Product Manual

ViraDuctin™ Adenovirus Transduction Reagent

Catalog Number

AD-201	500 µL (50 transductions in 35mm dish/6-well plate)

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
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Introduction

Recombinant adenoviruses have tremendous potential in both research and therapeutic applications. There are numerous advantages in using an adenovirus to introduce genetic material into host cells. The virus has been used to infect many mammalian cell types (both replicative and non-replicative) for high expression of the recombinant protein. Recombinant adenoviruses are especially useful for gene transfer and protein expression in cell lines that have low transfection efficiency with liposome. After entering cells, the virus remains epichromosomal (i.e. does not integrate into the host chromosome so does not activate or inactivate host genes). Recently, recombinant adenoviruses have been used to deliver RNAi into cells.

A limiting step in adenoviral uptake is the entry into target cells, which is mediated by the coxsackievirus–adenovirus receptor (CAR) and cellular αvβ integrins. In many cell types, these receptors are either absent or expressed at low levels, leading to poor transduction efficiency by adenovirus. For instance, most stem cells and fibroblast-type cells are poorly infected by adenoviral vectors because of low expression of CAR.

ViraDuctin™ Adenovirus Transduction Reagent is a proprietary formulation for the high level adenovirus transduction on cells with no or low levels of CAR. After forming a complex with virus in culture medium, ViraDuctin™ provides the following advantages:

- Higher transduction efficiency in cells with no or low levels of CAR.
- Increase in both the total number of transduced cells and the level of transgene expression per cell.
- Ideal for transduction of nonpermissive cells such as hematopoietic cells and stem cells.

Related Products

1. AD-100: 293AD Cell Line
2. AD-200: ViraDuctin™ Adenovirus Transduction Reagent, 10 Transductions
3. VPK-099: ViraBind™ Adenovirus Miniprep Kit
4. VPK-109: QuickTiter™ Adenovirus Titer Immunoassay Kit
5. VPK-252: RAPAd® CMV Adenoviral Expression System
6. LTV-200: ViraDuctin™ Lentivirus Transduction Kit
7. RV-200: ViraDuctin™ Retrovirus Transduction Reagent
8. AAV-200: ViraDuctin™ AAV Transduction Kit

Materials Not Supplied

1. Adenoviral Stock Solution
2. Cells and cell culture growth medium
3. 37ºC Incubator

Storage
Store 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.

Protocol
The following transduction protocol is written for adherent cells in a 6-well plate or 35 mm culture dish. Refer to the below table for the appropriate dispensing volumes of other plate formats. For suspension cells, the transduction method can be adapted accordingly.

Culture Dish	96-well	24-well	12-well	6-well or 35 mm	60-mm	10-cm
ViraDuctin™ Adenovirus Transduction Reagent (µL)	0.5	2	5	9	18	54
Serum-free Medium (µL)	17	67	167	300	600	1800
Culture Medium Containing FBS (µL)	25	100	250	450	900	2700
Total Transduction Mixture (µL)	42.5	169	422	759	1518	4554
Additional Culture Medium Containing FBS (µL)	100	400	1000	1800	3600	10800

Table 1: Dispensing Volumes of Different Plate Formats

1. The day before transduction, trypsinize and count the cells, plating 0.2-2 x 10^5 cells in 2.0-3.0 mL complete culture medium per well of a 6-well plate. Incubate cells at 37°C overnight.
2. On the day of transduction, warm up the ViraDuctin™ Adenovirus Transduction Reagent for 10 minutes at room temperature. Add 9 µL DIRECTLY into 300 µL of serum-free, antibiotic-free DMEM or RPMI in a polystyrene tube, mix by gentle pipetting and incubate 10 minutes at room temperature.
3. Add your adenovirus stock at desired MOI to the above mixture, mix by gentle pipetting and further incubate for 10 minutes at room temperature.
   Note: The volume of adenovirus added should be not greater than 50 µL.
4. Remove the prepared culture of cells from the incubator. Aspirate the culture medium and add 450 µL of culture medium containing FBS to each well.
5. Add the transduction mixture to the culture plate and gently rock the plate back and forth a few times. Incubate for 4 hrs at 37°C.
6. Without removing the transduction mixture, add 1.8 mL of culture medium containing FBS to each well. Incubate the transduced cells for 24-72 hrs and detect gene expression with desired method.

Note: For most commonly used cell lines, the cells can continue to incubate in the presence of the ViraDuctin™ reagent until the time of the gene expression assay. For

cell lines sensitive to ViraDuctin™ reagent, completely remove the transduction reagents after step 5 and add 2-3 mL of culture medium containing FBS to each well.

Example of Results

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

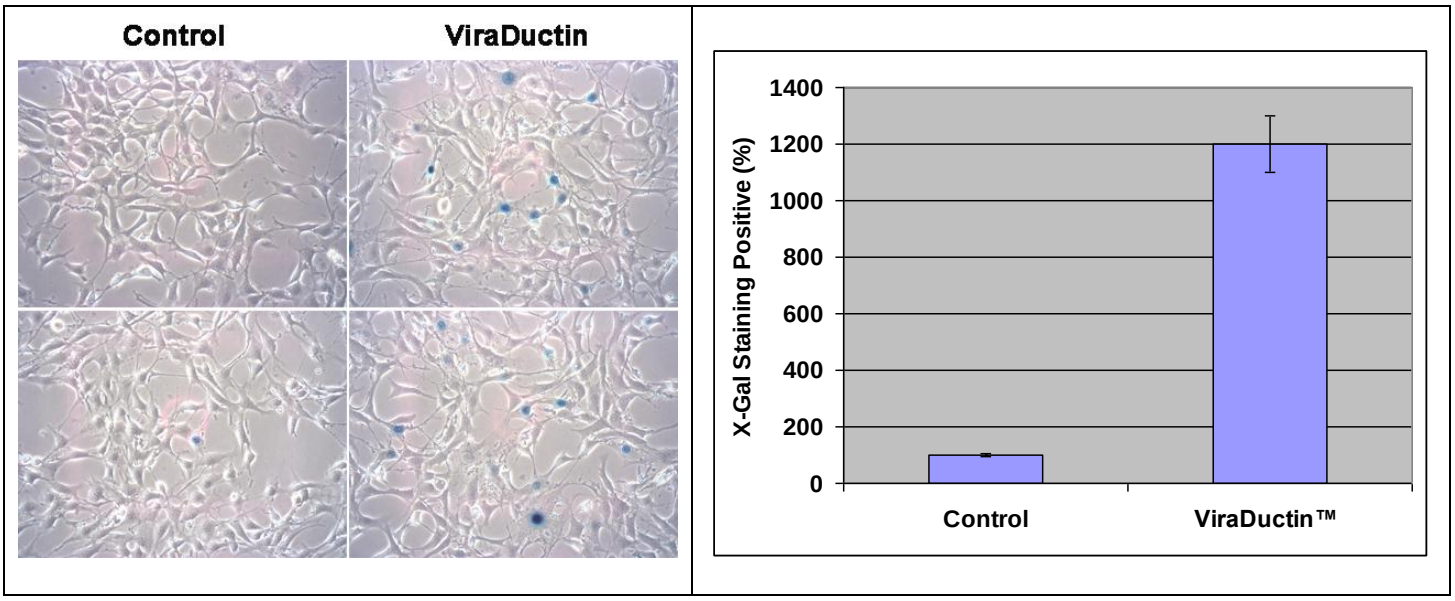

Figure 1: Enhanced Adenovirus Transduction by ViraDuctin™. 100,000 of NIH3T3 cells (low CAR level) were seeded in 6-well plate overnight. 50 MOI of recombinant β-Gal adenovirus (Cat.# ADV-002) was used to infect NIH3T3 cells for 48 hrs in the presence of ViraDuctin™ Adenovirus Transduction Reagent. Infected cells were scored as X-gal staining positive under microscope.

References

1. Bergelson JM, Cunningham JA, Droguett G, Kurt-Jones EA, Krithivas A, Hong JS, Horwitz MS, Crowell RL, Finberg RW. (1997) *Science* **275**, 1320-3.
2. Tomko, R. P., R. Xu, and L. Philipson. (1997) *Proc. Natl. Acad. Sci. USA* **94**, 3352-3356

Recent Product Citations

1. Liu, X. et al. (2020). TRIM62 knockout protects against cerebral ischemic injury in mice by suppressing NLRP3-regulated neuroinflammation. *Biochem Biophys Res Commun*. **529**(2):140-147. doi: 10.1016/j.bbrc.2020.06.014.
2. Dong, W. et al. (2018). Retinoic acid receptor α expression exerts an anti-apoptosis effect on PC12 cells following oxygen-glucose deprivation. *Exp Ther Med*. **16**(4):3525-3533. doi: 10.3892/etm.2018.6639.
3. Haidari, M. et al. (2014). Disruption of endothelial adherens junctions by high glucose is mediated by protein kinase C-β-dependent vascular endothelial cadherin tyrosine phosphorylation. *Cardiovasc Diabetol*. **13**:112.

4. Monick, M. et al. (2008). Constitutive ERK MAPK activity regulates macrophage ATP production and mitochondrial integrity. *J. Immunol.* **180**:7485-7496.
5. Ackerman, W. et al. (2007). Nuclear Factor-kappa B regulates inducible prostaglandin E synthase expression in human amnion mesenchymal cells. *Biol. Reprod.* **78**: 68-76.

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