus volume used, e.g., if 100 µl of virus is used for infection, add 10 µl of **Buffer A** and 10 µl of **Buffer B**.
- 7) Mix gently and add the virus mixture to cells, and infect for 2 hours at 37°C in a Co2 incubator.
- 8) Centrifuge the virus-cell tube at 300 x g for **30 minutes**
 (* For optimal results, we recommend centrifuging for no less than 30 minutes.)
- 9) Remove supernatant, add 1 ml fresh complete media.
- 10) Culture infected cells for 2-3 days to quantify viral infection.

Table 1: Scaleup recommendations for viral infection using Turbo-Infectin™

Cell number (For infection)	Cell Volume	Buffer A and B (10X)	Final Cell Culture (Vol)
2×10^5	100 µl	10 µl Buffer A + 10 µl Buffer B	1 ml
5×10^5	250 µl	25 µl Buffer A + 25 µl Buffer B	2.5 ml
1×10^6	500 µl	50 µl Buffer A + 50 µl Buffer B	5 ml
2×10^6	1 ml	100 µl Buffer A + 100 µl Buffer B	10 ml
5×10^6	2.5 ml	250 µl Buffer A + 250 µl Buffer B	25 ml
1×10^7	5 ml	500 µl Buffer A + 500 µl Buffer B	50 ml
5×10^7	25 ml	2.5 ml Buffer A + 2.5 ml Buffer B	250 ml
1×10^8	50 ml	5 ml Buffer A + 5 ml Buffer B	500 ml

References

Yoder A, Yu D, Dong L, Iyer SR, Xu X, Kelly J, et al. HIV envelope-CXCR4 signaling activates cofilin to overcome cortical actin restriction in resting CD4 T cells. *Cell*. 2008; 134(5):782-92. PubMed PMID: 18775311.

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