*Trans*IT-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

SPECIFICATIONS

Storage	Store *Trans*IT-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 × 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 *Trans*IT-VirusGEN®:DNA complexes (immediately before transfection)

1. Warm *Trans*IT-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 *Trans*IT-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 *Trans*IT-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
***Trans*IT-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 *Trans*IT-VirusGEN® required for transfection is dictated by the amount of DNA. Determine the best *Trans*IT-VirusGEN® Reagent:DNA ratio for each cell type. Start with 3 µl of *Trans*IT-VirusGEN® per 1 µg of DNA. Vary the concentration of *Trans*IT-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.

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*Trans*IT-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 × 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 CO_2 levels (e.g. 37°C, 5-8% CO_2, shaking).

B. Prepare *Trans*IT-VirusGEN® Reagent:DNA complexes

1. Seed cells at a density of 2 - 3 × 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 *Trans*IT-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 *Trans*IT-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 *Trans*IT-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 CO_2 levels (e.g. 37°C, 5-8% CO_2, 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 *Trans*IT-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
*Trans*IT-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.

*Trans*IT-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 *Trans*IT-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 × 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 *Trans*IT-VirusGEN®:DNA complexes (immediately before transfection)

1. Warm *Trans*IT-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 *Trans*IT-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 *Trans*IT-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% CO_2 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 $MgCl_2$).
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
***Trans*IT-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 *Trans*IT-VirusGEN® Reagent:DNA ratio for each cell type. Start with 2 µl of *Trans*IT-VirusGEN® per 1 µg of DNA. Vary the concentration of *Trans*IT-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.

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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 × 10^6 cells/ml the next day.
2. Incubate cells overnight at appropriate temperature and CO_2 levels (e.g. 37°C, 5-8% CO_2, shaking).

B. Prepare *Trans*IT-VirusGEN® Reagent:DNA complexes

1. Seed cells at a density of 2 - 3 × 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 *Trans*IT-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 *Trans*IT-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 *Trans*IT-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 CO_2 levels (e.g. 37°C, 5-8% CO_2, 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 $MgCl_2$).
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 *Trans*IT-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
*Trans*IT-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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