Product Manual

ViraDuctin™ AAV Transduction Kit

Catalog Number

AAV-201	50 transductions in 35 mm dish/6-well plate

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

The viral system includes vectors developed from retrovirus (RV), adenovirus (AdV), adeno-associated virus (AAV), lentivirus (LV), and herpes simplex virus (HSV). AAV belongs to the family of Parvoviridae, a group of viruses among the smallest of single-stranded and non-enveloped DNA viruses. There are eight different AAV serotypes reported to date.

Recombinant AAV-2 is the most common serotype used in gene delivery, and can be produced at high titers with a helper virus or Cell Biolabs' AAV Helper-Free System. AAV can infect both dividing and non-dividing cells and can be maintained in the human host cell, creating the potential for genome integration. Because AAV is a naturally defective virus, requiring provision of several factors in *trans* for productive infection, it is considered the safest viral vector to use. These factors make AAV an attractive vector for gene therapy.

The AAV transduction process includes viral binding and entry, intracellular trafficking, nuclear transport, and viral second strand DNA synthesis. The viral second strand DNA synthesis has been shown to be the rate limiting step, which leads to inefficient transduction by AAV vectors.

ViraDuctin™ AAV Transduction Kit is a proprietary formulation for the transduction of AAV. ViraDuctin™ provides the following advantages:

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

Related Products

1. AAV-100: 293AAV Cell Line
2. VPK-140: ViraBind™ AAV Purification Kit
3. VPK-141: ViraBind™ AAV Purification Mega Kit
4. VPK-145: QuickTiter™ AAV Quantitation Kit
5. LTV-200: ViraDuctin™ Lentivirus Transduction Kit
6. RV-200: ViraDuctin™ Retrovirus Transduction Kit

Kit Components

1. ViraDuctin™ AAV Transduction Reagent A (20X) (Part No. 320107): One sterile bottle – 5 mL.
2. ViraDuctin™ AAV Transduction Reagent B (100X) (Part No. 320108): One sterile tube – 1 mL.

Materials Not Supplied

1. AAV Stock Solution
2. Cells and cell culture growth medium
3. 37ºC Tissue Culture Incubator

Storage
Store kit components at -20ºC. Avoid multiple freeze/thaws by aliquoting.

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.

Culture Dish	96-well	24-well	12-well	6-well or 35 mm	60-mm	10-cm
ViraDuctin™ AAV Reagent A (20X) (µL)	5	25	50	100	250	500
ViraDuctin™ AAV Reagent B (100X) (µL)	1	5	10	20	50	100
Complete Culture Media (µL)	94	470	940	1880	4700	9400
Final Reagent Mixture (µL)	100	500	1000	2000	5000	10000

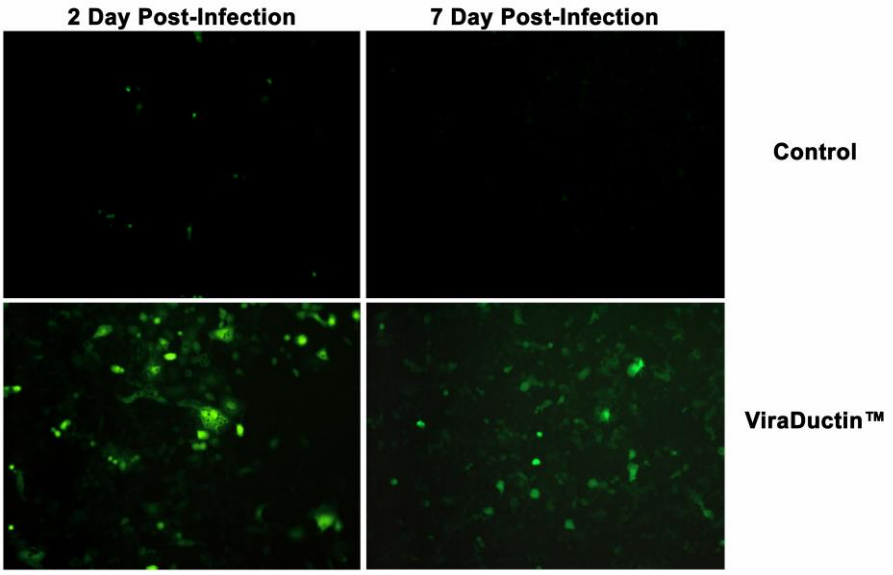
Table 1: Dispensing Volumes of Different Plate Formats

1. The day before transduction, trypsinize and count the cells, plating 1-4 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 to ensure firm attachment.
2. Warm the ViraDuctin™ AAV Transduction Reagents for at least 10 minutes at room temperature.
3. In a sterile tube, add 100 µL of ViraDuctin™ AAV Transduction Reagent A (20X) and 20 µL of ViraDuctin™ AAV Transduction Reagent B (100X), mix by inverting.
4. Incubate 5 minutes at room temperature.
5. Next, add 1.88 mL of complete culture medium to the ViraDuctin™ Reagent mixture, mix by inverting.
6. Incubate 5 minutes at room temperature.
7. Remove the culture medium from the overnight plated cells. Apply the entire volume of ViraDuctin™ Transduction Reagent mixture to the cells (~ 2 mL).
8. Incubate at 37°C overnight.
9. Carefully, aspirate the media from the cells.
10. Wash the cells twice with 2 mL of complete culture medium.
11. Add 1 mL of desired AAV-containing media to the plate well. Incubate at 37°C for 1-2 hours, gently swirling/mixing every 30 minutes.

12. Add an additional 1 mL of pre-warmed complete media per well, incubating at 37°C for 2-7 days.

Example of Results

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



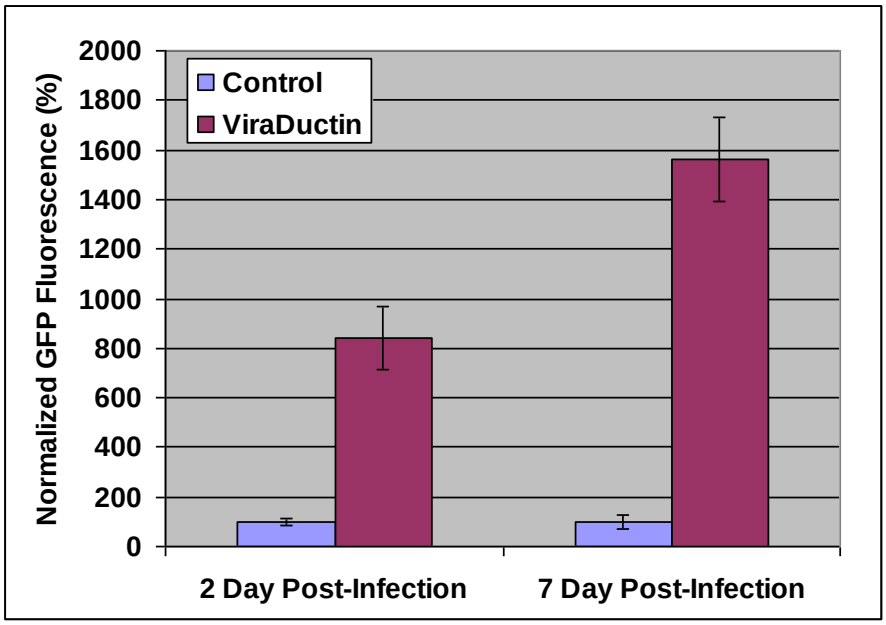


Figure 1: AAV-GFP Transduction Efficiency with ViraDuctin™ . HeLa cells were seeded at 50,000 cells/well in a 24-well plate overnight. Cells were then treated with ViraDuctin™ for 20 hours before infecting with AAV-GFP.

References

1. Rabinowitz, J, and Samulski, R. J. (1998). *Curr. Opin. Biotechnol*., **9**, 470-475.
2. Summer ford, C., and Samulski, R. J. (1999). *Nat. Med., 5, 587-588.*
3. Clark, K., Liu, X., McGrath, J., and Johnson, P. (1999). *Hum. Gene Ther*., **10**, 1031-1039.

Recent Product Citations

1. McQuillan, H.J. et al. (2022). Definition of the estrogen negative feedback pathway controlling the GnRH pulse generator in female mice. *Nat Commun*. **13**(1):7433. doi: 10.1038/s41467-022-35243-z.
2. Lau, C-H. et al. (2019). Targeted Transgene Activation in the Brain Tissue by Systemic Delivery of Engineered AAV1 Expressing CRISPRa. *Molecular Therapy: Nucleic Acid*. doi: 10.1016/j.omtn.2019.04.015.
3. Nazari, M. et al. (2014). AAV2-mediated follistatin overexpression induces ovine primary myoblasts proliferation. *BMC Biotechnol*. **14**:87.

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