VirusGEN® LV Transfection Kit

Quick Reference Protocol

Instructions for MIR 6760

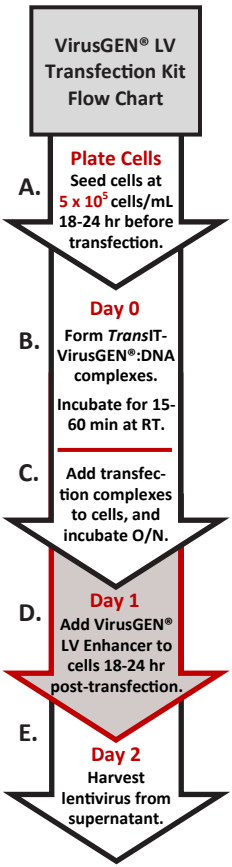
Full protocol, SDS and Certificate of Analysis available at mirusbio.com/virusgen

SPECIFICATIONS

Storage	Store *Trans*IT-VirusGEN® 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	6 months 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/virusgen*



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 packaging DNA premix and ___µl transfer plasmid DNA encoding the gene of interest. Mix gently by pipetting.
4. Add ___µl of *Trans*IT-VirusGEN® Reagent. Vortex gently to mix.
5. Incubate at room temperature for 15-60 minutes to allow transfection complexes to form.

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 overnight.

D. Add VirusGEN® LV Enhancer to transfected cells

1. Between 18-24 hours post-transfection, add ___mL of VirusGEN® LV Enhancer to cultures.
2. Incubate cultures for an additional 24-30 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.

If using individual packaging plasmids, we recommend a starting ratio of 4 µg *gag-pol* vector, 1 µg *rev* vector and 1 µg VSV-G vector. Premix the packaging plasmids with an equal amount of the transfer vector (e.g. 6 µg) to maintain a 1:1 (wt:wt) ratio of packaging to transfer plasmids.

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
Packaging DNA Premix (1 µg/µl)	1 µl	5 µl	7.5 µl
Transfer Plasmid DNA (1 µg/µl)	1 µl	5 µl	7.5 µl
*Trans*IT-VirusGEN® Reagent	6 µl	30 µl	45 µl
NOTE: Add VirusGEN® LV Enhancer 18-24 hours post-transfection.			
VirusGEN® LV Enhancer	200 µl	1.0 ml	1.5 ml

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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 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 packaging DNA premix and ___µl transfer plasmid DNA encoding the gene of interest. Mix completely.
5. Add ___µl of *Trans*IT-VirusGEN® Reagent. Vortex gently to mix.
6. Incubate for 15-60 minutes to allow transfection complexes to form.

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 overnight in an orbital shaker.

D. Add VirusGEN® LV Enhancer to transfected cells

1. Between 18-24 hours post-transfection, add ___mL of VirusGEN® LV Enhancer to cultures.
2. Incubate cultures for an additional 24-30 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.

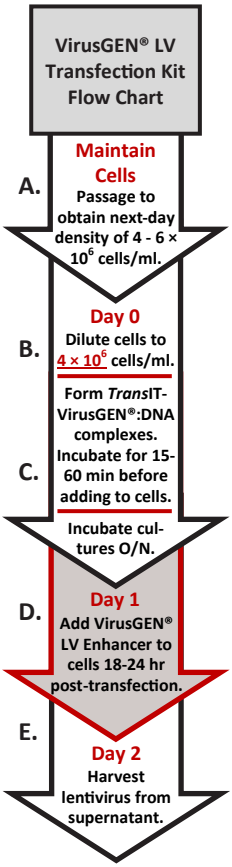

Table 2. Volume scaling worksheet for VirusGEN® LV Transfection Kit

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
Packaging DNA Premix (1 µg/µl)	0.5 µl	×	_____ml	=	_____µl
Transfer Plasmid DNA (1 µg/µl)	0.5 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

If using individual packaging plasmids, we recommend a starting ratio of 4 µg *gag-pol* : 1 µg *rev* : 1 µg VSV-G vectors. Premix the packaging plasmids with an equal amount of the transfer vector (e.g. 6 µg) to maintain a 1:1 (wt:wt) ratio of packaging to transfer plasmids.

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