LentiTran™ -
An Efficient Transfection Reagent for Lentivirus Packaging

Application Guide

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Package Content and Storage Conditions

SKU	Components	Storage Condition	Shipping Condition
TT400001	LentiTran, a transfection reagent specifically formulated for high titer lentivirus production, 0.5 mL	+4°C	Room temperature
TT400002	LentiTran, a transfection reagent specifically formulated for high titer lentivirus production, 1 mL	+4°C	Room temperature
TT400002P5	LentiTran, a transfection reagent specifically formulated for high titer lentivirus production, 5x1 mL	+4°C	Room temperature

NOTE: FOR RESEARCH PURPOSES ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USAGE.

Related Products

Lenti-ORF and Lenti shRNA
Lentivirus titration kit, p24 ELISA – Simplified workflow
Lenti packaging kit – Highly efficient, transfection reagent included
Lenti concentrator – Easily increase the viral titer by 100 folds
Lenti stabilizer – Preserve the viral infectivity during storage
Expression cDNA clones/vectors - tagged and untagged, ready for transfection
shRNA plasmids - Human, Mouse and Rat
CRISPR vectors, gene knockout kits

For technical assistance, contact OriGene at 1-888-267-4436 (301-340-3188 outside the US) or write to us at techsupport@origene.com

Introduction

LentiTran is a non-lipid polymer-based transfection reagent, that is specially formulated to generate lentivirus with extremely high titers from 293T cells. LentiTran effectively condenses DNA for highly efficient gene-delivery via endosomal uptake and protects the transfected DNA from lysosomal degradation.

The major advantages of LentiTran include:

- **High transfection efficiency in HEK293 cells.**
- **High titer lentivirus production**

- **Simple application**: Perform better with serum-containing ; no requirement for changes at time of transfection.

Experimental Procedures

Important Transfection Guidelines:

1. For high titer lentivirus production, 293T cell must be healthy. Please grow 293T cells per the supplier's instruction.
2. For high efficiency, transfect cells at a higher cell density at the time of transfection. ~90% confluency is highly recommended.
3. To lower cytotoxicity, transfect cells in the presence of serum (10%) and antibiotics.
4. Use serum-free DMEM to dilute LentiTran reagent and DNA. The diluent must be serum-free. Opti-MEM cannot be used.

Procedures for Transfecting 293T Cells:

The following protocol is for transfection in 10 cm dish. For other culture formats, scale up or down per culture dish's surface.

Step 1. Cell Seeding (see Table 1):

Cells should be plated 18 to 24 hrs prior to transfection so that the monolayer cell density reaches to the optimal ~90% confluency at the time of transfection. Complete culture media with serum and antibiotics are freshly added to each well 30~60 min before transfection.

Note: High serum levels (>5%) with antibiotics usually do not have inhibitory effect on transfection efficiency. For some specific 293 cells, maximal transfection efficiencies are observed in the presence of serum and antibiotics. We recommend using complete serum/antibiotics-containing media initially.

Table 1. A Guideline for Seeding 293T Cells Prior to Transfection in Different Culture Formats.

Culture Dishes	Surface Area (cm2)	Number of Cells to Seed
T75 Flask	75	$3.0 – 6.0 \times 10^6$
100 mm Dish	58	$2.2 – 4.4 \times 10^6$
60 mm Dish	21	$0.9 – 1.8 \times 10^6$
35 mm Dish	9.6	$3.5 – 7.0 \times 10^5$
6-well Plate	9.6	$4.0 – 8.0 \times 10^5$

12-well Plate	3.5	1.5 – 3.0 x 10^5
24-well Plate	1.9	0.8 – 1.6 x 10^5
48-well Plate	1.0	4.0 – 8.0 x 10^4

Step 2. Transfection

The following protocol is for transfection in 10 cm dish. For other culture formats, scale up or down per culture dish's surface.

1. Change fresh media: 30~60 minutes before transfection, replace with 8 mL of complete media containing serum and antibiotics.
2. For each dish, dilute a total 12 µg of DNA (6.0 µg lenti-vector plasmid plus 6.0 µg lentivirus packaging mix) into 1 mL of serum-free DMEM. Gently pipette up and down or vortex briefly to mix.
3. Add 40 µl of LentiTran reagent into the diluted DNA (not the reverse order). Gently pipette up and down or vortex briefly to mix. Incubate for 10 minutes at room temperature to allow transfection complexes to form.

   Note: Never use Opti-MEM to dilute LentiTran reagent and DNA, it contains serum and will disrupt transfection complex formation.

   Note: Never keep the LentiTran/DNA complexes longer than 20 minutes at room temperature.

4. Add the mixture prepared in step 3 drop-wise onto cells. Gently rock the plate back-and-forth and from side-to-side to distribute the complex evenly. Incubate cells at 37°C.
5. 12~18 hours post transfection, remove transfection complex-containing media and replace with fresh complete serum/antibiotics containing media.
6. Harvest the first batch of lentivirus supernatants 48 hrs post transfection (24 hrs after changing media at step 5) and store at 4°C. Add 8 mL of refresh complete culture media to cells and continue incubate cells at 37°C.
7. Harvest the second batch of lentivirus supernatants 72 hrs post transfection and combine with the first batch of virus.

Filter through a 0.45µm filter to remove cellular debris or Spin down at 2500 RPM for 10-15 mins to remove cellular debris.

Lentivirus Titration

The un-titered virus supernatants can be used to transduce your cells of interest. You may need to measure the viral titer before transduction. Virus titration will show how

successful your lentivirus production is and will help to trouble shoot if viral transduction fails. Therefore, virus titration is necessary. In addition, if your cells are hard to transduce and optimization is needed by using different MOI (Multiplicity Of Infection), the lentiviral titer needs to be measured. OriGene's simplified p24 ELISA kit (cat# TR30038) can be used to measure the viral titer.

The viral titer from the raw virus supernatants is usually 10^6 -10^7 TU/mL**. The viral supernatant is now ready for most transduction applications. If necessary, further concentration can be applied.
** If the fragment between LTRs in your lenti vector is larger, the viral titer may be lower.

If a higher titer is needed, the lentiviral particles can be quickly concentrated using OriGene's lenti concentrator (cat# TR30025), no ultracentrifuge needed. If you need ultra-high titer of lentivirus or for *in vivo* animal studies, OriGene provide custom lentivirus production service.

Lentiviral Particles Storage

Lentiviral particles are enveloped viruses and tend to lose infectivity during storage, even at -80°C. OriGene developed a lentiviral storage buffer, Lentivirus Stabilizer (cat# TR30039), which can preserve the infectivity for at least one year at - 80°C.

You can become a lentivirus production expert from OriGene's lentivirus production guide web page: https://www.origene.com/products/cdna-clones/lentiviral-particles/lentivirus-production