# Lenti-X<sup>™</sup> Lentiviral Systems & Tools

**Designed for High Titers** 





# Lenti-X Lentiviral Systems and Tools

Designed for high titers and high-efficiency transduction

# Highlights

- Achieve very high titers of 10<sup>7</sup>–10<sup>8</sup> infectious units (IFU) per ml
- Obtain highly infective VSV-G or ecotropic pseudotyped lentivirus in 48 hr
- Infect an entire plate of target cells with as little as 10 µl of viral supernatant
- Novel five-vector split-packaging technology ensures virus is safe



# Surpasses competitor transduction efficiency by several orders of magnitude



Figure 1. High infectivity of supernatants produced by Lenti-X Packaging System. Lenti-X (Panel A) and a packaging system from a competitor (Panel B) were each used to generate virus containing a vector system for expressing the ZsGreen1 fluorescent protein. 10  $\mu$ I of supernatant from each system was used to transduce HeLa cells. ZsGreen1-positive cells were quantified by flow cytometry. Lenti-X transduced the majority of cells, whereas the other system transduced only a small percentage of the cells.

# Comprehensive and integrated product offering to meet your lentiviral transduction needs

Lenti-X lentiviral systems and tools from Clontech provide an industry-leading set of integrated tools for lentiviral transductions: from the Lenti-X HTX high-titer lentiviral packaging systems to an optimized cell line to accessory products that enhance or accelerate transduction efficiency







Figure 2. The 4th generation Lenti-X HTX Packaging System. A lentiviral vector and the Lenti-X HTX Packaging Mix are co-transfected into Lenti-X 293T cells using the highly efficient Xfect transfection reagent. High titer supernatants are ready for use after 48 hr.

# Key System Components

#### **Optimized Lenti-X Vectors**

Contain **cPPT/CTS** (central polypurine tract/central termination sequence) and **WPRE** (woodchuck hepatitis virus post-transcriptional regulatory element) that enhance transduction and gene expression.

#### Optimized Lenti-X HTX Packaging Mix

Essential viral components on a proprietary suite of vectors, premixed in specific ratios to maximize virus production.

#### **Tetracycline Transactivation**

High-level expression of key lentiviral packaging components is produced by **Tet-Off**® transactivation of tetracycline-responsive promoter elements (TREs).

#### **Optimized Transfections**

Nanoparticle-based **Xfect**<sup>™</sup> Transfection Reagent *(included)* transfects Lenti-X 293T Cells with over 95% efficiency.

#### **Instant Titer Test**

Determine the optimal harvest time for lentiviral supernatants in seconds with Lenti-X GoStix<sup>™</sup> (three tests included).

#### Specialized Lenti-X 293T Cell Line

Highly transfectable cells that yield lentiviral titers as high as 10<sup>8</sup> IFU/mI. (*Not included; sold separately*)

#### **Ecotropic Receptor Boosters**

Allow you to increase the infection efficiency of any cell type, including human cells and cells that are resistant to viral infection.



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### Lenti-X Concentrator

#### Increase titer without ultracentrifugation

- Easy protocol mix and spin
- Scalable to any volume or titer
- 100X concentration with 90% recovery

Add Lenti-X Concentrator reagent to clarified viral supernatant, incubate for 30 min to overnight at 4°C, and spin. It's that easy!

### Lenti-X Accelerator

#### Fast and highly efficient transduction

- Magnetic bead-based—no polybrene
- For lentivirus & MMLV retrovirus
- Ideal for sensitive cell types (e.g. stem cells)
- Transduction time of 5 min

With the Lenti-X Accelerator, a magnetic field concentrates virus-bound magnetic beads at the cell monolayer surface. This reduces the exposure time of sensitive target cells to viral supernatant to just 5 min—compared to overnight transduction with polybrene. The Lenti-X Accelerator starter kit includes a magnetic plate.

### RetroNectin® Reagent

#### Transduction of hard-to-infect cell types

- Multivalent molecule simultaneously binds virus particles and cell surface proteins
- · Widely used for hematopoietic cells and stem cells
- Increases transduction of hard-to-infect cells

Use RetroNectin-coated tissue culture plates to enhance lentivirus-mediated gene transduction into mammalian cells. The multivalent RetroNectin peptide allows for simultaneous binding of cells and lentivirus, bringing them into close physical proximity. The RGDS-containing domain (green) binds the cell surface integrin receptor VLA-5, the heparin-binding domain (blue) binds many types of virus particles, and the CS-1 sequence (orange) binds the VLA-4 cellular integrin receptor.

#### Lenti-X Maxi Purification Kit High yield & purity

- Gentle gravity column-based protocol
- Does not damage lentiviral particles
- Results in better transduction efficiency
- Concentrates virus up to 10-fold

The Lenti-X gravity column-based method is extremely effective, and preserves virus much better than syringe filter-based methods. Just bind, wash, and elute.











## Lenti-X GoStix—Instant Lentiviral Titration

#### Get a YES or NO in only 10 minutes

- Distinguish between good and bad preps
- Know when to harvest lentiviral supernatants

Want to know if you have enough lentivirus? The Lenti-X GoStix protocol can tell you in 10 minutes. Simply apply 20 µl of your lentiviral supernatant to the sample well, add 4 drops of buffer, and wait for the indicator bands to appear.

### Lenti-X p24 Rapid Titer Kit

#### **Rapid titer quantification**

- Fast and easy ELISA-based titration protocol •
- Collect supernatant, lyse, bind, wash, and detect
- Correlate p24 capsid protein content to viral titer

Lentiviral supernatant is bound to anti-p24 coated wells and detected using a combination of biotinylated anti-p24 secondary antibody, streptavidin-HRP, and a color reagent. Titration by provirus is also available.

### Lenti-X qRT-PCR Titration Kit

#### Accurate titration in 4 hr

- Fast and accurate titration using SYBR® Green chemistry
- Harvest, titer, and infect in a single day
- Obtain results in 4 hr

Set up your gRT-PCR reaction with purified RNA from harvested lentiviral supernatant. Determine the viral genome content from a calibrated RNA standard curve.

### **Ecotropic Receptor Boosters**

#### Transduce human cells with ecotropic virus

- Temporarily increase viral receptor density ٠ on your target cells
- Transduce more cell types, with equal efficiency

Our Ecotropic Receptor Booster consists of a vial of concentrated exosome-like vesicles that are densely coated with the mCAT-1 receptor protein. When the booster vesicles are applied to your cells they fuse with the plasma membrane, resulting in an increased level of receptor proteins on the cell surface.



ecotropic receptor

Target cells lack

**Receptor Boosters** are concentrated ecotropic receptors on exosomelike particles

**Receptor Boost**ers fuse with target cells to increase the





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Streptavidin HRI Anti-p24 (biotinylated) Lentiviral p24 Anti-p24-coated wel Lentiviral packaging cells Anti-p24-coated plate (12 x 8-well strips)



retroviral supernatant

Viral RNA purification & DNase I treatment







Data analysis

concentration of ecotropic receptor

tion efficiency

www.clontech.com/lenti-X





#### **Constitutive cDNA Expression**

Express your gene of interest from a strong CMV promoter, and select for lentiviral integration using antibiotic selection.



#### **EF-1 Alpha Promoter**

Express your gene of interest from an EF-1 alpha promoter in cell types where the CMV promoter can be silenced over time, such as hematopoietic or stem cells.





FP = fluorescent protein (mCherry or ZsGreen1)

pLVX-EF1α-FP-N1\*\*



#### **Bicistronic Expression**

Coexpress your gene of interest and an antibiotic or fluorescent selectable marker from the same transcript using these IRES-containing lentiviral vector systems.

pLVX-IRES-Puro/Neo/Hyg\*



FP = fluorescent protein (mCherry, tdTomato, or ZsGreen1)

#### **Fluorescent Protein Expression**

Clone your gene in-frame with one of Clontech's many fluorescent proteins (FP) and monitor the expression and subcellular localization of your protein of interest.



FP = fluorescent protein (AcGFP1, AmCyan1, DsRed-Express2, DsRed-Monomer, mCherry, tdTomato, or ZsGreen1)

Vectors available as part of an expression system.

\*\* Vectors available separately.

#### **Tetracycline-Inducible Expression**

Tight control and high fold inducibility using the Tet-One™, Tet-On® 3G or Tet-Express<sup>™</sup> vector systems.



#### shRNA Expression

Clone your shRNA downstream of a U6 promoter to knock down gene expression in hard-to-transfect cells.



#### **ProteoTuner™ Protein Control**

Clone your gene downstream of a ProteoTuner destabilization domain (DD) and control the stability of your protein with Shield1, a small molecule ligand, which protects the DD fusion protein from being degraded.



#### **Promoter Reporters**

Monitor the activity of your favorite promoter using a reporter system that expresses bright fluorescent proteins or secreted luciferase.



FP = fluorescent protein (AmCyan1, ZsGreen1, or tdTomato)

Cat. #	Product	Package Size	
Lentiviral Packagir	ng Systems		
631247 631249	Lenti-X HTX Packaging System	20 rxns 60 rxns	
631251	Lenti-X HTX Ecotropic Packaging System	20 rxns	
632180	Lenti-X 293T Cell Line	1 ml	
Lentiviral Vector Systems (visit www.clontech.com/lenti-X for a complete list of lentiviral vectors)			
632164*	Lenti-X Expression System	each	
631253*	Lenti-X Expression System (EF1alpha version)	each	
632182*	Lenti-X Bicistronic Expression System (Hyg)	each	
632181*	Lenti-X Bicistronic Expression System (Neo)	each	
632183*	Lenti-X Bicistronic Expression System (Puro)	each	
631844	Lenti-X Tet-One Inducible Expression System	each	
631847	Lenti-X Tet-One Inducible Expression System (Puro)	each	
631187*	Lenti-X Tet-On 3G Inducible Expression System	each	
631189*	Lenti-X Tet-Express Inducible Expression System	each	
632177*	Lenti-X shRNA Expression System	each	
632154/(632155)	pLVX-AcGFP1-N1/(C1) Vector	10 µg	
632558/(632557)	pLVX-AmCyan1-N1/(C1) Vector	10 µg	
632560/(632559)	pLVX-DsRed-Express2-N1/(C1) Vector	10 µg	
631983/(631984)	pLVX-EF1alpha-AcGFP1-N1/(C1) Vector	10 µg	
631987	pLVX-EF1alpha-IRES-mCherry Vector	10 µg	
631982	pLVX-EF1alpha-IRES-ZsGreen1 Vector	10 µg	
631986/(631985)	pLVX-EF1alpha-mCherry-N1/(C1) Vector	10 µg	
631237	pLVX-IRES-mCherry Vector	20 µg	
631238	pLVX-IRES-tdTomato Vector	20 µg	
632187	pLVX-IRES-ZsGreen1	10 µg	
632562/(632561)	pLVX-mCherry-N1/(C1) Vector	10 µg	
632563/(632564)	pLVX-tdTomato-N1/(C1) Vector	10 µg	
632565/(632566)	pLVX-ZsGreen1-N1/(C1) Vector	10 µg	
Lentiviral Transduction Tools			
631231 631232	Lenti-X Concentrator	100 ml 500 ml	
631233 631234	Lenti-X Maxi Purification Kit	2 preps 5 preps	
631245	Lenti-X Maxi Purification Kit (with Rack)	5 preps	
631235	Lenti-X qRT-PCR Titration Kit	200 rxns	
631239	Lenti-X Provirus Quantitation Kit	200 rxns	
631243 631244	Lenti-X GoStix	20 tests 50 tests	
631254	Lenti-X Accelerator Starter Kit	each	
631256 631257	Lenti-X Accelerator	400 μl 1,000 μl	
631471	Ecotropic Receptor Booster	20 rxns	
632200	Lenti-X p24 Rapid Titer Kit	96 rxns	
T110A	RetroNectin Precoated Dish	10 dishes	
T100A T100B	RetroNectin Recombinant Human Fibronectin Fragment	0.5 mg 2.5 mg	

T100B

Notes: C-terminal fusion vectors and associated Cat. Nos. are indicated in parenthesis. Asterisk (\*) marked Cat. Nos. include the Lenti-X HTX Packaging System (20 rxn).

Please check www. clontech.com for current pricing.

#### Notice to Purchaser

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