
Product Manual

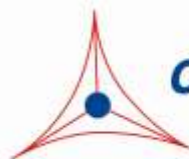
ViraDuctin™ Lentivirus Transduction Kit

Catalog Number

LTV-201

200 transductions (24-well plate)

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures



CELL BIOLABS, INC.
Creating Solutions for Life Science Research

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. LTV-300: GFP Lentivirus Control
5. RV-200: ViraDuctin™ Retrovirus Transduction Kit
6. VPK-107: QuickTiter™ Lentivirus Titer Kit (Lentivirus-Associated HIV p24)
7. VPK-108-H: QuickTiter™ Lentivirus Quantitation Kit (HIV p24 ELISA)
8. VPK-112: QuickTiter™ Lentivirus Quantitation Kit
9. VPK-211-PAN: ViraSafe™ Universal Lentivirus Expression System (Promoterless), Pantropic

Kit Components

1. ViraDuctin™ Lentivirus Transduction Reagent A (100X) (Part No. 320101): One sterile tube – 1.0 mL
2. ViraDuctin™ Lentivirus Transduction Reagent B (100X) (Part No. 320102): One sterile tube – 1.0 mL
3. ViraDuctin™ Lentivirus Transduction Reagent C (8X) (Part No. 320103): One sterile bottle – 15 mL

Materials Not Supplied

1. Lentiviral Stock Solution
2. Cells and cell culture growth medium
3. 37°C Incubator

Storage

Store all kit components at 4°C. 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.

Protocol

The following transduction protocol is 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 $0.2-2 \times 10^5$ 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.

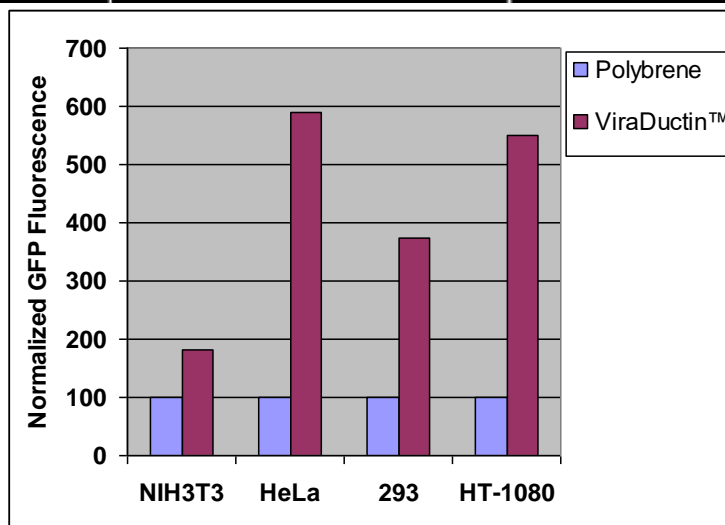
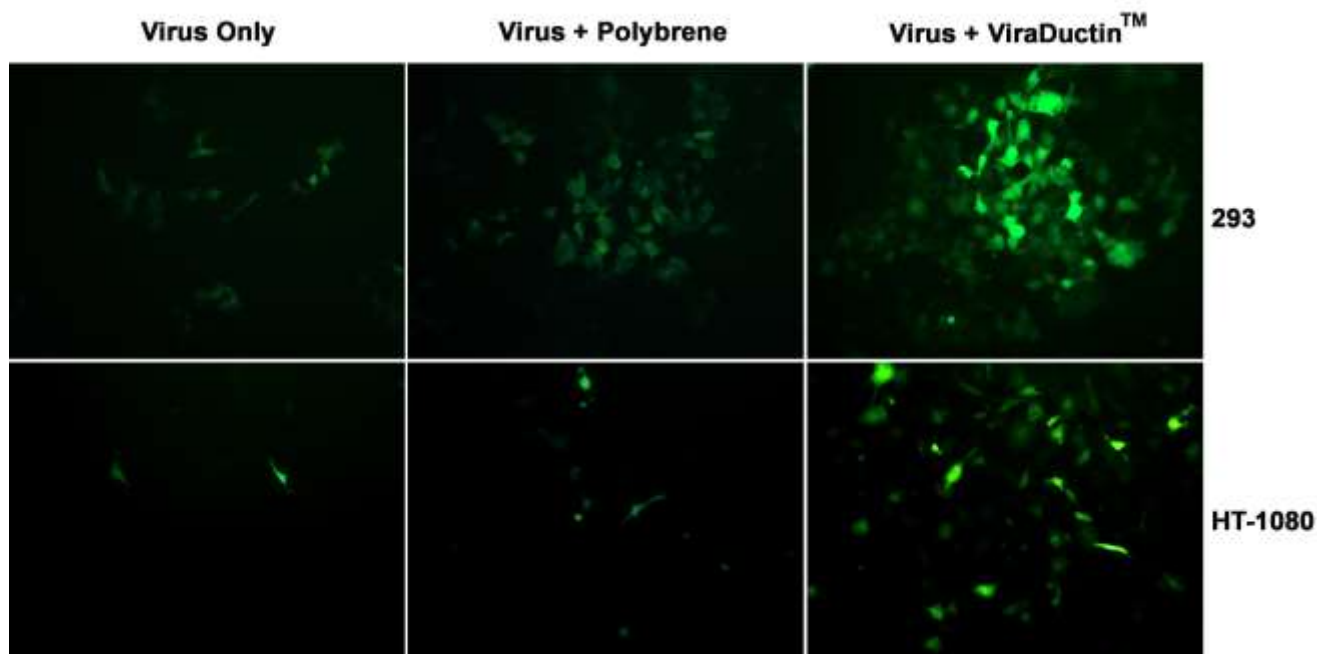


Figure 1: Comparison of the Transduction Efficiency of ViraDuctin™ vs. Polybrene®. Targets cells are seeded at 50,000 cells/well in a 24-well plate overnight. Cells were infected with GFP lentivirus in the presence of Polybrene or ViraDuctin™ for 48 hrs.

Recent Product Citations

1. Tang, C.P. et al. (2021). GCN2 kinase activation by ATP-competitive kinase inhibitors. *Nat Chem Biol.* doi: 10.1038/s41589-021-00947-8.
2. Gao, Y. et al. (2021). The contribution of neuropilin-1 in the stability of CD4+ CD25+ regulatory T cells through the TGF- β 1/Smads signaling pathway in the presence of lipopolysaccharides. *Immun Inflamm Dis.* doi: 10.1002/iid3.551.

3. Xie, W. et al. (2021). Pterostilbene accelerates wound healing by modulating diabetes-induced estrogen receptor β suppression in hematopoietic stem cells. *Burns Trauma*. doi: 10.1093/burnst/tkaa045.
4. Johansson, P. et al. (2020). A Patient-Derived Cell Atlas Informs Precision Targeting of Glioblastoma. *Cell Rep*. **32**(2):107897. doi: 10.1016/j.celrep.2020.107897.
5. Nakamura, M. et al. (2020). MicroRNA-22 enhances radiosensitivity in cervical cancer cell lines via direct inhibition of c-Myc binding protein, and the subsequent reduction in hTERT expression. *Oncology Letters*. **19**:2213-2222. doi: 10.3892/ol.2020.11344.
6. Bokelmann, M. et al. (2020). Utility of primary cells to examine NPC1 receptor expression in Mops condylurus, a potential Ebola virus reservoir. *PLoS Negl Trop Dis*. **14**(1):e0007952. doi: 10.1371/journal.pntd.0007952.
7. Huang, C. et al. (2019). EphA2-to-YAP Pathway Drives Gastric Cancer Growth and Therapy Resistance. *Int J Cancer*. doi: 10.1002/ijc.32609.
8. Zhao, R.B. et al. (2018). High-throughput sequencing analysis of lncRNAs in hippocampus tissues with hypoxic-ischemic brain damage. *Int J Clin Exp Pathol*. **11**(11):5265-5277.
9. Kim, S.C. et al. (2018). Establishment and Characterization of Paired Primary and Peritoneal Seeding Human Colorectal Cancer Cell Lines: Identification of Genes That Mediate Metastatic Potential. *Transl Oncol*. **11**(5):1232-1243. doi: 10.1016/j.tranon.2018.07.014.
10. Kraniak, J.M. et al. (2018). Development of 3D culture models of plexiform neurofibroma and initial application for phenotypic characterization and drug screening. *Experimental Neurology*. **299**, Part B: 289-298.
11. Ghosh, N. & Banerjee, E.R. (2017). A Review on Various Tissue Engineering Techniques to Induce Differentiation of Pluripotent Stem Cells. *Medical Glory*. **1**(2):130-149.
12. Fang, X. et al. (2016). IKK α -mediated biogenesis of miR-196a through interaction with Drosha regulates the sensitivity of cancer cells to radiotherapy. *Cell Death Differ*. doi:10.1038/cdd.2016.32.
13. Osorio, L. A. et al. (2015). SNAIL transcription factor increases the motility and invasive capacity of prostate cancer cells. *Mol Med Rep*. **13**:778-786.
14. Kandasamy, K. et al. (2015). Changes in endothelial Cx43 expression inversely correlates with microvessel permeability and VE-cadherin expression in endotoxin challenged lungs. *Am J Physiol Lung Cell Mol Physiol*. doi: 10.1152/ajplung.00211.2014.
15. Abel, E. V. et al. (2014). The Notch pathway is important in maintaining the cancer stem cell population in pancreatic cancer. *PLoS One*. **9**:e91983.
16. Ozelo, M.C. et al. (2014). Omental implantation of BOECs in hemophilia dogs results in circulating FVIII antigen and a complex immune response. *Blood*. **123**:4045-4053.
17. Rossello, R.A. et al. (2013). Mammalian genes induce partially reprogrammed pluripotent stem cells in non-mammalian vertebrate and invertebrate species. *eLife Sci*. **2**:e00036.
18. Zemskova, M. et al. (2010). p53-dependent induction of prostate cancer cell senescence by the PIM1 protein kinase. *Mol. Cancer Res*. **8**:1126-1141.
19. McEachron, T.A. et al. (2010). Protease-activated receptors mediate crosstalk between coagulation and fibrinolysis. *Blood*. **116**:5037-5044.

Notice to Purchaser

This product is sold for research and development purposes only and is not to be incorporated into products for resale without written permission from Cell Biolabs. The patented technology is covered by an exclusive license. By the use of this product you accept the terms and conditions of all

applicable Limited Use Label Licenses. You may contact our Business Development department at busdev@cellbiolabs.com for information on sublicensing this technology.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com

©2007-2022: Cell Biolabs, Inc. - All rights reserved. No part of these works may be reproduced in any form without permissions in writing.