VirusGEN® LV Transfection Kit

Quick Reference Protocol

Instructions for MIR 6760, 6765, 6792

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

SPECIFICATIONS

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

PROTOCOL FOR LENTIVIRUS GENERATION IN ADHERENT HEK 293 CELL CULTURES

Full protocol and additional documentation available at *mirusbio.com/virusgen*

VirusGEN® LV Transfection Kit Workflow

A. Plate Cells
Seed cells at **5 × 10^5** cells/ml 18 - 24 hr before transfection.

B. 0 hr
Form complexes in VirusGEN® LV Complex Formation Solution in a volume that is 5-10% of cell culture volume.

Per ml of culture, add
1. **DNA:** 0.5 - 1.5 µg
2. ***Trans*IT-VirusGEN®:** 1.5 - 4.5 µl.

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

C. Add transfection complexes to cells.

D. 5 - 24 hr
Add **VirusGEN® LV Enhancer** in a volume that is 5-10% of cell culture volume.

E. 48 hr
Harvest lentivirus from supernatant.

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

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

A. Maintain cells
1. Approximately 18-24 hours prior to transfection, plate cells at a starting density of 4 - 6 × 10^5 cells/ml. Cultures should be 80-95% confluent at the time of transfection.
2. Incubate cells overnight at appropriate temperature and CO_2 levels.

B. Prepare *Trans*IT-VirusGEN® Reagent:DNA complexes
1. Warm *Trans*IT-VirusGEN® Reagent to room temperature and vortex.
2. Place ___ml of VirusGEN® LV Complex Formation Solution in a sterile tube.
3. Add ___µl of the total plasmid DNA to the tube. Mix gently by pipetting.
4. Add ___µl of *Trans*IT-VirusGEN® Reagent. Vortex gently to mix.
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 in complete growth medium
1. Add *Trans*IT-VirusGEN® Reagent:DNA complexes (from Step B) to cells.
2. Gently rock culture vessel for even distribution of complexes.
3. Incubate transfected cultures until addition of the VirusGEN® LV Enhancer.

D. Add VirusGEN® LV Enhancer to transfected cells
1. Between 5-24 hours post-transfection, add ___mL of VirusGEN® LV Enhancer to cultures.
   NOTE: Mirus recommends comparing transfection +/- Enhancer because, with some cell lines and vector constructs, equivalently high titer can be obtained *without* the Enhancer component.
2. Incubate cultures for an additional 24-43 hours prior to virus harvest (i.e. harvest lentivirus a total of 48 hours post-transfection).

E. Harvest virus
1. Following the 48-hour incubation, harvest cell supernatant containing recombinant lentivirus particles.
2. Filter through a 0.45 µm PVDF filter to remove any cell debris.
3. Flash-freeze aliquots of lentivirus in cryotubes 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
LV Complex Formation Solution	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
NOTE: Add VirusGEN® LV Enhancer 5-24 hours post-transfection.			
VirusGEN® LV Enhancer	200 µl	1.0 ml	1.5 ml

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Product Guarantee	When properly stored and handled, *Trans*IT-VirusGEN® Transfection Reagent is guaranteed for 1 year from the date of purchase, and VirusGEN® LV Complex Formation Solution and VirusGEN® LV Enhancer are guaranteed for 6 months from the date of purchase.

PROTOCOL FOR LENTIVIRUS GENERATION IN SUSPENSION HEK 293 CELL CULTURES

Full protocol and additional documentation available at *mirusbio.com/virusgen*

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

A. Maintain cells

1. Passage suspension HEK 293 cells 18-24 hours prior to transfection to obtain a density of 4 - 6 × 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 (e.g. 37°C, 5-8% CO_2, shaking).

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

1. Immediately prior to transfection, seed cells at a density of 2 - 4 × 10^6 cells/ml.
2. Warm *Trans*IT-VirusGEN® Reagent to room temperature and vortex.
3. Place ___ml of VirusGEN® LV Complex Formation Solution 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. Vortex gently to mix.
6. Incubate for 15-30 minutes to allow transfection complexes to form. Do not vigorously agitate again once formed.

C. Distribute complexes to cells in complete growth medium

1. Add *Trans*IT-VirusGEN® Reagent:DNA complexes (from Step B) to cells.
2. Incubate transfected cultures in an orbital shaker until addition of the VirusGEN® LV Enhancer.

D. Add VirusGEN® LV Enhancer to transfected cells

1. Between 5-24 hours post-transfection, add ___mL of VirusGEN® LV Enhancer to cultures.
2. Incubate cultures for an additional 24-43 hours prior to virus harvest (i.e. harvest lentivirus a total of 48 hours post-transfection).

E. Harvest virus

1. Following the 48-hour incubation, centrifuge cells in a sterile tube at 300 × g for 5 minutes. Do NOT dispose of the supernatant.
2. Transfer the virus-containing supernatant into a new sterile tube.
3. Filter through a 0.45 µm PVDF filter to remove any cell debris.
4. Flash-freeze aliquots of lentivirus in cryotubes and store at -80°C.

Table 2. Volume scaling worksheet for VirusGEN® LV Transfection Kit

Starting conditions per milliliter of complete growth medium					
	Per 1 ml		Total culture volume		Reagent quantities
LV Complex Formation Solution	0.1 ml	×	_____ml	=	_____ml
Total Plasmid DNA (1 µg/µl)	1 µl	×	_____ml	=	_____µl
*Trans*IT-VirusGEN® Reagent	3 µl	×	_____ml	=	_____µl
NOTE: Add VirusGEN® LV Enhancer 18-24 hours post-transfection.					
VirusGEN® LV Enhancer	0.1 ml	×	_____ml	=	_____ml

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.

VirusGEN® LV Transfection Kit Workflow

A. Maintain Cells
Passage cells regularly; ensure >95% viability before transfection.

B. 0 hr
Dilute cells to **2 - 4 × 10^6** cells/ml.

Form complexes in VirusGEN® LV Complex Formation Solution in a volume that is 5-10% of cell culture volume.

Per ml of culture, add
1. **DNA:** 0.5 - 1.5 µg
2. ***Trans*IT-VirusGEN®:** 1.5 - 4.5 µl.

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

C. Add transfection complexes to cells.

D. 5 - 24 hr
Add **VirusGEN® LV Enhancer** in a volume that is 5-10% of cell culture volume.

E. 48 hr
Harvest lentivirus from supernatant.

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