

LentiGlo

PRODUCT APPLICATIONS GUIDE

**2008**

**Pluristem innovations**

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## Lentiviral Vectors

Lentiviral vectors (LVs) are viral-based gene delivery systems that can stably deliver genes or RNAi into primary cells or cell lines with up to 100% efficiency. LVs bind to target cells using an envelope protein which allows for release of the LV RNA containing the gene or gene silencing sequence into the cell. The LV's RNA is then converted into DNA using an enzyme called reverse transcriptase by a process called reverse transcription. The DNA pre-integration complex then enters the nucleus and integrates into the target cell's chromosomal DNA (see figure below).

Gene delivery is stable because the target gene or gene silencing sequence is integrated in the chromosome and is copied along with the DNA of the cell every time the cell divides. One of the discriminating features of LVs is their ability to integrate into non-dividing cells, in contrast to other vectors that either don't integrate efficiently into chromosomal DNA (e.g. non-viral, Adenoviral and Adenoviral-Associated vectors) or can only integrate upon cell division (e.g. conventional Retroviral vectors)

## Lentiviral Vector Applications

- Creation of stable cell lines
- Expression of genes in primary cells
- Gene of RNAi delivery into neurons or hard to transfect cell types
- Gene Therapy Applications
- RNAi expressing cell lines—stable knockdown of gene expression
- Efficient generation of transgenic animals
- Animal experiments that require localized gene delivery
- Detection and localization of proteins in live cells
- Drug discovery—creation of cell lines that express reporter genes in response to chemical stimulants
- Rapid production of proteins from cell lines

### **LentiGlo™ Lentiviral Vector System**

LentiGlo is a novel, highly efficient Lentiviral vector system in which the gene encoding Gaussia luciferase, expressed under control of the CMV promoter has been engineered into a proprietary optimized HIV-1 based Lentiviral vector that can be modified to accommodate additionally any desired gene, RNAi, promoter, and post-transcription or insulator element. Also, LentiGlo is free from the intellectual property (IP) constraints of other Lentiviral vector systems as this vector

There are two types of LentiGlo vectors, currently offered by Pluristem Innovations. The first are those that are engineered to express the gaussia luciferase alone under control of the CMV promoter(Lenti-Gluc) or to express both Gaussia luciferase (under control of the CMV promoter) along with a GFP (Lenti-Gluc-GFP), that has been subcloned downstream of an IRES.

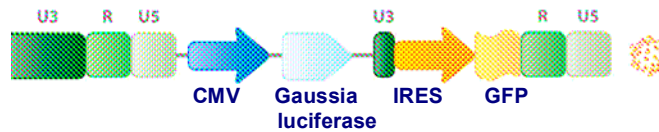
The second category of vectors are the LentiGlo custom vectors:

The GFP gene can be removed and replaced with an siRNA to inhibit a specific gene, or replaced with another gene of interest, and those that are designed to express a specific gene in target cells. Below you will find examples of a LentiMax vector that either silences or expresses a gene of interest. With LentiMax, you are in control of your vector design. You select the type of promoter to drive the expression of your sequence of interest

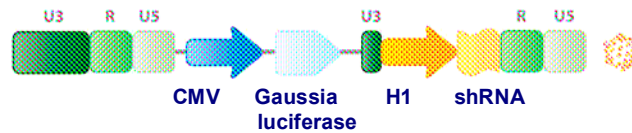
The **LentiGlo-Gluc** and the LentiGlo-Gluc-GP vectors are both priced at \$1500 and provide sufficient viral particles to transduce 500,000 to 1000,000 cells.

The **Lenti-Custom** vectors (custom made) are priced at \$1500 and provide sufficient viral particles to transduce 500,000 to 1000,000 cells

## LentiGlo Lenti-Gluc expression vector



## LentiGlo gene silencing vector



**Figure 1.**

Examples of LentiGlo Lentiviral vectors.

### LentiGlo Lenti-Gluc expression vector

In the LentiGlo luciferase expression vectors, the Lentiviral vector particles are pseudotyped with a Mokola virus envelope protein. The Gene Expressing vectors (top) express either gaussia luciferase alone or in combination with a GFP reporter. The Gaussia luciferase gene is expressed from the CMV promoter; the turbo-GFP gene is expressed from the same mRNA as the first gene and is separated from an Internal Ribosome Entry Sequence (IRES) element. Optionally, a post-transcriptional element can be inserted downstream of the GFP gene. In this case the LV particles are pseudotyped with the VSVG protein.

### LentiGlo gene silencing vector

The gene silencing vector (bottom) expresses Gaussia luciferase under control of the CMV promoter and a shRNA targeted to a gene of interest. The shRNA sequence is driven by the H1 promoter

## Vector Safety

LentiGlo™ vectors have been designed to maximize their biosafety features, which include:

- A deletion in the enhancer of the U3 region of 3'LTR ensures self-inactivation of the lentiviral construct after transduction and integration into genomic DNA of target cells
- Lack of accessory genes in the lentiviral vector manufacturing process

- The vector and helper constructs contain no significant areas of homology, minimizing their chance for recombination
- None of the HIV-1 genes (gag, pol, rev) will be expressed in transduced cells, as they are expressed from packaging plasmids lacking packaging signal. Therefore, the lentiviral particles that are generated are replication-incompetent
- Lentiviral vector particles will carry only a copy of your gene or gene silencing sequence of interest

### **Safety in the Laboratory**

Lentiviral vectors have been used in hundreds of laboratories around the world without incident. Whenever conducting experiments using lentiviral vectors, it is recommended that they be performed under standard Biosafety Level 2 (BSL-2) laminar flow hood. Details on requirements for creating a BSL-2 work environment are available in the U.S. Department of Health and Human Services publication *Biosafety in Microbiological & Biomedical Laboratories*, 4th edition.

### **Safety in the Clinic**

The LentiGlo vectors are sold for research purposes only and not intended for human use. However, we can supply LentiGlo vectors that would be suitable for human clinical trials

Lentiviral vectors are currently being evaluated in at least five clinical trials. To date, lentiviral vectors have been shown to be safe when introduced into humans, as no adverse events have been reported (Dropulic and June, 2006).

Lentiviral vectors have a distinct safety advantage over traditionally used Murine Leukemia Virus (MuLV) Retroviral

Vectors. MuLVs have caused insertional oncogenesis in human clinical trials. In contrast, lentiviral vectors have several distinct safety advantages over MuLV vectors:

- Lentiviral vectors are not known to cause oncogenesis (i.e., show signs of genotoxicity), in contrast to MuLVs
- The lentiviral vector Long Terminal Repeats (LTR) do not contain highly active transcriptional enhancers, rather amplify mRNA expression by the Tat protein binding to the TAR element, facilitating elongation of mRNA. The highly active transcriptional enhancers of MuLV vectors are believed to be one of the factors involved in oncogenic genotoxicity resulting from insertional mutagenesis
- The self-inactivating nature of LentiMax™ vector increases their safety
- There are 40 million people infected with the AIDS virus, a lentivirus. Even though this represents billions of integration events, including events in patients which contain disease progression by anti-retroviral drug therapy, there is not a single documented case of oncogenesis due to an integration event by a Lentivirus
- Recent studies in mice have shown that while MuLV vectors are genotoxic (due to insertional mutagenesis), lentiviral vectors show no such genotoxicity (Montini, et. al., *Nature Biotechnology*, 2006)

## General Transduction Protocols

### Adherent Cell Lines, Standard 6-well plate

*Transduction efficiency between cell lines will vary.* Lentigen has collected data for many popular cell lines. Although it is possible to deduce the proper MOI required for the desired efficiency based on Lentigen's data, the optimal protocol for each unique LentiMax™ vector can only be achieved through trial and error. Lentigen suggests an initial MOI of 50-100. It may also be necessary to add a second dose of vector 24 hours post transduction to increase efficiency. Consult the table below for specific recommendations based on the target cell type. Please call Lentigen Technical Service with any questions or concerns regarding optimal transduction.

### Suggested Lentiviral MOIs for particular cell types:

CELLS	CELLTYPE/SPECIES
POLYCATION	
SUGGESTED MOI	
	HT 1080
	Human Fibrosarcoma
1 to 10	6 µg/ml polybrene
	Human Embryonic Kidney
	293
	3 µg/ml polybrene
5 to 10	
	Human Epithelial Carcinoma
	3 µg/ml polybrene
5 to 10	
	HeLa
	None
	Rat
	Cortical Neurons
10 to 25	
	Neural Stem Cells
	Rat
10 to 100	
	None

**10 to 35**

**6-10 µg/ml DEAE Dextran**

**Mouse  
B Cells**

**1. Plate the cell line on a tissue culture plate and allow the cells to adhere before performing the transduction.**

The desired confluency for transduction is 70% to 90%. The length of time required to achieve this level of confluency will vary by the cell line. Using 293T as an example, seeding 1.2 million cells in a standard 6-well plate will yield close to 90% confluency within 18 hours. If using a recently thawed cell line, you should allow at least one passage after thaw before performing a transduction. Also, avoid using cell lines with less than 80% viability since maximum transduction efficiency is achieved using highly viable cells.

**2. Replace the media with 1 mL of fresh, pre-warmed media.**

If using polybrene, add it to the media beforehand at a concentration of 8 µg/mL. Cellular toxicity to polybrene should be determined beforehand as some cells are sensitive to the compound (See the section labeled “Polybrene” in this guide for more details).

**3. Add LentiMax™ vector and gently disperse the vector, avoiding harsh movements that may dislodge the cells. The MOI and volume will vary between cell lines. Return the plate(s) to the incubator. Note: You may achieve higher transduction efficiencies by removing the plate from the incubator every half-hour and gently rocking the plate by hand, using care to ensure media is not transferred between wells. After rocking the plate for approximately 15-20 seconds, place it back in the incubator.**

The optimal MOI depends on both the permissiveness of the cell line to transduction by lentiviral vectors and also the vector titer. Some highly permissive cell lines may require significantly less volume to achieve high transduction levels. Experiments using varying MOI should be performed to determine the most efficient volumes to use. Serial dilutions of high titer vectors (greater than 10<sup>9</sup> TU/mL) may also be required.

**4. (Optional) For titer experiments, count the total number of cells in a control well. Record this number for titer calculations.**

**5. (Optional) After a 4 hour incubation, wash 2X with media, and replace the media with 4 mL of fresh media.**

Some cells are sensitive to the prolonged exposure to lentiviral vectors and polybrene. Determine if your cell line is sensitive to these conditions and adjust the incubation time accordingly. If your cell line is not sensitive, the vector and/or polybrene can remain in culture until the next scheduled media change, between 4 and 18 hours after transduction.

**6. (Optional; for difficult to transduce cells only) Add an equal volume of vector (as previously added) 24 hours after initiating transduction. Gently disperse the media as in step 3.**

It may be necessary to change the media based on the pH, growth kinetics of the cell line, etc. If it is necessary to replace the media, add 1 mL before dispensing the second dose. If a second dose is added, the time frame for completing the assay must be shifted by one day. **16** [www.lentigen.com](http://www.lentigen.com)

**7. Change the media within 4-18 hours after the last transduction and add 4 mL of fresh, pre-warmed media.**

The window for replacing the media will vary by cell line. Hearty cell lines, such as 293T, can survive 18 hours of incubation in the presence of vector without compromising the culture. Some cell lines will require media change at 4 hours so as to limit any toxicity due to prolonged exposure to the vector. Experiments should be performed to determine the optimal incubation time period.

**8. Incubate the transduced cells at 37°C and 5% CO<sub>2</sub> for 24 to 72 hours.**

If cells are transduced with genes expressing a fluorescent marker such as GFP, highly transduced cells will begin expressing detectable levels of the protein within 24 hours. Fluorescence can be observed using a fluorescent microscope using the appropriate filter against the marker. The Lentigen website contains examples of highly transduced cells expressing GFP protein.

**9. Assay the cells a minimum of 48 hours post transduction to determine the transduction efficiency.**