

# VirusGEN® AAV Transfection Kit

## Quick Reference Protocol

Instructions for MIR 6745, 6750, 6755

Full protocol, SDS and Certificate of Analysis available at [mirusbio.com/virusgen](http://mirusbio.com/virusgen)



### SPECIFICATIONS

<b>Storage</b>	Store <i>TransIT</i> -VirusGEN® Transfection Reagent at -10 to -30°C, tightly capped. <b>Before each use</b> , warm to room temperature and vortex gently. Store VirusGEN® AAV Complex Formation Solution and Enhancer at 2 to 10°C.
<b>Product Guarantee</b>	When properly stored and handled, <i>TransIT</i> -VirusGEN® Transfection Reagent is guaranteed for 1 year from the date of purchase, and VirusGEN® Complex Formation Solution and Enhancer is guaranteed for 6 months from the date of purchase.

### ► PROTOCOL FOR ADENO-ASSOCIATED VIRUS (AAV) GENERATION IN SUSPENSION HEK 293 CULTURES



Full protocol and additional documentation available at [mirusbio.com/virusgen](http://mirusbio.com/virusgen)

#### VirusGEN® AAV Transfection Kit Workflow

#### A. Maintain Cells

Passage cells regularly and ensure they are >95% viable before transfection.

#### B. Day 0

Dilute cells to  $2 - 3 \times 10^6$  cells/ml.

Form transfection complexes in either VirusGEN® AAV CFS&E or PBS; use a volume that is 5-10% of cell culture volume.

Per ml of culture, add  
1. **DNA:** 1 - 2 µg  
2. **TransIT-VirusGEN®:** 1.5 - 3 µl.

Incubate stationary for **15 - 30 min.**

#### C. Add transfection complexes to cells.

#### D. Day 2 - 3

Harvest AAV **48 - 72 hr** post-transfection.

Total Plasmid DNA refers to the combined mass of packaging plasmids and the transfer plasmid containing the gene-of-interest. Premix the plasmids together prior to adding to the complex formation medium.

### Fill in volumes below based on total culture volume (Table 1).

#### A. Maintain cells

1. Passage suspension HEK 293 cells 18-24 hours prior to transfection to obtain a density of  $2 - 4 \times 10^6$  cells/ml the next day. Do NOT proceed with transfection if cells are not doubling every 24 hours or are < 95% viable.
2. Incubate cells overnight at appropriate temperature and CO<sub>2</sub> levels.

#### B. Prepare *TransIT*-VirusGEN® Reagent:DNA complexes

1. At time of transfection, seed cells to a density of  $2 - 3 \times 10^6$  cells/ml.
2. Warm *TransIT*-VirusGEN® Reagent to room temperature and vortex.
3. Place \_\_\_ ml of VirusGEN® AAV Complex Formation Solution and Enhancer (CFS&E) or PBS in a sterile tube.
4. Add \_\_\_ µl of the total plasmid DNA to the tube. Mix gently by pipetting.
5. Add \_\_\_ µl of *TransIT*-VirusGEN® Reagent. Vortex gently to mix.
6. Incubate at room temperature for 15-30 minutes to allow transfection complexes to form. Do not vigorously agitate complexes again once formed.

#### C. Distribute complexes to cells in complete growth medium

1. Add *TransIT*-VirusGEN® Reagent:DNA complexes (from Step B) to cells.
2. Incubate cultures in appropriate conditions (i.e. 37°C, 5% CO<sub>2</sub>, shaking) for 48-72 hours prior to AAV harvest.

#### D. Harvest virus

1. Following the 48-72 hour incubation, prepare 10X Cell Lysis Buffer (500 mM Tris pH 8, 10% Tween® 20, 20 mM MgCl<sub>2</sub>).
2. Transfer the total volume of cell suspension (\_\_\_ ml) to a sterile conical tube or appropriate vessel.
3. Add 0.1X volume (\_\_\_ ml) of 10X Cell Lysis Buffer and 100 U/ml (\_\_\_ µl) of Benzonase®. Mix completely and incubate at 37°C for 1.5 hours with shaking.
4. Add 0.1X volume (\_\_\_ ml) of 5 M NaCl. Mix completely and incubate at 37°C for 30 minutes with shaking.
5. Centrifuge the mixture at 4,100 × g for 10 minutes to remove cell debris.
6. Transfer the AAV-containing supernatant to a new tube. Store at -80°C.

Table 1. Volume scaling worksheet for VirusGEN® AAV Transfection Kit

	Starting conditions per milliliter of complete growth medium		
	Per 1 ml	Total culture volume	Reagent quantities
VirusGEN® AAV CFS&E or PBS	0.1 ml	× _____ ml	= _____ ml
Total Plasmid DNA (1 µg/µl)	2 µl	× _____ ml	= _____ µl
<i>TransIT</i> -VirusGEN® Reagent	3 µl	× _____ ml	= _____ µl

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**Reagent Agent<sup>®</sup>**

Reagent Agent<sup>®</sup> is an online tool designed to help determine the best solution for nucleic acid delivery based on in-house data, customer feedback and citations.

Learn more at: [mirusbio.com/ra](https://www.mirusbio.com/ra)

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