*Trans*IT®-Lenti Transfection Reagent

Quick Reference Protocol

Instructions for MIR 6600, 6603, 6604, 6605, 6606, 6610

Full protocol, SDS and Certificate of Analysis available at mirusbio.com/6600

SPECIFICATIONS

Storage	Store *Trans*IT®-Lenti 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 293T CELL CULTURES

Full protocol and additional documentation available at ***mirusbio.com/6600***

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 x 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 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 packaging plasmid premix and ___µl transfer plasmid DNA encoding the gene of interest (GOI). Mix gently by pipetting.
4. Transfer the total volume of packaging premix + transfer plasmid mixture to the tube containing OptiMEM® I Reduced-Serum Medium. Mix gently by pipetting.
5. Add ___µl *Trans*IT®-Lenti to the diluted DNA mixture. Mix gently by pipetting.
6. Incubate at room temperature for 10 minutes.

C. Distribute complexes to cells

1. Add *Trans*IT®-Lenti: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
Packaging DNA Premix (1 µg/µl stock)	1.0 µl	5.0 µl	7.5 µl
***Trans*IT®-Lenti Reagent**	6 µl	30 µl	45 µl

Transfection Optimization

The amount of *Trans*IT®-Lenti required for transfection is dictated by the amount of DNA. Determine the best *Trans*IT®-Lenti:DNA ratio for each cell type. Start with 3 µl of *Trans*IT®-Lenti per 1 µg of 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 the TransIT®-Lenti Reagent full protocol.

Mirus Bio LLC

www.mirusbio.com | techsupport@mirusbio.com | U.S. Toll Free: 844.MIRUSBIO (844.647.8724) | Direct: +1.608.441.2852

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PROTOCOL FOR LENTIVIRUS GENERATION IN SUSPENSION HEK 293 CELL CULTURES

Full protocol and additional documentation available at ***mirusbio.com/6600***

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.
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 x 10^6 cells/ml immediately prior to transfection.
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 packaging plasmid premix and ___µl transfer plasmid DNA encoding the gene of interest (GOI). Mix gently by pipetting.
5. Transfer the total volume of packaging premix + 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 x 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 (Lentivirus Generation)					
	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
Packaging DNA Premix (1ug/ul stock)	0.5 µl	×	_____ml	=	_____µl
*Trans*IT®-Lenti Reagent	3 µl	×	_____ml	=	_____µl

NOTE: *Trans*IT®-Lenti Reagent was optimized using a pre-mix of lentivirus packaging vectors. 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

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