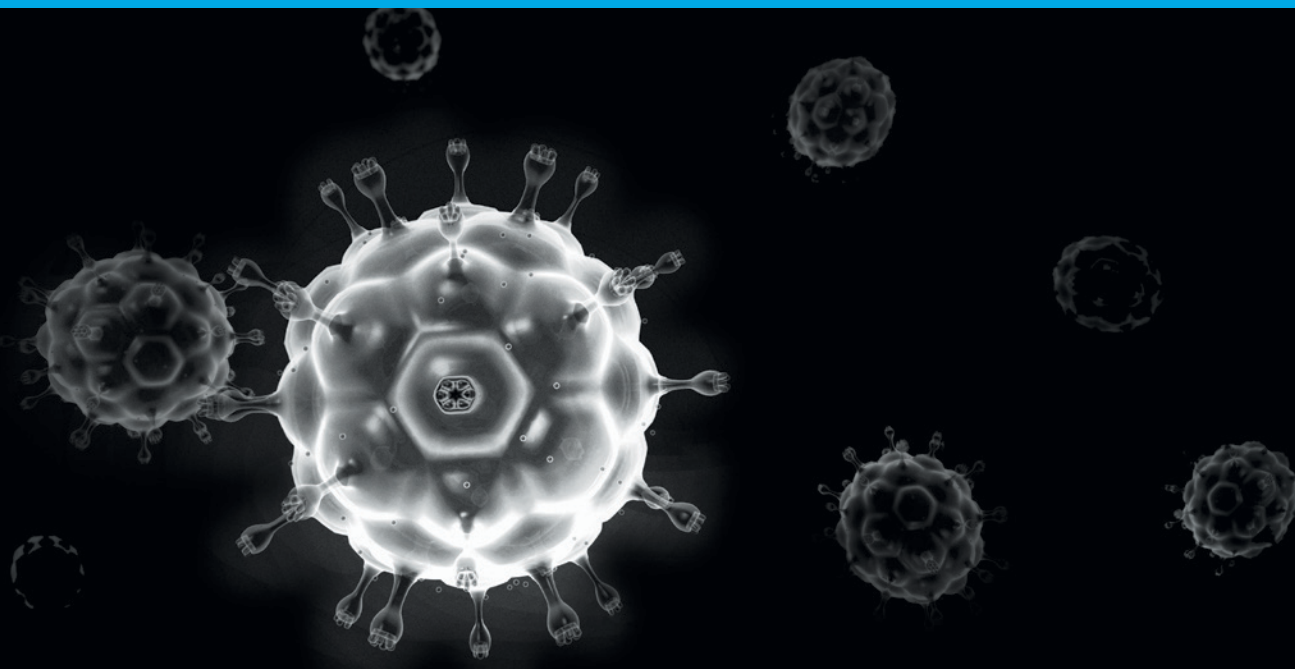


# Lentiviral Packaging & Expression Systems

Mammalian Expression Systems



## Lenti-X™ — What is Your X-Factor?

eXtra high titers

eXtra control

eXtra safety



Clontech

## Lenti-X™ HT—No Packaging System Generates Higher Titers (pages 3–4)

Our new packaging system generates the highest titers of any commercially available packaging mix.

### Here's why:

- **Optimized Composition** – Extra packaging components are included in the mix to yield increased viral titers.
- **Optimized Ratios** – All ratios of viral components were systematically tested to identify the conditions that generate a superior system.
- **Tetracycline Transactivation**—High-level expression of several key packaging components is produced by Tet-Off® transactivation of tetracycline-responsive promoter elements (TREs). In many cell lines, including HEK 293 cells, the tetracycline transactivator generates absolute expression levels much higher than those produced by the CMV promoter (Yin *et al.* 1996).
- **Optimized Transfections**—Calcium phosphate-based transfection methods yield high transfection efficiencies in 293T cells, resulting in expression levels that are higher than those produced by lipid-based transfection. Clontech has taken this one step further with **Lentiphos™ HT**—a transfection system perfected for 293T packaging cells (see page 10). After testing a broad range of conditions, we devised a system with highly optimized pH, buffer compositions, salt concentrations, and volumes that allow you to transfect >99% of your 293T cells with ease. Follow the simple protocol and achieve unprecedented transfection success.

Why Not  
Get a LOT  
eXtra?



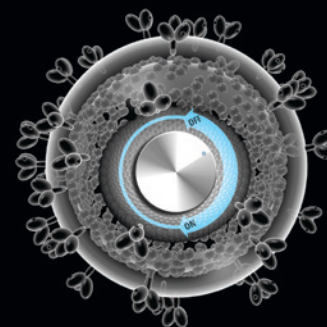
Lenti-X™ HT  
Packaging System

## Lenti-X Vector Systems—All the Necessary Features (pages 5–9)

Our popular **Tet-On®** and **Tet-Off Advanced Inducible Expression Systems** (see pages 5–7) and **Living Colors® Green and Red Fluorescent Fusion Proteins** (see page 9) are now available for delivery in Lenti-X format. Our new lentiviral expression systems utilize Lenti-X Vectors that contain the following elements for improved expression and vector function:

- **WPRES**—All Lenti-X vectors contain the woodchuck hepatitis virus post-transcriptional regulatory element (WPRES) that is believed to promote RNA processing events and enhance nuclear export. The WPRES element both enhances packaging of viral genomic transcripts to increase titers and bolsters expression of your included transgene (Zufferey *et al.* 1999).
- **cPPT**—This central polypurine tract (cPPT) increases nuclear importation of the viral genome (Zennou *et al.* 2000) during target cell infection, resulting in improved vector integration and more efficient transduction.
- **RRE**—The Rev-responsive element (RRE) enhances gene expression and viral titers by allowing the transport of unspliced RNA out of the nucleus (Cochrane *et al.* 1990).
- **IRES**—Many of our vectors also contain an internal ribosome entry site (IRES) from EMCV that permits expression of a drug resistance marker and another gene from a single transcript. You can be confident that any drug resistant cell also expresses your fusion protein (**Lenti-X Living Colors Vectors**) or the Tet-Advanced transactivators (**Lenti-X Tet-Advanced System Vectors**).

Why Not  
Get More  
Control?



Lenti-X™ HT  
Tetracycline Inducible  
Expression Systems

### References

1. Cochrane, A.W. *et al.* (1990) *Proc. Natl. Acad. Sci. USA* **87**(3):1198–1202.
2. Yin, D. X. *et al.* (1996) *Anal. Biochem.* **235**:195–201.
3. Zennou, V. *et al.* (2000) *Cell* **101**(2):173–185.
4. Zufferey, R. *et al.* (1999) *J. Virol.* **73**(4):2886–2892.

# High-Efficiency Lentiviral Packaging

The Lenti-X™ HT Packaging System produces the highest titers available for any lentiviral vector

- Generates titers that are 25–50-fold higher than other packaging systems
- Obtain VSV-G pseudotyped lentivirus in 48 hours
- Virus is safe and replication-incompetent, due to the unique design of the packaging constructs
- Infect entire plate of target cells with as little as 10 µl of viral supernatant

Recombinant lentiviruses represent the latest generation of powerful, multipurpose vectors for delivering genes into almost any mammalian cell type, including primary cultures, nondividing cells, and stem cells. Clontech's **Lenti-X HT Packaging System**, in combination with our Lenti-X System vectors, produces the highest titers of any lentiviral system: up to  $5 \times 10^8$  infectious units (IFU) per ml of unconcentrated supernatant. Our packaging system outproduces titers advertised for other lentiviral systems by up to 50-fold.

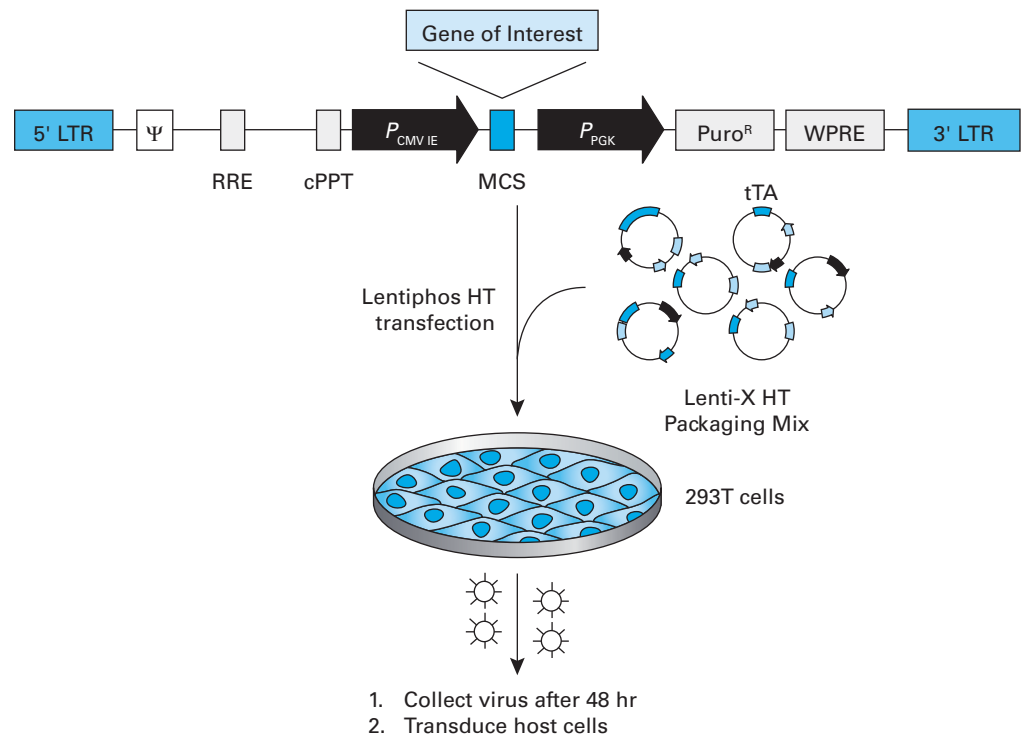
## Synergistic Performance

The keys to these outstanding levels of virus production lie in a tripartite synergism of highly optimized system components (Figure 1).

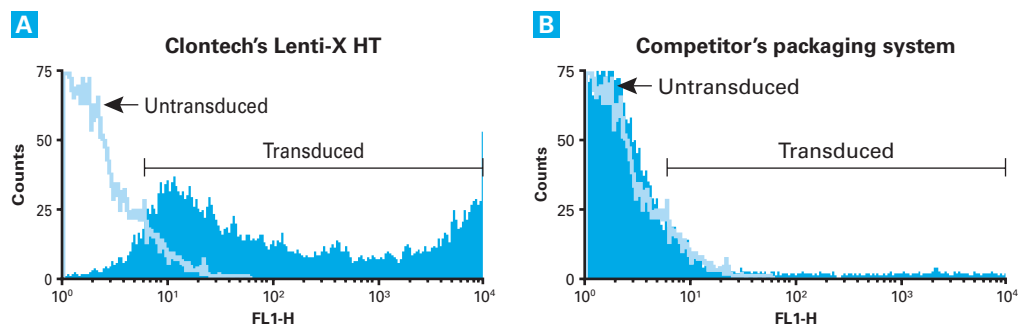
First, our novel Lenti-X HT Packaging Mix provides the entire complement of essential lentiviral packaging components on a proprietary suite of vectors that collectively expresses high levels of Gag-Pro, Tat, Rev, RT, IN, and VSV-G proteins (1). Several of these proteins are expressed from split genes for added safety, and the plasmids are combined in ideal ratios to maximize virus production.

Second, to raise titers even higher, we included a vector to express our Tet-Off transactivator (tTA), which drives high-level expression of critical viral proteins from tetracycline-responsive promoters.

Third, our optimized **Lentiphos™ HT** transfection system transfers the Lenti-X HT Packaging Mix of plasmids, combined with your lentiviral vector, into 293T cells with unprecedented efficiency. The concerted effects of these advanced components allow 293T cells to produce the highest amounts of safe, replication-incompetent lentivirus from any of our Lenti-X System vectors, or from any other lentiviral vector.



**Figure 1. The Lenti-X HT Packaging System.** A lentiviral vector (e.g. pLVX-Puro) and the Lenti-X HT Packaging Mix are cotransfected into 293T cells using the highly efficient Lentiphos HT transfection system. High titer supernatants are ready for use 48 hr after transfection.



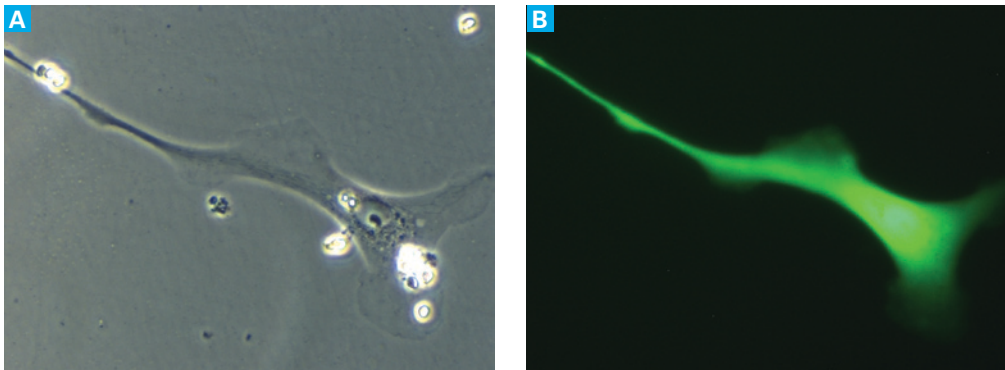
**Figure 2. High infectivity of supernatants produced by the Lenti-X HT Packaging System.** The Lenti-X HT Packaging System (Panel A) and a packaging system from a competitor (Panel B) were each used to generate viral supernatants from their respective lentiviral system vector that was engineered to express the ZsGreen1 fluorescent protein. As little as 10 µl of supernatant from the Lenti-X HT Packaging System transduced the majority of these HeLa cells, whereas 10 µl of supernatant from the other system transduced only a small percentage of the cells. Transduced cells were quantified by flow cytometry.

## Superior Lenti-X Vectors

To produce the highest titers of virus having superior expression and transduction capabilities, use any of our Lenti-X System vectors (e.g., **pLVX-Puro**; Figure 1). These vectors not only include all the required viral genome processing sequences, but also contain elements to improve expression and overall vector function. A WPRE element, which promotes RNA processing and

nuclear export, both enhances packaging of viral genomic transcripts to increase titers and bolsters expression of your inserted transgene (2). The cPPT element in Lenti-X vectors increases nuclear import of the viral genome following infection, resulting in improved vector integration and more efficient transduction (3).

# High-Efficiency Lentiviral Packaging...continued



**Figure 3. Transduction of neural progenitor cells by Lenti-X lentivirus.** Recombinant lentivirus for expressing ZsGreen1 was produced from the Lenti-X HT Packaging System and used to transduce normal human neural progenitor cells. A single transduced cell is shown under phase contrast microscopy (**Panel A**) and fluorescence microscopy (**Panel B**).

## Safe & Abundant Lentivirus Production

While lentiviral expression systems hold great promise for transducing genes into previously inaccessible cells and tissues, their safe use relies on a split-gene packaging strategy to prevent the development of self-replicating viral genomes through recombination. The Lenti-X HT Packaging Mix is a proprietary combination of expression elements that together produce all the necessary virus components to efficiently package any recombinant lentiviral expression vector transcript into infectious virus. Since the genes for these proteins reside on separate plasmids and lack homology with the lentiviral vector, their combined transfer or recombination into the packaged vector sequence is extremely unlikely. For added safety and unlike most other commercially available systems, Gag-Pol activity is provided via a split-gene strategy that thoroughly prevents viral replicative functions from being transferred to target cells (1). Thus, target cells infected with your recombinant virus will always lack the necessary replication machinery to subsequently produce new virus.

## Highest Titers & Neural Transduction

Transient high-level production of these viral components in 293T cells enables high titers of VSV-G pseudotyped lentivirus to be produced 48–72 hours after transfection.

Supernatant titers are sufficiently high to be used as is, without a need to concentrate them prior to target cell infection. For example, as little as 10  $\mu$ l of viral supernatant is able to transduce the vast majority of cells in a HeLa cell culture (Figure 2). Lentiviruses produced from the Lenti-X HT System also retain their inherently broad tropism and are fully capable of transducing cells derived from a neural lineage (Figure 3).

## Super-Efficient & Viable Packaging Cell Transfections

An essential feature of our high-titer Lenti-X HT Packaging System is its highly optimized transfection protocol that delivers a complete set of packaging vectors, along with the desired Lenti-X expression vector, into virtually every cell in a packaging culture (see page 10). As a result, each cell becomes a virus-producing factory. The extremely efficient Lentiphos HT transfection system reproducibly transfects 293T cells at efficiencies approaching 99%, so that up to  $5 \times 10^8$  IFU/ml can be produced using a single 10 cm plate of 293T cells. In addition, our novel transfection technique, which is a modified version of calcium-phosphate and is optimized for 293T cells, is less toxic than lipid-based methods, so greater numbers of viable 293T cells remain to produce more virus during the packaging process.

The Lenti-X HT Packaging System is an essential component of any lentiviral expression system and can be used with most lentiviral vectors. It easily produces high-titer lentiviral supernatants suitable for safe use with virtually any downstream application.

## References

1. Wu, X. *et al.* (2000) *Mol. Ther.* **2**(1):47–55.
2. Zufferey, R. *et al.* (1999) *J. Virol.* **73**(4): 2886–2892.
3. Zennou, V. *et al.* (2000) *Cell* **101**(2):173–185.

Product	Size	Cat. No.	Price
Lenti-X HT Packaging System	20 rxns	632160	\$876.00
	40 rxns	632161	\$1,226.00
Lentiphos HT	20 rxns	632151	\$264.00

Prices are subject to change without notice.

## Components

- Lenti-X™ HT Packaging Mix
- Lentiphos™ HT

## Related Products

- Lenti-X™ DsRed-Monomer Fluorescent Vectors (Cat. Nos. 632152 & 632153)
- Lenti-X™ AcGFP1 Fluorescent Vectors (Cat. Nos. 632154 & 632155)
- Lenti-X™ Expression System (Cat. No. 632164)
- Lenti-X™ Tet-On® Advanced Inducible Expression System (Cat. No. 632162)
- Lenti-X™ Tet-Off® Advanced Inducible Expression System (Cat. No. 632163)

# Inducible Lentiviral Gene Expression Systems

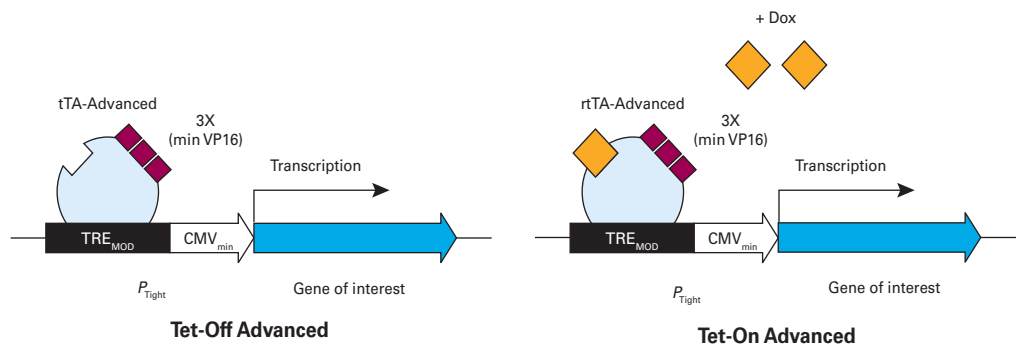
## New Lenti-X™ Tet-On® Advanced and Tet-Off® Advanced Inducible Expression Systems

- Highly efficient lentiviral delivery for establishing our Tet-Advanced inducible systems in any cell type
- Includes the Lenti-X HT Packaging System for high viral titers
- Outstanding induction control and extremely low basal expression

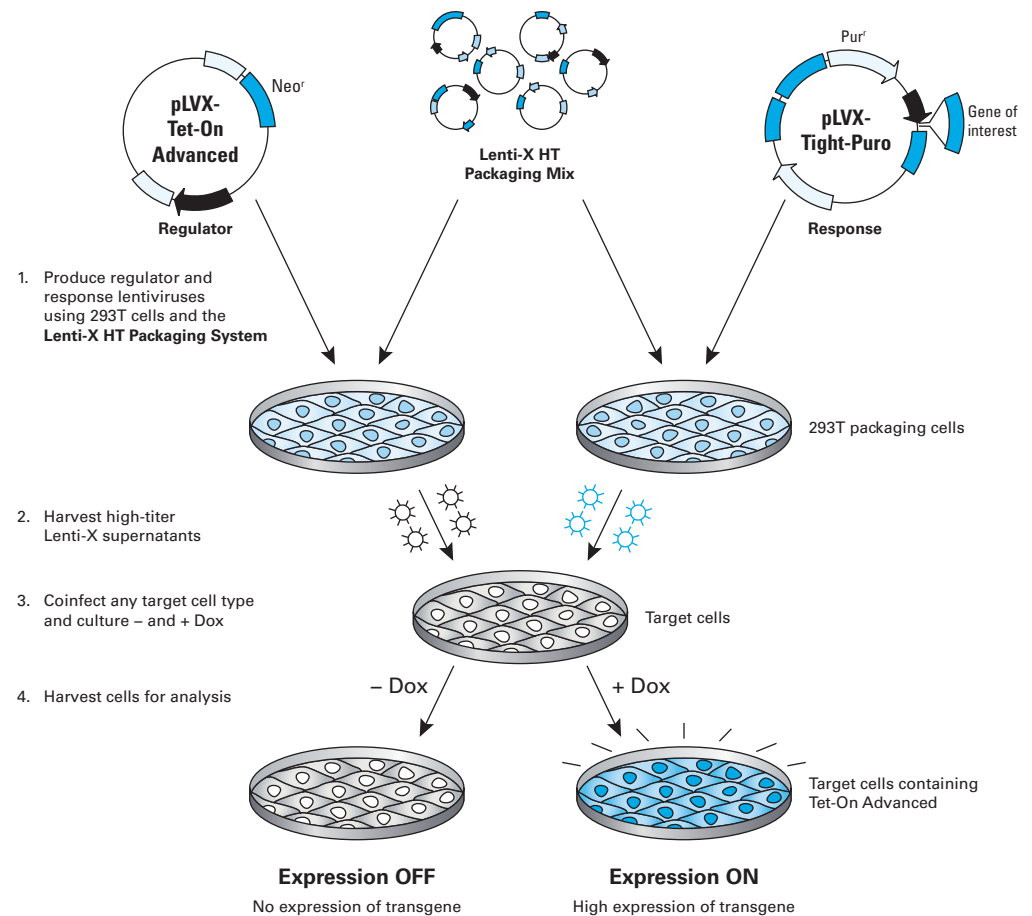
The Lenti-X Tet-On & Tet-Off Advanced Inducible Expression Systems combine the broad cellular tropism of lentiviral gene transfer technology with the power and versatility of our state-of-the-art tetracycline-regulated gene expression systems (Figure 1). These lentiviral vectors were developed in order to enable our popular inducible gene expression systems to be established in a wide variety of dividing and nondividing cell types from tissues such as liver, brain, muscle, and stem cells. Each system consists of two lentiviral vectors: a regulator vector that stably expresses either the Tet-On Advanced (rtTA-Advanced) or Tet-Off Advanced (tTA-Advanced) transcriptional activator, and a response vector (pLVX-Tight-Puro) that controls the expression of your gene of interest. Also included are a control response vector for luciferase expression, and our **Lenti-X HT Packaging System** (see pages 3–4).

### Produce High Titers of Lenti-X Tet-Advanced Lentivirus

Our Lenti-X HT Packaging System produces exceptionally high titers of safe, nonreplicating lentivirus from each of the Lenti-X Tet-Advanced System Vectors. The keys to this incredible level of virus production lie in our highly optimized **Lentiphos™ HT** transfection system (see page 10), combined with our Lenti-X HT Packaging Mix. The latter is a proprietary mixture of viral and nonviral expression constructs optimized for high-level expression of critical lentiviral packaging and replication proteins. Each Lenti-X Tet-Advanced System Vector, along with the Lenti-X Packaging Mix, is cotransfected into 293T packaging cells with almost 100% efficiency. This results in the production of supernatants with viral titers so high, they can be used directly without prior concentration. This not only assures high-efficiency transduction, but saves you much time and effort in setting up your inducible system. In our hands, as little as 10 µl of an unconcentrated Lenti-X HT supernatant was able to transduce the vast majority of HeLa cells in a 6-well plate culture (see page 3, Figure 2).

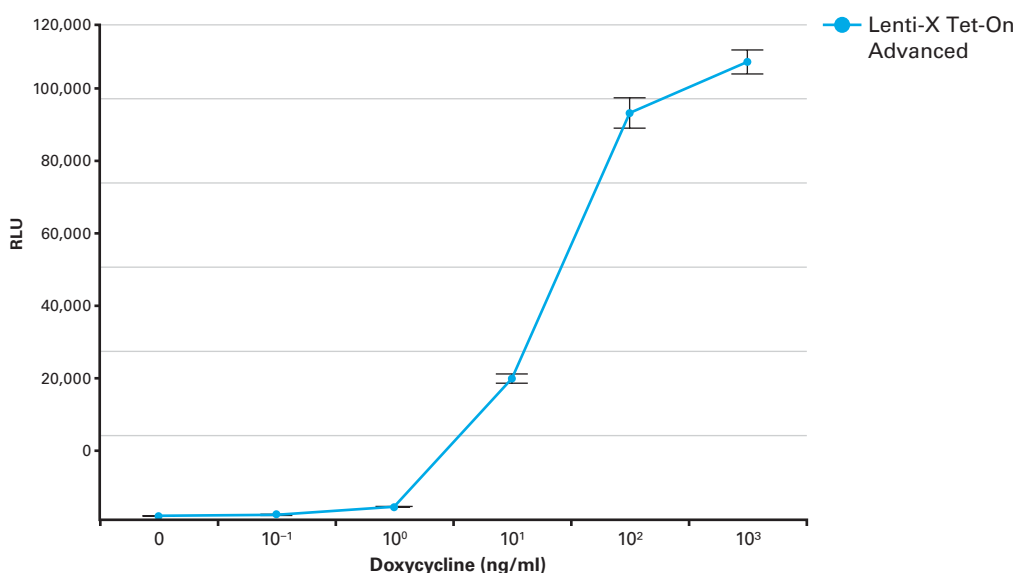


**Figure 1. Induced expression in the Tet-Off Advanced and Tet-On Advanced Systems.** The Tet-controlled transactivators are fusion proteins that contain a DNA-binding TetR domain joined to three minimal transcription activation domains from HSV VP16. Each transactivator has been optimized for expression in mammalian cells. In Tet-Off Advanced Systems, the basal state is maintained in the presence of doxycycline (Dox), and induced by its withdrawal. Tet-On Advanced Systems are activated in the presence of Dox. System induction produces high-level transcription of your gene from  $P_{Tight}$ .



**Figure 2. Establishing an inducible gene expression system with Lenti-X Tet-On Advanced.** Use the Lenti-X HT Packaging System and 293T cells to generate high-titer lentiviral supernatants from the pLVX-Tet-On Advanced Vector, and from the pLVX-Tight-Puro Vector containing your gene of interest. Simultaneously coinfect cultures of your target cells with the two lentiviruses (~8 hr). Then, after culturing for an additional 48–72 hr (+ and – Dox), harvest the cells for analysis. These same procedures also apply to the Lenti-X Tet-Off Advanced System, but the effects of Dox are reversed.

# Inducible Lentiviral Gene Expression Systems...continued



**Figure 3. The Lenti-X Tet-Advanced Systems are highly inducible.** Using equal amounts of high-titer supernatants, HeLa cells cultured at the indicated concentrations of Dox were cotransduced for 8 hr with a LVX-Tight-Puro-Luc lentivirus and a LVX-Tet-On Advanced lentivirus. Cultures were harvested after 48 hr and assayed for luciferase activity. Luciferase was expressed at very high levels, while the basal/uninduced expression level was very low.

## Tet-Systems Overview

Clontech's Tet-Advanced Systems implement superior versions of the inducible gene expression strategies of Gossen & Bujard (1), and include several major improvements described by Urlinger, *et al.* (2–4; Figure 1). To establish an inducible system, high-titer lentiviral supernatants produced from a pLVX-Tet Advanced Vector (Tet-On or Tet-Off) and from a pLVX-Tight-Puro Vector containing your gene of interest (controlled by the  $P_{\text{Tight}}$  inducible promoter), are used to cotransduce your target cells (Figure 2). Having high titers of virus ensures that the MOIs used during coinfection will be sufficiently high to transduce the majority of your cells with both viruses. Such efficiency allows you to proceed directly with gene induction experiments without selecting for stable transductants. Alternatively, you may choose to select doubly-transduced cells with G418 and puromycin. Cells transduced with a Tet-On Advanced System will initiate rapid and robust transcription of your gene in response to doxycycline (Dox) treatment, while cells containing a Tet-Off Advanced System will produce high levels of gene expression in the absence of Dox.

## Outstanding System Performance

We used the Lenti-X Tet-On Advanced System to establish inducible luciferase expression in HeLa cells in order to demonstrate its powerful induction properties. High-titer lentiviral supernatants were generated for the regulator vector and for a pLVX-Tight-Puro response vector harboring a luciferase cDNA (LVX-Tight-Puro-Luc). The LVX-Tight-Puro-Luc supernatant was mixed with an equal volume of the LVX-Tet-On Advanced supernatant, and then used to infect HeLa cells cultured in the presence or absence of Dox. The low basal activity and outstanding induction properties of the highly inducible luciferase expression system are shown in Figure 3.

## Coinfection Strategies

Based on our experience with the **Retro-X™ Tet-Advanced Inducible Expression Systems**, it is also possible to optimize the induction characteristics of cells transduced with these inducible Lenti-X Systems to meet specific expression needs. Infecting target cells with viral supernatant at ratios that favor the response vector (LVX-Tight-Puro) over the corresponding regulator vector (LVX-Tet-On or Tet-Off Advanced) offer maximum overall expression of your gene of interest. In contrast, supernatant ratios that favor the regulator vector ensure the absolute lowest basal expression levels and will achieve maximum fold-induction (5).

## Single- & Double-Stable Cell Lines

When using immortalized cell lines, the regulator and response lentiviruses in these Lenti-X Tet-Advanced Systems can be used independently and sequentially to produce extremely useful stable cell lines. Cells transduced with a Lenti-X Tet-On or Tet-Off Advanced virus can be selected with G418 to generate a multipurpose, single-stable Tet-Advanced host cell line that serves as a recipient for different  $P_{\text{Tight}}$ -controlled gene constructs. Transduction of a single-stable Tet-responsive host cell line with a LVX-Tight-Puro lentivirus, and subsequent selection with puromycin, produces a double-stable inducible cell line containing both elements of the respective system.

# Inducible Lentiviral Gene Expression Systems...continued

## The Lentiviral Advantage

The versatile transduction features of lentivirus are now coupled to both of our powerful Tet-Advanced Inducible Expression Systems. Thus, researchers can readily establish inducible expression systems in cells and tissues that have been historically resistant to standard gene delivery techniques of transfection and retroviral infection, such as differentiated primary cells and stem cells.

Lentiviral delivery also ensures that intact, single vector copies integrate efficiently and independently into the host cell's genome, resulting in a high frequency of functional transductants. Using high titers of infectious virus further assures that a maximum number of cells will be transduced, which often eliminates the need to perform antibiotic selection to enrich for stable transformants.

Whether you wish to install these highly responsive inducible systems in primary cell cultures or in cells that transfect poorly, or elect to take advantage of the efficiency of lentiviral gene delivery, Clontech's Lenti-X Tet-Advanced Gene Expression Systems, each including the Lenti-X HT Packaging System, are designed to meet your needs.

## References

1. Gossen, M. & Bujard, H. (1992) *Proc. Natl. Acad. Sci. USA* **89**(12):5547–5551.
2. Urlinger, S., et al. (2000) *Proc. Natl. Acad. Sci. USA* **97**(14):7963–7968.
3. Inducible Gene Expression Systems (January 2007) *Clontechniques* **XXII**(1):1–2.
4. Tet-On® Advanced Inducible Gene Expression System (July 2006) *Clontechniques* **XXI**(2):1–3.
5. Inducible Retroviral Gene Expression Systems (July 2007) *Clontechniques* **XXII**(3):2–3.

Product	Size	Cat. No.	Price
Lenti-X Tet-On Advanced Inducible Expression System	each	632162	\$1,634.00
Lenti-X Tet-Off Advanced Inducible Expression System	each	632163	\$1,634.00
Tet System Approved FBS	500 ml	631106	\$265.00
Tet System Approved FBS, US-Sourced	500 ml	631101	\$390.00
Puromycin	25 mg	631305	\$71.00
	100 mg	631306	\$176.00
G418	1 g	631307	\$100.00
	5 g	631308	\$375.00

Prices are subject to change without notice.

## Components

- pLVX-Tet-On Advanced Vector or pLVX-Tet-Off Advanced Vector
- pLVX-Tight-Puro Vector
- pLVX-Tight-Puro-Luc Control Vector
- Lenti-X™ HT Packaging Mix
- Lentiphos™ HT
- Tet System Approved FBS
- User Manual (PT3985-1 or PT3986-1)
- Protocol-at-a-Glance (PT3984-2)

## Related Products

- Lenti-X™ Expression System (Cat. No. 632164)
- Lenti-X™ HT Packaging System (Cat. Nos. 632160 & 632161)
- Retro-X™ Tet®-On Advanced and Tet®-Off Advanced Inducible Expression Systems (Cat. Nos. 632104 & 632105)

# Lentiviral Expression System

Obtain high-level expression in virtually any cell type with our complete Lenti-X™ Expression System

- Optimized lentiviral vector and packaging system for high titers and high expression
- Transfer genes into dividing and nondividing cells and stem cells
- Puromycin resistance allows rapid selection of transduced cells
- Safe, replication-incompetent virus

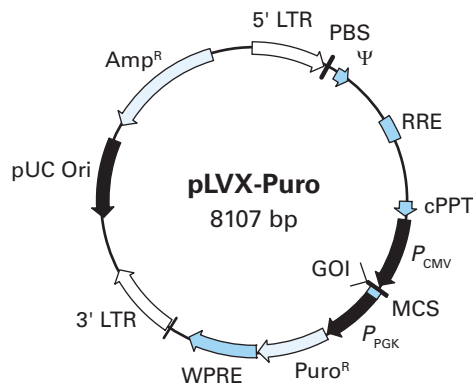
Recombinant lentiviruses derived from HIV-1 are able to deliver genes into almost any mammalian cell type, including primary cultures, dividing or nondividing cells, and stem cells. Clontech has developed a highly advanced lentiviral expression system that provides the broad cellular tropism of VSV-G pseudotyped lentivirus, high viral titers, and excellent transgene expression. The **Lenti-X Expression System**, which includes the **pLVX-Puro** expression vector and our **Lenti-X HT Packaging System**, enables you to produce exceptionally high titers of safe, replication-incompetent lentivirus from your customized pLVX-Puro vector (Figure 1).

## Superior Lenti-X Vectors

Like all our Lenti-X vectors, pLVX-Puro not only carries the LTRs and packaging sequence required for lentivirus production and replication, but it also contains elements that improve transgene expression, titer, and overall vector function. Its WPRE element, believed to promote RNA processing events and nuclear export, imparts a dual benefit (1). First, it acts within the context of viral genomic transcripts to enhance vector packaging and increase the titers of viral supernatants produced from 293T packaging cells. Second, it boosts expression of your gene of interest in target cells by facilitating the production of mature mRNA from transcripts initiated by the vector's internal CMV promoter. Lenti-X vectors also contain a cPPT element that increases nuclear importation of the viral genome during target cell infection, resulting in improved vector integration and more efficient transduction (2).

## High-Efficiency Packaging

Our Lenti-X HT Packaging System produces outstanding viral titers due to a synergism of highly optimized components (3; pages 3–4). The Lenti-X HT Packaging Mix safely provides all the essential lentiviral packaging and replication gene products *in trans* on a proprietary



**Figure 1. Map of pLVX-Puro.** The vector contains the lentiviral-specific LTRs and packaging sequence ( $\Psi$ ); a multiple cloning site (MCS) to insert your gene of interest (GOI); puromycin resistance; and WPRE and cPPT elements to boost packaging, viral titers, and transgene expression.

suite of separate vectors. Selected plasmids in the mixture generate high expression levels for critical viral proteins as a result of Tet-Off<sup>®</sup> transactivation. For added safety, a split gag-pol gene delivery strategy thoroughly prevents viral replicative functions from being transferred to target cells (3). Finally, the included **Lentiphos™ HT** transfection reagents transfer the Lenti-X HT Packaging Mix, along with your pLVX-Puro vector, into 293T cells with unprecedented efficiency (see page 10). The resulting high-titer viral supernatants can be used directly, without concentration.

## High Titers & Rapid Selection

We used the Lenti-X Expression System to generate a high-titer pLVX-Puro supernatant, serial dilutions of which were used to infect naïve cultures of 293T cells (Figure 2). After replating the infected cells on 10 cm dishes and selecting transductants with puromycin, the resulting colonies of stable transductants were stained for detection. Cells infected with only 0.1  $\mu$ l of supernatant produced hundreds of colonies, while colonies from cells infected with 1  $\mu$ l virtually covered the entire plate. These results demonstrate the high titer and infectivity of a typical pLVX-Puro supernatant.

The Lenti-X Expression System is a comprehensive system for preparing recombinant lentivirus to express any cDNA in any cell type susceptible to lentivirus transduction. It easily produces high-titer lentiviral supernatants suitable for safe use with virtually any downstream application.

Product	Size	Cat. No.	Price
Lenti-X Expression System each		632164	\$1,096.00
Puromycin	25 mg	631305	\$71.00
	100 mg	631306	\$176.00

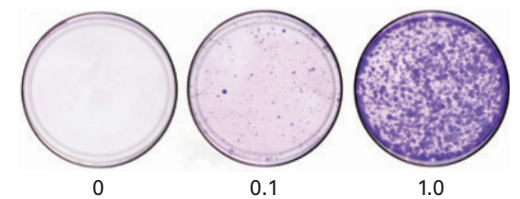
Prices are subject to change without notice.

## Components

- pLVX-Puro Vector
- Lenti-X™ HT Packaging Mix
- Lentiphos™ HT
- Lenti-X™ Lentiviral Expression Systems User Manual (PT3983-1)

## Related Products

- Lenti-X™ Fluorescent Vectors (Cat. Nos. 632152, 632153, 632154 & 632155)
- Lenti-X™ HT Packaging System (Cat. Nos. 632160 & 632161)



**Figure 2. Puromycin selection of transduced cells.** 293T cells were infected with the indicated volumes ( $\mu$ l) of pLVX-Puro supernatant and selected with puromycin for 9 days to allow the formation of colonies, which were then stained with crystal violet.

## References

1. Zufferey, R. *et al.* (1999) *J. Virol.* **73**(4):2886–2892.
2. Zennou, V. *et al.* (2000) *Cell* **101**(2):173–185.
3. Wu, X. *et al.* (2000) *Mol. Ther.* **2**(1):47–55.

# Fluorescent Lentiviral Expression Vectors

## Lentiviral delivery system for your fluorescent fusion proteins

- Efficient delivery into dividing and nondividing cells, including primary cells
- Easily achieve stable, long-term expression
- Visualize your protein of interest as a fluorescent fusion

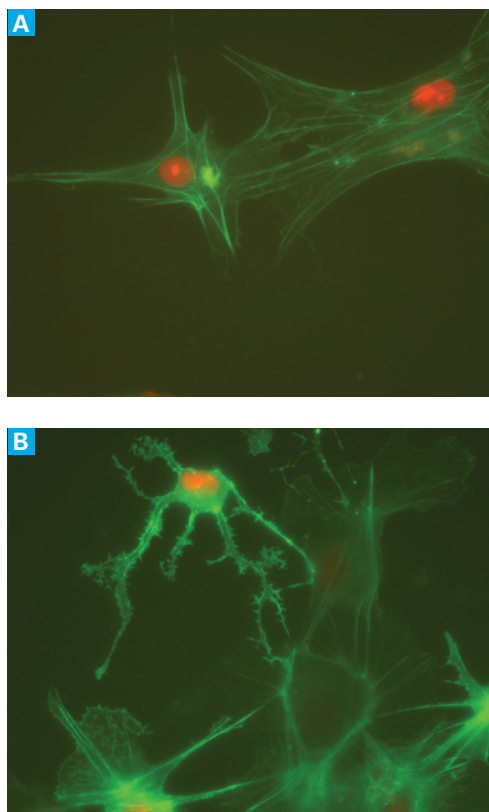
Clontech's new **Lenti-X™ Living Colors® Vectors** contain all of the features necessary to effectively express your fluorescently-tagged protein of interest in hard-to-transfect cells. Express your gene of interest, fused at the N- or C-terminus to either our monomeric red (DsRed-Monomer) or monomeric green (AcGFP1) fluorescent proteins (1–3). Both DsRed-Monomer and AcGFP1 have been specifically engineered to provide outstanding performance when expressed as fusions with other proteins.

The Lenti-X Living Colors Vectors are ideal for multicolor applications such as flow cytometry or fluorescence microscopy, and can serve as markers of packaging cell transfection and target cell infection. They contain all of the features necessary to efficiently deliver your gene of interest to dividing and nondividing hard-to-transfect cells, as well as to cultured cell lines and primary cells (Figure 1).

### Fluorescent Fusion Proteins

The DsRed-Monomer and AcGFP1 proteins are ideal tools for monitoring gene expression and intracellular protein trafficking. Because of their distinct spectra, these fluorescent proteins can be used for multicolor labeling and direct visualization applications (Figure 1; 1–5).

The monomeric nature of the DsRed-Monomer protein has been confirmed by FPLC gel filtration chromatography and pseudonative gel electrophoresis, both of which yield results consistent with its calculated molecular weight of 26.8 kDa, based on amino acid sequence (1). Similarly, the monomeric nature of AcGFP1 has been confirmed by three independent methods: FPLC gel filtration chromatography, pseudonative gel electrophoresis, and sucrose density gradient ultracentrifugation. All the results agree with its calculated molecular weight, 26.9 kDa (2).



**Figure 1. Visualization of human neural progenitor cells coinfecting with equivalent MOIs of pLVX-AcGFP1-Actin and pLVX-DsRed-Monomer-Nuc.** Neurospheres were differentiated on laminin, then labeled by infection with pLVX-AcGFP1 and pLVX-DsRed-Monomer. Actin labeling with AcGFP1 allows clear visualization of the cytoskeletal structure. Nuclear labeling with DsRed-Monomer permits identification of cell position in both images (Panels A & B). MOI = multiplicity of infection.

DsRed-Monomer and AcGFP1 fusion proteins are able to localize to compartments and structures that cannot accommodate oligomeric tags. Both proteins are extremely stable, and are ideal for subcellular localization studies, as shown in Figure 1. With these vectors, you can visualize biological processes as they occur and easily track your protein of interest to a specific subcellular organelle or structure.

### Lentiviral Expression

The Lenti-X Living Colors Vectors can be used to establish fluorescent fusions in a wide variety of cell lines, primary cell cultures, stem cells, and nondividing cells, which may be resistant to standard gene delivery techniques such as

Product	Size	Cat. No.	Price
pLVX-DsRed-Monomer-N1 Vector	10 µg	632152	*\$676.00
pLVX-DsRed-Monomer-C1 Vector	10 µg	632153	*\$676.00
pLVX-AcGFP1-N1 Vector	10 µg	632154	*\$676.00
pLVX-AcGFP1-C1 Vector	10 µg	632155	*\$676.00
Lenti-X HT Packaging System	20 rxns	632160	\$876.00
	40 rxns	632161	\$1,226.00
Lentiphos HT	20 rxns	632151	\$264.00

Prices are subject to change without notice.

\* Not-for-Profit Entities. For-Profit Entities: \$1331.

### Related Products

- Living Colors® DsRed Polyclonal Antibody (Cat. No. 632496)
- Living Colors® A.v. Monoclonal Antibody (JL-8) (Cat. Nos. 632380 & 632381)

transfection or retroviral infection. These vectors are designed for use with our **Lenti-X HT Packaging System**, which provides the entire complement of essential lentiviral packaging components, in ratios that maximize virus production (see pages 3–4).

### References

1. BD Living Colors® DsRed-Monomer Fluorescent Protein (January 2005) *Clontechiques* **XX**(1):2–4.
2. BD Living Colors® AcGFP1 Fluorescent Protein (January 2005) *Clontechiques* **XX**(1):5–6.
3. BD Living Colors® Fluorescent Protein Vectors (October 2005) *Clontechiques* **XX**(2):18–20.
4. Living Colors® Fluorescent Protein Vectors (April 2006) *Clontechiques* **XXI**(1):16–17.
5. Retro-X™ Living Colors Fusion Vectors (July 2006) *Clontechiques* **XXI**(2):20.

# Transfection Reagent for High-Titer Lentivirus

Optimized Lentiphos™ HT provides unsurpassed transfection efficiency and viability

- Transfects up to 99% of your 293T lentivirus packaging cultures
- Generates extremely high titers of lentivirus
- Optimized protocol for 293T cells

Our scientists at Clontech have created one of the most efficient transfection methods yet devised for generating high titers of lentivirus. The modified and optimized calcium phosphate-based **Lentiphos HT** system is easy to use and successfully transfects up to 99% of 293T cells for lentiviral packaging (see pages 3–4). This incredibly high transfection efficiency, coupled with inherently low toxicity, transforms entire 293T cell cultures into virus-producing factories. Lentiphos HT is an essential part of our **Lenti-X™ HT Packaging System**, but we are making it available separately to enable users to improve their 293T cell transfection capabilities and enhance their own lentiviral packaging systems.

## Comprehensive Transfection Efficiency & Viability

To demonstrate the efficiency and viability benefits of Lentiphos HT, 293T cells were cotransfected with our Lenti-X HT Packaging Mix and a ZsGreen1 lentiviral expression vector using either Lentiphos HT or a competitor's lipid-based system (Figure 1). Lentiphos HT transfection converted >99% of the cells into ZsGreen1-positive cells, produced much higher lentiviral titers (>5 x 10<sup>8</sup> IFU/ml), and maintained better posttransfection cell viability than the lipid-based transfection method. While initial transfection rates were high with both methods, the lipid-based transfection exhibited significant toxicity, reduced cell numbers and viability, and ultimately, lower titer values (see page 3, Figure 2). Lentiphos HT-transfected cultures retained excellent viability, which ensured continued production of virus from virtually every cell in the culture.

Product	Size	Cat. No.	Price
Lentiphos HT	20 rxns	632151	\$264.00

Price is subject to change without notice.

### Components

- Lentiphos1 Solution
- Lentiphos2 Solution
- Sterile water
- Protocol-at-a-Glance (PT3984-2)

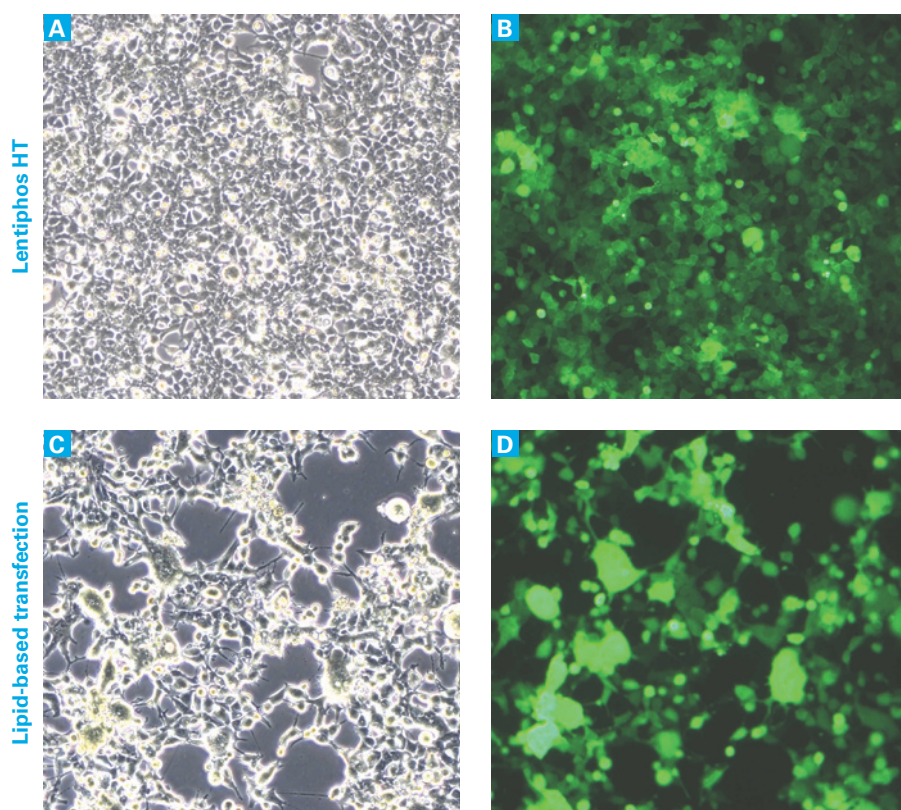
### Related Products

- Lenti-X™ HT Packaging System (Cat. Nos. 632160 & 632161)
- Lenti-X™ Expression System (Cat. No. 632164)
- Lenti-X™ Tet-On® & Tet-Off® Advanced Inducible Expression Systems (Cat. Nos. 632162 & 632163)

## Improve Your Lentiviral System

Producing the highest possible viral titers is critical for lentiviral systems that target difficult-to-transduce cell populations. Lentiphos HT enables you to maximize the viral titers of your lentiviral packaging system by ensuring the highest transfection efficiencies with the least toxicity. Designed to be used with plasmid mixtures, this highly effective method ensures that transfected cells receive the full complement of plasmids required to produce infectious virus from your lentiviral construct.

Lentiphos HT also provides significant benefits for users who wish to improve the transfection efficiencies of other cell culture systems.



**Figure 1. The Lenti-X HT Packaging System preserves 293T cell viability and produces more lentivirus.** Equivalent 293T-based cell cultures were transfected with either the Lenti-X System and Lenti-X ZsGreen1 Vector (Panels A & B), or with a competitor's lipid-based transfection/packaging system and their lentiviral ZsGreen1 vector (Panels C & D). After 48 hr, the cells were visualized using phase contrast microscopy (Panels A & C) and fluorescence microscopy (Panels B & D). The Lenti-X culture produced a titer of 5.1 x 10<sup>8</sup> IFU/ml, whereas the competitor's system produced a 25-fold lower titer.

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## Licensing Statements

### Adenoviral System 1 Expression Products

This product is covered under U.S. Patent No. 6,303,362.

### bGH Poly A

Please see the bGH poly A licensing statement at [www.clontech.com/licensing](http://www.clontech.com/licensing)

### CMV Sequence

The CMV promoter is covered under U.S. Patent Nos. 5,168,062 and 5,385,839 assigned to the University of Iowa Research Foundation.

### cPPT Element

Please see the cPPT licensing statement at [www.clontech.com/licensing](http://www.clontech.com/licensing)

### DsRed-Monomer

This product is covered under U.S. Patent No. 7,250,298.

### IRES Sequence

Use of the IRES sequence is covered by U.S. Patent No. 4,937,190 and is limited to use solely for research purposes. Any other use of the IRES sequence requires a license from Wisconsin Alumni Research Foundation.

### Lentiviral Expression Products

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Living Colors® Products AcGFP1, DsRed, HcRed, AsRed, AmCyan, ZsGreen, ZsYellow and their variants:

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