Fourth Generation Lentiviral Packaging Systems

The Lenti-X fourth generation packaging systems are optimized for viral yield, ease-of-use, and safety. Use these systems to produce ultra-high titers of lentivirus. There are two pseudotype (envelope protein) options to choose from depending on your desired tropism:

- **VSV-G lentiviral packaging**—Produces VSV-G pseudotyped lentivirus, which readily infects virtually all types of cells. A non-integrating version is also available.
- **Ecotropic lentiviral packaging**—Produces lentivirus pseudotyped with the MLV ecotropic envelope glycoprotein, which limits transduction to mouse and rat cells.

### High Lentiviral Titer

Our **Lenti-X packaging systems** generate lentiviral titers that far exceed most other commercially available packaging systems—you can expect titers of $10^7$–$10^8$ infectious units (IFU) per ml, that’s 25 times what other popular systems generate.

**How is such high titer achieved?**

- **Optimized composition**—Lentiviral packaging and nonviral components provided as a proprietary suite of 5 vectors (Figure 1), premixed in the ideal ratio to maximize virus production.
- **Tetracycline transactivation**—High-level expression of packaging components using Tet-system promoters.
- **Optimized transfection**—Xfect Transfection Reagent consistently results in >95% transduction efficiency for HEK 293T cells, allowing high expression of packaging components.
- **Specialized Lenti-X 293T cell line**—Highly transfectable cells selected to provide titers as high as $10^8$ IFU/ml.

![Figure 1. Clontech’s Lenti-X HTX Packaging Systems consist of 5 separate vectors (A). High expression of essential viral components are driven by the Tet-Off and Tat transactivators. The pol gene is fused to vpr to ensure transport of the reverse transcriptase/integrase protein into the recombinant viral particle. Other 3rd generation systems (B) do not contain separate gag and pol sequences. Not all vector elements are shown.](image-url)
**How much packaged lentivirus can be obtained from a single Lenti-X HTX packaging reaction?**

A single Lenti-X HTX packaging reaction generates 10 ml of unconcentrated lentiviral supernatant at $10^7$–$10^8$ IFU/ml. Leading competitor systems produce far less (Figure 2).

![Figure 2](image.png)

**Figure 2.** Lenti-X (Panel A) and a competitor’s packaging system (Panel B) were each used with Lenti-X 293T cells to generate lentiviral vectors that express ZsGreen1. The supernatant was used for transduction of HeLa cells. 10 µl of the Lenti-X packaging reaction was able to transduce the majority of the cells, whereas 10 µl of the competitor’s supernatant transduced only a small percentage of cells.

The maximum titer obtained by our scientists is ~5 x $10^8$ IFU/ml (unconcentrated supernatant), as determined by analysis of ZsGreen1 expression from a pLVX backbone by flow cytometry. The cPPT/CTS, RRE, and WPRE sequences and the wild-type 5' LTRs on pLVX vectors contribute to higher titers. In 13 independent packaging reactions performed with 10 different fluorescent protein constructs, the average titer obtained for pLVX vectors packaged using the Lenti-X system was 1.85 x $10^8$ IFU/ml, as determined by flow cytometric analysis of transduced HeLa cells.

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**Easy-to-Use Systems for Lentiviral Packaging**

**Lenti-X Packaging Single Shots (VSV-G)** provide an extremely simple and consistent one-step method for producing high-titer VSV-G pseudotyped lentivirus. The single tube format consists of individual vials containing lyophilized Xfect Transfection Reagent premixed with an optimized formulation of Lenti-X lentiviral packaging plasmids. High-titer virus is produced by simply reconstituting this mixture with your lentiviral vector of choice in sterile water and adding it to 293T cells in a 10-cm dish.

In addition to Lenti-X Packaging Single Shots, we also offer packaging systems for generating VSV-G pseudotyped lentivirus and ecotropic pseudotyped lentivirus in a traditional format. These kits include packaging mix and Xfect Transfection Reagent as separate liquid components.

**Click here to view all available Lenti-X Packaging products >>**

**What type of cells should be used for packaging?**
The Lenti-X Packaging Systems are optimized for use with Lenti-X 293T Cells, a clone selected at Clontech for its ability to generate high-titer lentivirus HEK 293 and 293FT cells can be used but will produce lower titers of packaged lentiviral particles (Figure 2).

![Graph showing titer comparison between Lenti-X 293T, HEK 293T, and 293FT cells.]

Figure 3. Lenti-X 293T cells produce over 6 times more virus than 293FT cells, and up to 30 times more virus than the HEK 293 cells.

**What type of lentiviral vectors are compatible with this system?**

We have not tested all vectors, but in principle, any HIV-1-based vector should be efficiently packaged using this system, resulting in high titer lentivirus. For example, a ZsGreen1 cassette in the pLenti6/V5 vector (Invitrogen)—a vector that lacks the WPRE and cPPT/CTS sequence elements and utilizes self-inactivating (SIN) LTRs—titers of \( >3 \times 10^7 \) IFU/ml were obtained as determined by flow cytometry. Since the Lenti-X system utilizes Tat transactivation, lentiviral vectors that utilize HIV-1 5' LTRs (such as pLVX vectors) will generate higher titers than those containing other LTRs. Vectors lacking WPRE and/or cPPT/CTS sequences yield consistently lower titers than Clontech pLVX vectors.

**Note:** Use of vector systems other than Clontech’s may require additional rights from third parties. You should evaluate whether permission from any third party is required for your intended use.

**What is the cloning/packaging capacity of an HIV-1 based lentiviral vector?**

Wild type lentiviruses contain \(~9.7\) kb of genome including both LTRs. Artificially creating a genome larger than this will result in unstable viral particles and a dramatic drop in viral titer. For recombinant lentiviruses such as those generated using Lenti-X systems, much of the viral genome has been replaced with other useful sequences such as selection markers or fluorescent proteins but enough space remains for cloning transgenes.

- The 3' LTR of pLVX-Puro ends at 5.4 kb, so \(~4.3\) kb of space remains for you to clone in your gene.
- The 3' LTR of pLVX-IRES-tdTomato ends at 6.3 kb, so \(~3.4\) kb of space remains for you to clone in your gene.
Improved Safety Profile

Although all lentiviral vectors should be handled in at least a BSL-2 laboratory, biosafety issues can be greatly mitigated by carefully considering the nature of the transgene insert and by ensuring that viral replication is restricted to specific packaging cells that provide these essential functions, in trans. For lentiviral transfer vectors to generate DNA-mobilizing, replication-competent lentivirus (RCL), several additional viral sequences must be acquired from the packaging plasmids via recombination (i.e., the structural genes \textit{gag-pro} and \textit{env}; and the reverse transcriptase/integrase gene, \textit{pol}). In 3rd-generation systems, \textit{gag-pro} and \textit{pol} sequences are in the same transcriptional unit, while \textit{env} (VSV-G) is on a separate plasmid. Therefore, in these systems, generation of RCL requires two recombination events. With Lenti-X Packaging Systems, \textit{gag-pro} and \textit{pol} are further separated onto two plasmids (Figure 1), such that three low-frequency recombination events would be needed to generate RCL. Click here for more safety information.

Available Products for Lentiviral Packaging

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