*Trans*IT® Lentivirus System

Quick Reference Protocol

Instructions for MIR 6650 and 6655

Full protocol, SDS and Certificate of Analysis available at mirusbio.com/6650

SPECIFICATIONS

Storage	Store *Trans*IT®-Lenti Transfection Reagent, Lentivirus Packaging Mix Powered by MISSION® Genomics and *Transduce*IT™ Reagent tightly capped at -20°C. ***Before each use***, warm to room temperature and vortex gently.
Product Guarantee	*Trans*IT®-Lenti Reagent, Lentivirus Packaging Mix Powered by MISSION® Genomics and *Transduce*IT™ Reagent are guaranteed for 1 year from date of purchase, when properly stored and handled.

PROTOCOL FOR LENTIVIRUS GENERATION IN ADHERENT HEK 293T CELL CULTURES

Full protocol and additional documentation available at ***mirusbio.com/6650***

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).
   For HEK 293T/17 cultures: 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. DO NOT transfect cells at a lower confluency, as this may lead to high cellular toxicity and lower virus titers.

B. Prepare *Trans*IT®-Lenti:DNA complexes (immediately before transfection)

1. Warm *Trans*IT®-Lenti Reagent to room temperature and vortex gently.
2. Place ___µl of OptiMEM® I Reduced-Serum Medium in a sterile tube.
3. In a separate tube, combine ___µl Lentivirus Packaging Mix Powered by MISSION® Genomics and ___µl transfer plasmid DNA encoding the gene of interest (GOI). Mix gently by pipetting.
4. Transfer the total volume of Lentivirus Packaging Mix + transfer plasmid mixture to the tube containing OptiMEM® I Reduced-Serum Medium. Mix gently by pipetting.
5. Add ___µl *Trans*IT®-Lenti Reagent to the diluted DNA mixture. Mix gently by pipetting.
6. Incubate at room temperature for 10 minutes to allow transfection complexes to form.

C. Distribute complexes to cells

1. Add *Trans*IT®-Lenti Reagent: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. It is not necessary to replace complete growth medium with fresh medium post-transfection.

D. Harvest and storage of lentivirus

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
Serum-free medium	200 µl	1.0 ml	1.5 ml
Transfer DNA (1 µg/µl stock)	1.0 µl	5.0 µl	7.5 µl
Lentivirus Packaging Mix* (0.1 µg/µl stock)	10 µl	50 µl	75 µl
***Trans*IT®-Lenti Reagent**	6 µl	30 µl	45 µl

*Lentivirus Packaging Mix Powered by MISSION® Genomics.

Transfection Optimization

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

For additional transfection optimization tips, see TransIT® Lentivirus System full protocol.

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PROTOCOL FOR LENTIVIRUS GENERATION IN SUSPENSION HEK 293 CELL CULTURES

Full protocol and additional documentation available at ***mirusbio.com/6650***

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

A. Maintenance of cells

1. Passage suspension HEK 293 cells 18-24 hours before 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®-Lenti Reagent:DNA complexes

1. Seed cells at a density of 2 × 10^6 cells/ml immediately prior to transfection. DO NOT proceed with transfections if cells are not doubling normally and at high viability.
2. Warm *Trans*IT®-Lenti Transfection Reagent to room temperature and vortex gently.
3. Place ___ml of serum-free medium (e.g. Opti-MEM® I Reduced-Serum Medium) in a sterile tube.
4. In a separate tube, combine ___µl Lentivirus Packaging Mix Powered by MISSION® Genomics and ___µl transfer plasmid DNA encoding the gene of interest (GOI). Mix gently by pipetting.
5. Transfer the total volume of Lentivirus Packaging Mix + transfer plasmid mixture to the tube containing serum-free medium. Mix gently by pipetting.
6. Add ___µl of *Trans*IT®-Lenti Reagent. Mix gently by pipetting.
7. Incubate at room temperature for 10 minutes to allow transfection complexes to form.

C. Distribute complexes to cells in complete growth medium

1. Add *Trans*IT®-Lenti 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

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 um 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 in suspension HEK 293 cell cultures.

Starting conditions per milliliter of complete growth medium					
	Per 1 ml		Total culture volume		Reagent quantities
Serum-free Complex Medium	0.1 ml	×	_____ml	=	_____ml
Transfer Plasmid DNA (1 µg/µl stock)	0.5 µl	×	_____ml	=	_____µl
Lentivirus Packaging Mix Powered by MISSION® Genomics (0.1 µg/µl stock)	5 µl	×	_____ml	=	_____µl
*Trans*IT®-Lenti Transfection Reagent	3 µl	×	_____ml	=	_____µl

NOTE: The *Trans*IT® Lentivirus System was optimized in Freestyle™ 293-F cells. If using alternative suspension 293 cells, determine the best *Trans*IT®-Lenti Reagent:DNA ratio per cell type by varying the amount of *Trans*IT®-Lenti Reagent from 2-4 µl per 1 µg total DNA (transfer plasmid DNA + Lentivirus Packaging Mix).

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