Product Manual

ViraDuctin™ Lentivirus Transduction Kit

Catalog Number

LTV-200	40 transductions (24-well plate)

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

The rate at which lentiviral vectors bind to and infect cells is mostly controlled by diffusion. During lentivirus infection, only a small fraction of the lentiviral vectors can transduce target cells. Virion adsorption is the limiting step of this process. The use of polycations, such as Polybrene®, is standard in many lentiviral infection protocols owing to the observations of improved infection efficiency. Its mechanism of action is thought to involve neutralization of electrostatic repulsion between virion and cell membranes, enhancing attachment.

ViraDuctin™ Lentivirus Transduction Kit is a proprietary formulation for the transduction of lentivirus. After forming a complex with lentivirus in culture medium, ViraDuctin™ provides the following advantages:

- Higher transduction efficiency in many cell types compared to reagents such as Polybrene
- Easy to use
- Ideal for transduction of nonpermissive cells such as primary cells and stem cells

Related Products

1. AAV-200: ViraDuctin™ AAV Transduction Kit
2. AD-200: ViraDuctin™ Adenovirus Transduction Reagent
3. LTV-100: 293LTV Cell Line
4. RV-200: ViraDuctin™ Retrovirus Transduction Kit
5. VPK-107: QuickTiter™ Lentivirus Titer Kit (Lentivirus-Associated HIV p24)

Kit Components (shipped at room temperature)

1. ViraDuctin™ Lentivirus Transduction Reagent A (100X) (Part No. 320001): One sterile tube – 200 µL.
2. ViraDuctin™ Lentivirus Transduction Reagent B (100X) (Part No. 320002): One sterile tube – 200 µL.
3. ViraDuctin™ Lentivirus Transduction Reagent C (8X) (Part No. 320003): Two sterile tubes – 1.5 mL each.

Materials Not Supplied

1. Lentiviral Stock Solution
2. Cells and cell culture growth medium

Storage

Store all kit components at 4ºC. DO NOT FREEZE.

Safety Considerations

Remember that you will be working with samples containing infectious virus. Follow the recommended NIH guidelines for all materials containing BSL-2 organisms.

Transduction Protocol

The following transduction protocols are written for a 24-well format. Refer to the below table for the appropriate dispensing volumes of other plate formats.

Culture Dish	96-well	24-well	12-well	6-well	60-mm	10-cm
Lentivirus/Culture Media (µL)	100	500	1000	2000	5000	10000
Reagent A (100X) (µL)	1	5	10	20	50	100
Reagent B (100X) (µL)	1	5	10	20	50	100
Final Transduction Volume (µL)	102	510	1020	2040	5100	10200
Reagent C (1X) (µL)	100	500	1000	2000	5000	10000

Table 1: Dispensing Volumes of Different Plate Formats

I. Transduction of Adherent Cells

1. The day before transduction, trypsinize and count the cells, plating 0.2-2 x 10^5 cells in 0.5 mL complete culture medium per well of a 24-well plate. Incubate cells at 37°C overnight.
2. On the day of transduction, thaw your lentiviral stock and dilute the lentiviral stock into complete culture medium to a final volume of 0.5 mL in a sterile tube. Mix by inverting; do not vortex. You may prepare serial dilutions if desired.
3. Add 5 µL of ViraDuctin™ Lentivirus Transduction Reagent A (100X), mix by inverting. Immediately add 5 µL of ViraDuctin™ Lentivirus Transduction Reagent B (100X) and mix by inverting.
4. Incubate 30 minutes at 37°C.
5. Remove the culture medium from the cells. Apply all lentivirus/ ViraDuctin™ complexes to cells. Refer to the literature to determine the proper MOI for your specific cell.
6. Incubate at 37°C overnight.
7. Remove the media containing virus and replace with 0.5 mL of complete culture medium.
8. Dilute the appropriate amount of ViraDuctin™ Lentivirus Transduction Reagent C (8X) to 1X with complete culture medium (for example, add 70 µL of 8X Reagent C to 490 µL of complete culture medium).
9. To completely remove the transduction complex, remove the culture medium and replace with 500 µL of the diluted ViraDuctin™ Lentivirus Transduction Reagent C (1X) in each well; gently rock the plate for 30-60 seconds. IMMEDIATELY aspirate the medium containing ViraDuctin™ Lentivirus Transduction Reagent C and replace with 0.5 ml of complete culture medium. Wash twice with complete culture medium to remove any residue complex.
10. 48-72 hrs after transduction, proceed with desired method of detection including functional analysis, immunofluorescence, and western blot. To select stable cell clones, replace medium with fresh medium containing antibiotic every 3-4 days until antibiotic-resistant colonies can be identified.

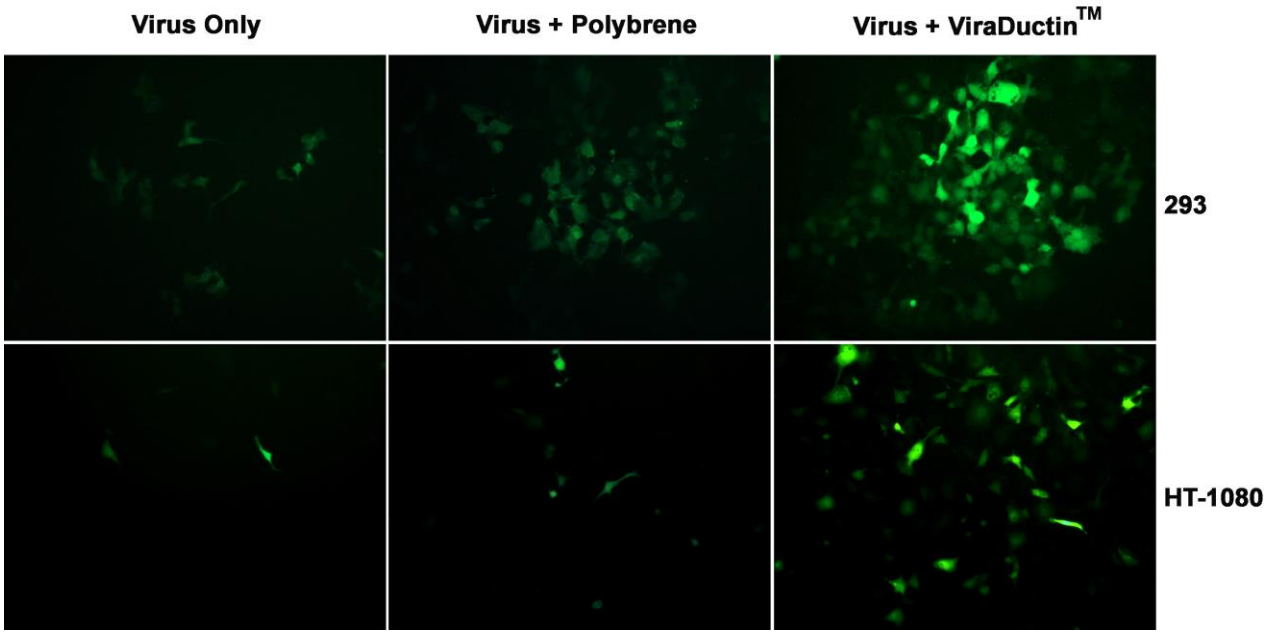
II. Transduction of Suspension Cells

1. On the day of transduction, thaw your lentiviral stock and dilute the lentiviral stock into complete culture medium to a final volume of 0.5 mL in a sterile tube. Mix by inverting; do not vortex. You may prepare serial dilutions if desired.

2. Add 5 µL of ViraDuctin™ Lentivirus Transduction Reagent A (100X), mix by inverting. Immediately add 5 µL of ViraDuctin™ Lentivirus Transduction Reagent B (100X) and mix by inverting.
3. Incubate 30 minutes at 37°C.
4. Pellet your suspension cells for 5 minutes at 1000 g and remove supernatant. Resuspend cell pellet by adding lentivirus/ ViraDuctin™ complexes. Refer to the literature to determine the proper MOI for your specific cell.
5. Incubate at 37°C overnight.
6. Centrifuge for 5 minutes at 1000 g; remove the media containing virus and replace with 0.5 ml of complete culture medium.
7. Dilute the appropriate amount of ViraDuctin™ Lentivirus Transduction Reagent C (8X) to 1X with complete culture medium (for example, add 70 µL of 8X Reagent C to 490 µL of complete culture medium).
8. To completely remove the transduction complex, centrifuge for 5 minutes at 1000 g and remove the supernatant. Add 500 µL of the diluted ViraDuctin™ Lentivirus Transduction Reagent C (1X) to each well and gently rock the plate for 30-60 seconds.
9. Centrifuge for 5 minutes at 1000 g; IMMEDIATELY aspirate the medium containing ViraDuctin™ Lentivirus Transduction Reagent C and resuspend in 0.5 ml of complete culture medium. Repeat twice to remove any residue complex.
10. 48-72 hrs after transduction, proceed with desired method of detection including functional analysis, immunofluorescence, and western blot. To select stable cell clones, replace medium with fresh medium containing antibiotic every 3-4 days until antibiotic-resistant colonies can be identified.

Example of Results

The following figures demonstrate typical purification results. One should use the data below for reference only. This data should not be used to interpret actual results.



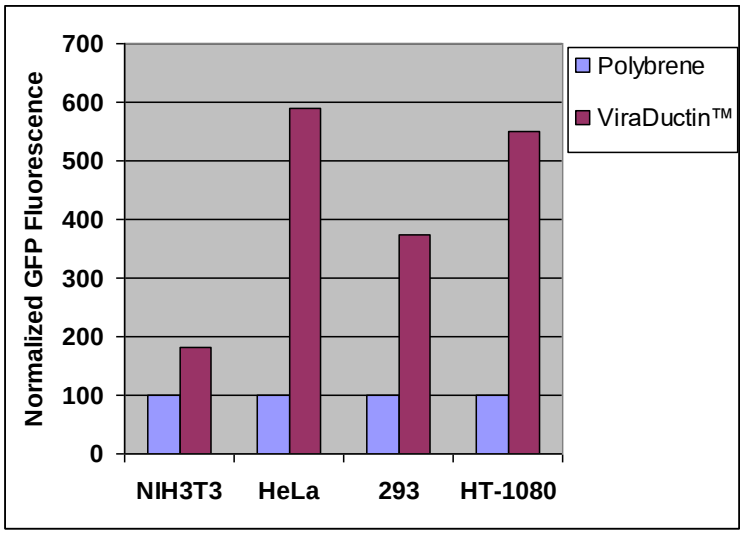


Figure 1: Comparison of the Transduction Efficiency of ViraDuctin™ vs. Polybrene®. Targets cells are seeded at 50,000 cells/well in a 24-well plate overnight. Cells were infected with GFP lentivirus in the presence of Polybrene or ViraDuctin™ for 48 hrs.

Recent Product Citations

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Notice to Purchaser

Warranty

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