# *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*

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## SPECIFICATIONS

| | |
|---|---|
| **Storage** | Store *Trans*IT®-Lenti Transfection Reagent tightly capped at –20°C.<br>***Before each use***, warm to room temperature and vortex gently. |
| **Product Guarantee** | 6 months from the date of purchase, when properly stored and handled. |

## LENTIVIRUS TRANSFECTION PROTOCOL

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% 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 full protocol.*

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## ▸ LENTIVIRUS TRANSDUCTION AND TITERING PROTOCOL

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

The following procedure describes how to transduce HEK 293T/17 cells grown in a 24-well plate with a GFP reporter lentivirus to determine functional lentivirus titers. The number of wells needed will depend on the number of dilutions required for testing each lentivirus stock.

**A. Plate cells approximately 18-24 hours prior to transduction.**

1. Plate HEK 293T/17 cells at approximately 2.0 x $10^5$ cells/ml in 0.5 ml complete growth medium per well in a 24-well plate. NOTE: Plate at least 2 extra wells to trypsinize and count at the time of transduction. This is critical to determine an accurate functional titer post-transduction.
2. Culture cells overnight. Cells should be ≥40% confluent at the time of transduction.

**B. Transduce with GFP-encoding recombinant lentivirus**

1. Dilute *Transduce*IT™ or hexadimethrine bromide to a working concentration of 16 µg/ml in pre-warmed complete growth medium (e.g. add 6.25 µl of a 10 mg/ml solution into 10 ml growth medium).
2. Gently remove half of the medium from each test well (plated in section A) using a micropipettor.
3. Immediately add 250 µl *Transduce*IT™ or hexadimethrine bromide working solution to each well.
4. Determine the appropriate dilutions of lentivirus stock to test using the following guidelines, and immediately add dilutions to separate wells.
   NOTE: To obtain an accurate titer, it is desirable to have less than 20% GFP positive cells at 72 hours post-transduction. This minimizes the number of cells transduced by 2 different viruses. If higher transduction levels are desired, more virus may be required.

   ***For titers expected to be ≤ 5.0 x $10^7$ TU/ml:*** Do not dilute the lentivirus stock. Add 1 µl, 3 µl and 5 µl of undiluted lentiviral stock to separate wells.
   ***For titers expected to be ≥ 5.0 x $10^7$ TU/ml:*** Dilute the lentivirus stock 10-fold in complete growth media. Add 1 µl, 3 µl and 5 µl of the diluted lentivirus stock to separate wells.
5. Trypsinize and count the cells in 2 untransduced wells (see NOTE in A.1) to obtain an accurate cell concentration at the time of transduction.
6. Incubate the remaining test wells at 37°C in 5% $CO_2$ for 72 hours post-transduction.

**C. Cell Harvest and Analysis**

1. Gently wash cells with 1X PBS and immediately add 100 µl of trypsin to each well.
2. Incubate cells at 37°C and closely monitor cell rounding and detachment.
3. After cells have rounded, immediately add 400 µl of complete growth media to each well to neutralize the trypsin and resuspend the cells.
4. Transfer 100 µl of cell suspension from each well to separate wells in a non-treated 96-well plate (or similar vessel) that is compatible with your flow cytometer.
5. Add 150 µl of complete growth medium to each well to dilute the cells. This is required to obtain accurate flow cytometry results with some instruments.
6. Analyze for GFP expression by flow cytometry.
7. Calculate the functional titer of the lentivirus stock using the following equation:

$$\text{Titer (transducing units/ml)} = \frac{\text{Number of target cells (Count at day 2, transduction) x [\% of GFP positive cells/100]}}{\text{(Volume of Lentivirus Stock in ml)}}$$


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