



SPECIFICATIONS

Storage	Store TransIT-VirusGEN® Transfection Reagent tightly capped at -20°C. Before each use , warm to room temperature and vortex gently.
Product Guarantee	1 year from the date of purchase, when properly stored and handled.

► **PROTOCOL FOR LENTIVIRUS GENERATION
IN ADHERENT HEK 293 CELL CULTURES**



Full protocol and additional documentation available at mirusbio.com/6700

Fill in volumes below based on culture vessel used for transfection (Table 1).

- A. Plate cells approximately 18-24 hours prior to transfection**
1. Plate cells in ___ ml complete growth medium (per well or flask).
Plate cells at a density of $4.0 - 5.0 \times 10^5$ cells/ml
 2. Culture overnight. Cells should be 80-95% confluent on day of transfection. Transfecting cells at a lower confluency may lead to high cellular toxicity and lower virus titers.
- B. Prepare TransIT-VirusGEN®:DNA complexes (immediately before transfection)**
1. Warm TransIT-VirusGEN® Transfection Reagent to room temperature and vortex gently.
 2. Place ___ µl of PBS (e.g. Millipore Sigma Cat. No. D8537) or serum-free media in a sterile tube.
 3. Add ___ µl of the total plasmid DNA to the tube. Mix completely.
 4. Add ___ µl of TransIT-VirusGEN® to the DNA mixture. Mix by pipetting, vortexing or inversion.
 5. 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**
1. Add TransIT-VirusGEN®:DNA complexes drop-wise to different areas of the well.
 2. Gently rock plate or vessel for even distribution of complexes.
 3. Incubate 48 hours prior to lentivirus harvest. NOTE: It is not necessary to replace complete growth medium with fresh medium post-transfection.
- D. Virus harvest and storage**
1. Harvest cell supernatant containing recombinant lentivirus particles.
 2. Filter virus-containing supernatant through a 0.45 µm PVDF filter to remove any cells.
 3. Immediately flash freeze aliquots in cryogenic tubes and store at -80°C.

Table 1. Recommended starting conditions

Culture vessel	6-well plate	10-cm dish	T75 flask
Surface area	9.6 cm ²	59 cm ²	75 cm ²
Complete growth medium	2.0 ml	10 ml	15 ml
PBS or serum-free medium	200 µl	1.0 ml	1.5 ml
Total Plasmid DNA (1 µg/µl)	2 µl	10 µl	15 µl
TransIT-VirusGEN® Reagent	6 µl	30 µl	45 µl

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.

► **Transfection Optimization**

The amount of TransIT-VirusGEN® required for transfection is dictated by the amount of DNA. Determine the best TransIT-VirusGEN® Reagent:DNA ratio for each cell type. Start with 3 µl of TransIT-VirusGEN® per 1 µg of DNA. Vary the concentration of TransIT-VirusGEN® from 2-4 µl per 1 µg of DNA to find the optimal ratio.

For additional transfection optimization tips, see the TransIT-VirusGEN® [full protocol](#).

TransIT-VirusGEN® Transfection Reagent

Quick Reference Protocol for Lentivirus Generation

Instructions for MIR 6700, 6703, 6704, 6705, 6706, 6710, 6720, 6740

Full protocol, SDS and Certificate of Analysis available at mirusbio.com/6700



► PROTOCOL FOR LENTIVIRUS GENERATION IN SUSPENSION HEK 293 CELL CULTURES



Full protocol and additional
documentation available at
mirusbio.com/6700

Fill in volumes below based on total culture volume (Table 2).

A. Maintenance of cells

1. Passage suspension HEK 293 cells 18-24 hours prior to transfection to ensure that cells are actively dividing at the time of transfection and to obtain a density of $2 - 3 \times 10^6$ cells/ml the next day. DO NOT proceed with transfection if cells are not doubling every 24 hours or are < 95% viable by trypan blue exclusion.
2. Incubate cells overnight at appropriate temperature and CO₂ levels (e.g. 37°C, 5-8% CO₂, shaking).

B. Prepare TransIT-VirusGEN® Reagent:DNA complexes

1. Seed cells at a density of $2 - 3 \times 10^6$ cells/ml immediately prior to transfection. DO NOT proceed with transfections if cells are not doubling normally or are not at high viability.
2. Warm TransIT-VirusGEN® Transfection Reagent to room temperature and vortex gently.
3. Place ____ ml of PBS (e.g. Millipore Sigma Cat. No. D8537) or serum-free media in a sterile tube.
4. Add ____ µl of the total plasmid DNA to the tube. Mix completely.
5. Add ____ µl of TransIT-VirusGEN® Reagent to the DNA. Mix by pipetting, vortexing or inversion.
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 to cultured cells (prepared in Step B).
2. Shake cultures on an orbital shaker (e.g. 125 rpm when using a shaker with a 1.9 cm orbital throw) at appropriate temperature and CO₂ levels (e.g. 37°C, 5-8% CO₂, shaking).
3. Incubate transfected cultures for 48 hours prior to lentivirus harvest.

D. Virus harvest and storage

1. Following the 48-hour incubation, centrifuge cells in a sterile tube at 300 × g for 5 minutes. DO NOT dispose of the supernatant following centrifugation.
2. Transfer the virus containing supernatant into a new sterile tube.
3. Filter through a 0.45 µm PVDF filter (e.g. Millipore Steriflip-HV) to remove any cell debris.
4. Immediately flash-freeze aliquots of lentivirus in cryo-tubes and store at -80°C.

Table 2. Volume scaling worksheet for lentivirus generation using TransIT-VirusGEN® Reagent

Starting conditions per milliliter of complete growth medium (Lentivirus Generation)				
	Per 1 ml		Total culture volume	Reagent quantities
PBS or serum-free medium	0.1 ml	×	_____ ml	= _____ ml
Total Plasmid DNA (1 µg/µl)	1 µl	×	_____ ml	= _____ µl
<u>TransIT-VirusGEN®</u> Reagent	3 µl	×	_____ ml	= _____ µl

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.

TransIT-VirusGEN® Transfection Reagent

Quick Reference Protocol for AAV Generation

Instructions for MIR 6700, 6703, 6704, 6705, 6706, 6710, 6720, 6740
Full protocol, SDS and Certificate of Analysis available at mirusbio.com/6700



SPECIFICATIONS

Storage	Store TransIT-VirusGEN® Transfection Reagent tightly capped at -20°C. Before each use , warm to room temperature and vortex gently.
Product Guarantee	1 year from the date of purchase, when properly stored and handled.

► PROTOCOL FOR ADENO-ASSOCIATED VIRUS (AAV)
GENERATION IN ADHERENT HEK 293 CELL CULTURES



Full protocol and additional
documentation available at
mirusbio.com/6700

Fill in volumes below based on culture vessel used for transfection (Table 3).

- A. Plate cells approximately 18-24 hours prior to transfection**
1. Plate cells in ___ml complete growth medium to a density of 4.0 - 5.0 × 10⁵ cells/ml.
 2. Culture overnight. Cells should be 80-95% confluent on day of transfection. Transfecting cells at a lower confluency may lead to high cellular toxicity and lower virus titers.
- B. Prepare TransIT-VirusGEN®:DNA complexes (immediately before transfection)**
1. Warm TransIT-VirusGEN® Transfection Reagent to room temperature and vortex gently.
 2. Place ___µl of PBS (e.g. Millipore Sigma Cat. No. D8537) or serum-free media in a sterile tube.
 3. In a separate tube, combine all AAV plasmids per the manufacturer recommendations to a final concentration of 1 µg/µl. Mix thoroughly.
 4. Transfer ___µl of total plasmid DNA to the tube containing PBS. Mix completely.
 5. Add ___µl of TransIT-VirusGEN® to the DNA mixture. Mix by pipetting, vortexing or inversion.
 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**
1. Add TransIT-VirusGEN® Reagent:DNA complexes drop-wise to different areas of the well.
 2. Gently rock plate or vessel for even distribution of complexes.
 3. Incubate at 37°C in 5% CO₂ for 48-72 hours prior to AAV harvest.
- D. Virus harvest and storage**
1. Following the 48-72 hour incubation, prepare 10X Cell Lysis Buffer (500 mM Tris pH 8, 10% Tween® 20, 20 mM MgCl₂).
 2. Add 0.1X volume (___ml) of 10X Cell Lysis Buffer and 100 U/ml (___µl) of Benzonase® to each well or flask. Incubate at 37°C for 1.5 hours with shaking.
 3. Add 0.1X volume (___ml) of 5 M NaCl. Mix completely and incubate at 37°C for 30 minutes with shaking.
 4. Centrifuge the mixture at 4,100 × g for 10 minutes to remove cell debris.
 5. Transfer the AAV-containing supernatant to a new tube. Store at -80°C.

Table 3. Recommended starting conditions

Culture vessel	6-well plate	10-cm dish	T75 flask
Surface area	9.6 cm ²	59 cm ²	75 cm ²
Complete growth medium	2.0 ml	10 ml	15 ml
PBS or serum-free medium	200 µl	1.0 ml	1.5 ml
Total Plasmid DNA (1 µg/µl)	3 µl	15 µl	22.5 µl
TransIT-VirusGEN® Reagent	6 µl	30 µl	45 µl

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.

► Transfection Optimization

Determine the best TransIT-VirusGEN® Reagent:DNA ratio for each cell type. Start with 2 µl of TransIT-VirusGEN® per 1 µg of DNA. Vary the concentration of TransIT-VirusGEN® from 1-4 µl per 1-2 µg of DNA to find the optimal ratio.

For additional transfection optimization tips, see the TransIT-VirusGEN® [full protocol](https://mirusbio.com/6700).

TransIT-VirusGEN® Transfection Reagent

Quick Reference Protocol for AAV Generation

Instructions for MIR 6700, 6703, 6704, 6705, 6706, 6710, 6720, 6740
Full protocol, SDS and Certificate of Analysis available at mirusbio.com/6700



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



Full protocol and additional
documentation available at
mirusbio.com/6700

Fill in volumes below based on total culture volume (Table 4).

A. Maintenance of cells

- 1. Passage suspension HEK 293 cells 18-24 hours prior to transfection to ensure that cells are actively dividing at the time of transfection and to obtain a density of $2 - 3 \times 10^6$ cells/ml the next day.
- 2. Incubate cells overnight at appropriate temperature and CO₂ levels (e.g. 37°C, 5-8% CO₂, shaking).

B. Prepare TransIT-VirusGEN® Reagent:DNA complexes

- 1. Seed cells at a density of $2 - 3 \times 10^6$ cells/ml immediately prior to transfection. DO NOT proceed with transfections if cells are not doubling normally or are not at high viability.
- 2. Warm TransIT-VirusGEN® Transfection Reagent to room temperature and vortex gently.
- 3. Place ____ml of PBS (e.g. Millipore Sigma Cat. No. D8537) or serum-free media in a sterile tube.
- 4. In a separate tube, combine all AAV plasmids per the manufacturer recommendations to a final concentration of 1 µg/µl. Mix thoroughly.
- 5. Transfer ____µl of total plasmid DNA to the tube containing PBS. Mix completely.
- 6. Add ____µl of TransIT-VirusGEN® Reagent to the DNA. Mix by pipetting, vortexing or inversion.
- 7. 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 to cultured cells (prepared in Step B).
- 2. Shake cultures on an orbital shaker (e.g. 125 rpm when using a shaker with a 1.9 cm orbital throw) at appropriate temperature and CO₂ levels (e.g. 37°C, 5-8% CO₂, shaking).
- 3. Incubate transfected cultures for 48-72 hours prior to AAV harvest.

D. Virus harvest and storage

- 1. Following the 48-72 hour incubation, prepare 10X Cell Lysis Buffer (500 mM Tris pH 8, 10% Tween® 20, 20 mM MgCl₂).
- 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 4. Volume scaling worksheet for AAV generation using TransIT-VirusGEN® Reagent

Starting conditions per milliliter of complete growth medium (AAV Generation)				
	Per 1 ml		Total culture volume	Reagent quantities
PBS or serum-free medium	0.1 ml	×	_____ml	= _____ml
Total Plasmid DNA (1 µg/µl)	2 µl	×	_____ml	= _____µl
TransIT-VirusGEN® Reagent	3 µl	×	_____ml	= _____µl

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.

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