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 non-permissive 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. AD-200: ViraDuctin™ Adenovirus Transduction Reagent
6. LTV-200: ViraDuctin™ Lentivirus Transduction Kit
7. 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. 37°C Tissue Culture Incubator

**Storage**
Store kit components at -20°C. 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.

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

*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

Recent Product Citations

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