# I. Important

The solutions, reaction volumes and conditions described in this protocol have been highly optimized for the production of the highest titers of recombinant lentivirus. In order to obtain the best possible results, we recommend strict adherence to the culture sizes/volumes, amounts of DNA, and incubation times described below. The Lenti-X HTX System is optimized for use with Lenti-X Vectors, Lenti-X HTX Packaging Mixes, and the Lenti-X 293T Cell Line (Cat. No. 632180).

Transfections should be performed using **100-mm tissue culture plates**. Tet System Approved FBS (tetracycline-free) must be used with the Lenti-X HTX Packaging Mixes, in the transfection medium (Step 1) and in the medium used to collect the virus (Step 9).

*All of the following steps should be performed in a sterile tissue culture hood. Lentivirus requires the use of a Biosafety Level 2 facility. Pseudotyped lentiviruses packaged from HIV-1-based vectors are capable of infecting human cells. Know and use appropriate safety precautions.*

# II. Storage & Handling

- Thaw Xfect™ Polymer (100 µg/µl) at room temperature just prior to use. Once thawed, store Xfect Polymer at 4°C for up to twelve months.
- Thaw Xfect Reaction Buffer at room temperature just prior to use. Vortex after thawing. Once thawed, store Xfect Reaction Buffer at 4°C for up to twelve months.
- After each use make sure that the cap for the Xfect Polymer is closed tightly and return to the supplied foil pouch containing desiccant.

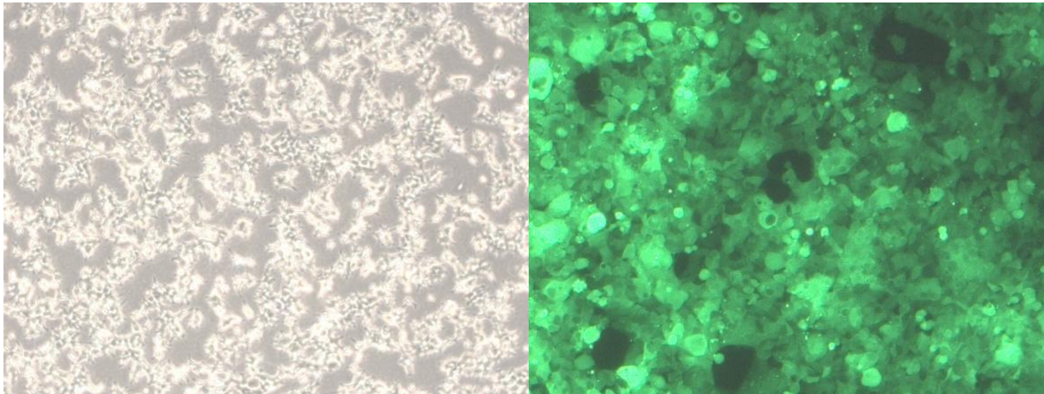

**Figure 1. Optimal density of Lenti-X 293T cells at point of transfection (left panel) and harvest time (right panel), shown here using a transfer vector containing ZsGreen1.**

## III. Transfection Protocol

**NOTE:** To achieve the highest titers it is critical to pay close attention to the transfection. You may want to perform a co-transfection with a lentiviral vector that contains a fluorescent protein. You should be able to achieve transfection efficiencies of greater than 90%.

1. Approximately 24 hr before transfection, seed 4–5 x $10^6$ Lenti-X 293T cells/100 mm plate, in 10 ml of growth medium. Make sure that the cells are plated evenly. Incubate at 37°C, 5% $CO_2$ overnight. Continue to incubate the cells until you are ready to add the transfection mixture in Step 7. The cells should be 80–90% confluent at the time of transfection.
2. Thoroughly vortex Xfect Polymer.
3. For each transfection sample, prepare two microcentrifuge tubes by adding reagents in the following order:

**Tube 1 (Plasmid DNA)**

| Volume | Component |
|---|---|
| 557 µl | Xfect Reaction Buffer |
| 36 µl | Lenti-X HTX Packaging Mix |
| 7 µl | Lenti-X Vector DNA (1 µg/µl) |
| 600 µl | Total volume |

**Tube 2 (Polymer)**

| Volume | Component |
|---|---|
| 592.5 µl | Xfect Reaction Buffer |
| 7.5 µl | Xfect Polymer |
| 600 µl | Total volume |

**NOTE:** It is **crucial** that the Xfect Polymer does not remain in aqueous solution for longer than 30 min at room temperature.

4. Vortex each tube well to mix.
5. Add the Polymer solution (Tube 2) to the Plasmid DNA solution (Tube 1) and vortex well at a medium speed for 10 sec.
6. Incubate each DNA-Xfect mixture for 10 min at room temperature to allow nanoparticle complexes to form.
7. Add the entire 1,200 µl of DNA-Xfect solution (5) dropwise to the cell culture medium from Step 1. Rock the plate gently back and forth to mix.

**NOTE:** It is **not** necessary to remove serum from your cell culture medium. It is normal for the medium to change color slightly upon addition of the DNA-Xfect solution.

8. Incubate the plate at 37°C.
9. After 4 hr to overnight, replace the transfection medium with 10 ml fresh complete growth medium (containing Tet System Approved FBS) and incubate at 37°C for an additional 24–48 hr. Virus titers will generally be highest 48 hr after the start of transfection. **Caution: discarded medium contains infectious lentivirus.**
10. Harvest the lentiviral supernatants and pool similar stocks, if desired. **Caution: supernatants contain infectious lentivirus.** Centrifuge briefly (500*g* for 10 min) or filter through a 0.45-µm filter to remove cellular debris.

**NOTE:** The filter used should be made of cellulose acetate, or polysulfonic (low protein binding), instead of nitrocellulose. Nitrocellulose binds proteins present in the membrane of lentivirus and destroys the virus.

11. Verify virus production using Lenti-X GoStix™ Plus and the smartphone app (see the Lenti-X GoStix Plus Protocol-at-a-Glance or takarabio.com/gostixhelp for details) or titrate the virus stock, then use the virus to transduce target cells, or store at –80°C.

**NOTE:** Titers can drop as much as 2- to 4-fold with each freeze-thaw cycle.

| Contact Us | |
|---|---|
| **Customer Service/Ordering** | **Technical Support** |
| tel: 800.662.2566 (toll-free) | tel: 800.662.2566 (toll-free) |
| fax: 800.424.1350 (toll-free) | fax: 800.424.1350 (toll-free) |
| web: takarabio.com | web: takarabio.com |
| e-mail: ordersUS@takarabio.com | e-mail: techUS@takarabio.com |


## Notice to Purchaser

Our products are to be used for research purposes only. They may not be used for any other purpose, including, but not limited to, use in drugs, *in vitro* diagnostic purposes, therapeutics, or in humans. Our products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without prior written approval of Takara Bio USA, Inc.

Your use of this product is also subject to compliance with any applicable licensing requirements described on the product's web page at takarabio.com. It is your responsibility to review, understand and adhere to any restrictions imposed by such statements.

**©2012 Takara Bio Inc. All Rights Reserved.**

All trademarks are the property of Takara Bio Inc. or its affiliate(s) in the U.S. and/or other countries or their respective owners. Certain trademarks may not be registered in all jurisdictions. Additional product, intellectual property, and restricted use information is available at takarabio.com.

This document has been reviewed and approved by the Quality Department.