

## pLenti-RFP-Puro Lentiviral Control Vector

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**CATALOG NUMBER:** LTV-403

**STORAGE:** -80°C

**QUANTITY AND CONCENTRATION:** 100 µL of bacterial glycerol stock

### **Background**

Lentivirus vector based on the human immunodeficiency virus-1 (HIV-1) has become a promising vector for gene transfer studies. The advantageous feature of lentivirus vector is the ability of gene transfer and integration into dividing and non-dividing cells. The pseudotyped envelope with vesicular stomatitis virus envelope G (VSV-G) protein broadens the target cell range. Lentiviral vectors have been shown to deliver genes to neurons, lymphocytes and macrophages, cell types that previous retrovirus vectors could not be used. Lentiviral vectors have also proven to be effective in transducing brain, liver, muscle, and retina *in vivo* without toxicity or immune responses. Recently, the lentivirus system is widely used to integrate siRNA efficiently in a wide variety of cell lines and primary cells both *in vitro* and *in vivo*.

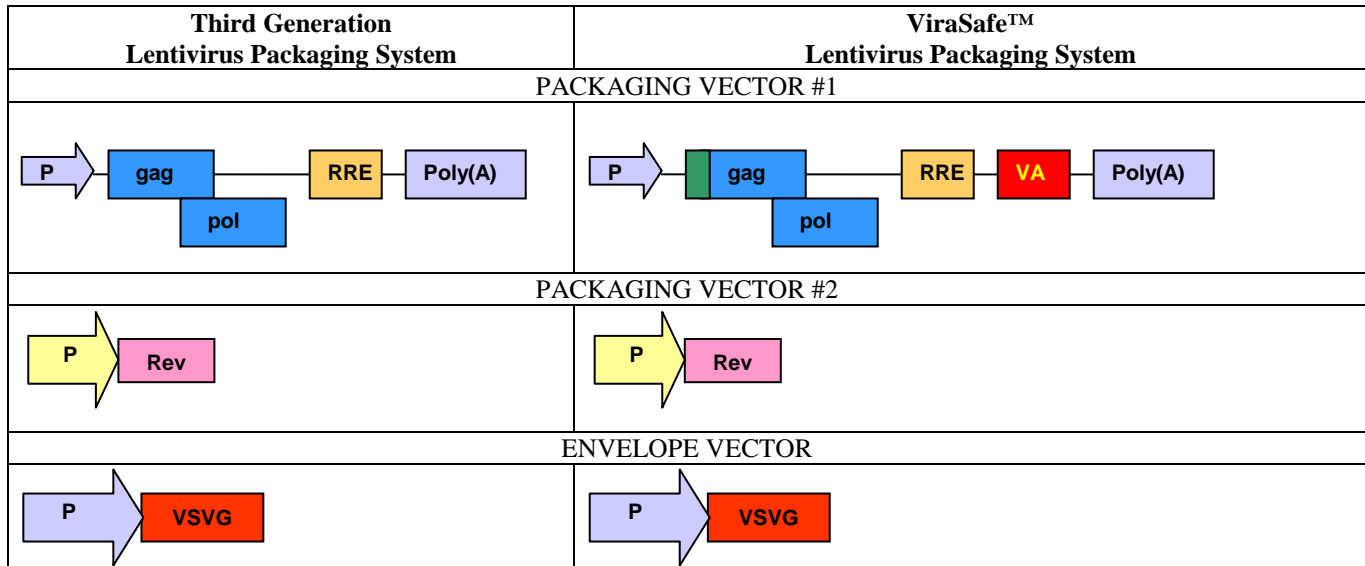
Lentivirus particles are produced from 293T cells through transient transfection of plasmids that encode for the components of the virion. Due to safety concerns regarding the infectious nature of HIV-1, recent lentiviral packaging systems have separated the viral components into 3 or 4 plasmids. However, these systems still present a small chance of generating replication-competent lentivirus upon recombination. In addition, most commercial lentiviral packaging systems provide plasmids containing the viral structure proteins in a premixed formulation, making it nearly impossible to optimize the ratio of the various plasmids for your particular experiment and host cell.

Lentiviral supernatant can be produced by cotransfecting 293T cells (Cat.# LTV-100) with pLenti-RFP-Puro and a lentivirus packaging mix such as Cell Biolabs' ViraSafe™ Lentiviral Packaging System (Cat.# VPK-206). Supernatants can be used directly or purified/concentrated if needed. For long term storage, store supernatant at -80°C in aliquots. The resulting control virus can also be used to generate RFP and puromycin stable cell lines, and stable clones can be selected by either red fluorescence sorting or puromycin resistance.

### **Related Products**

1. VPK-205: ViraSafe™ Lentiviral Packaging System, Ecotropic
2. VPK-206: ViraSafe™ Lentiviral Packaging System, Pantropic
3. VPK-107: QuickTiter™ Lentivirus Titer Kit (Lentivirus-Associated HIV p24)
4. VPK-090: ViraBind™ Lentivirus Concentration and Purification Kit
5. LTV-200: ViraDuctin™ Lentivirus Transduction Kit

**Unique Elements of the ViraSafe™ Lentivirus Packaging System (sold separately)**

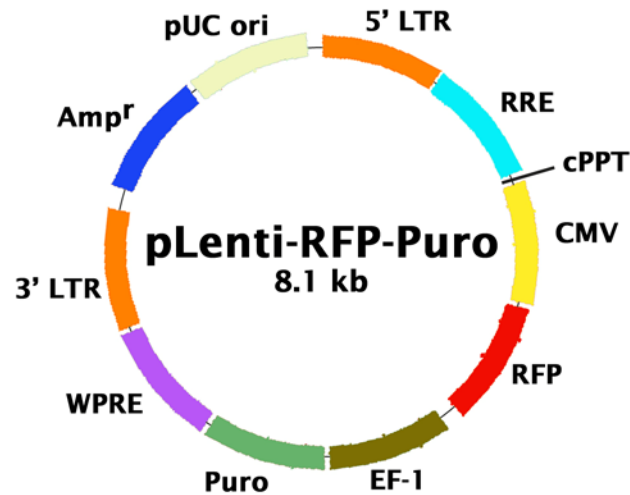


Vector Name	Element	Name	Benefits compared to 3 <sup>rd</sup> Generation System
<b>ELEMENTS ADDED</b>			
Packaging Vector #1		Codon Wobble	<ul style="list-style-type: none"> <li>Increased safety: reduces sequence homology</li> </ul>
		Adenovirus VA	<ul style="list-style-type: none"> <li>Increased viral titer</li> </ul>

**Safety Considerations**

Remember that you will be working with samples containing infectious virus. Follow the recommended NIH guidelines for all materials containing BSL-2 organisms. The ViraSafe™ Universal Lentiviral Expression System is designed to minimize the chance of generating replication-competent lentivirus, but precautions should still be taken to avoid direct contact with viral supernatants.

## pLenti-RFP-PuroVector



**Figure 1:** pLenti-RFP-Puro Lentiviral Control Vector

### Lentivirus Production

1. One day before transfection, plate sufficient 293T cells or 293LTV cells (cat.# LTV-100) to achieve 70-80% confluence on the day of transfection.
2. Transfect cells by Calcium Phosphate or other transfection reagents.

*Note: We suggest transfecting cells with FuGENE® Transfection Reagent (Roche Applied Science) or Lipofectamine™ Plus (Invitrogen). We recommend the ratio of vectors at 3:1:1:1 (pLenti: pCMV-VSV-G:pRSV-REV:pCgpV).*

3. Harvest lentiviral supernatant 36-72 hours after transfection. Supernatant can be harvested 2 or 3 times, every 12 hours. Keep it at 4°C over the collecting period.
4. Pool the collected supernatants, centrifuge 5 minutes at 1500 rpm to remove cell debris and filtrate on 0.22 µm.
5. Supernatants can be used directly or purified/concentrated if needed. For long term storage, store supernatant at -80°C in aliquots.

### Post-Packaging Considerations

Packaging your lentivirus is only the first step to ensuring successful expression of your gene. The following steps should be considered prior to infection of your host cell:

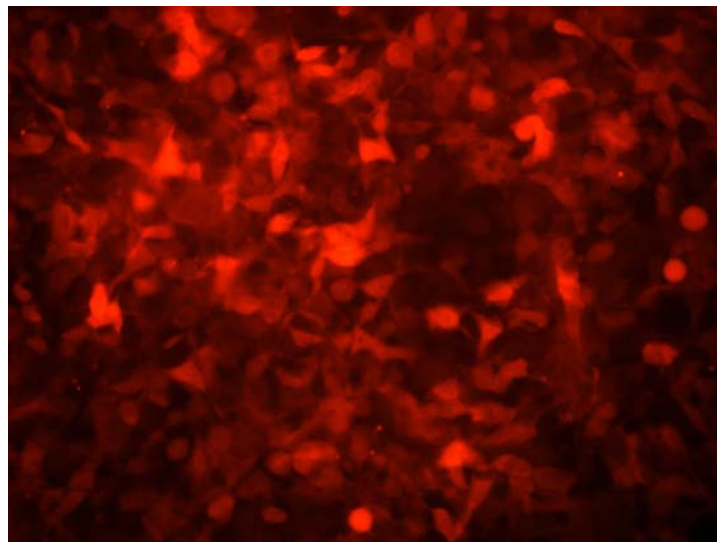
1. **Concentration and purification of your lentivirus:** Because of the latent nature of lentivirus, it is imperative that your virus be highly concentrated before infecting your host cell. Also, impurities from your viral supernatant can decrease the efficiency of infection. We recommend

using Cell Biolabs' ViraBind™ Lentivirus Concentration and Purification Kit (Catalog # VPK-090).

2. **Measure the titer of your lentivirus:** This is an important step to ensure consistent viral transduction into your host cell. However, QPCR or stable clone counting can take as much as 1-2 weeks to perform. Traditional p24 ELISA kits can greatly overestimate your lentiviral titer. Our advanced p24 ELISA, QuickTiter™ Lentivirus Titer Kit (Catalog # VPK-107), uses exclusive technology that eliminates free p24 from your supernatant, giving you much more accurate lentiviral titers. Results are obtained in 6-18 hours.
3. **Use transduction reagents to increase infection efficiency:** Many cells are difficult to infect with lentivirus, and without supplemental reagents transduction efficiencies can be low. Reagents such as Polybrene® can help, but are often insufficient. Cell Biolabs' proprietary reagents in our ViraDuctin™ Lentivirus Transduction Kit (Catalog # LTV-200) form a super-complex with your virus to increase transduction efficiencies by promoting virus and cell interaction.

### **Example of Results**

The following figure demonstrates typical results seen with Cell Biolabs pLenti-RFP-Puro lentiviral control vector. One should use the data below for reference only.



**Figure 2: RFP Expression in 293 cells:** Lentiviral supernatant is produced by cotransfecting 293T cells (Cat.# LTV-100) with pLenti-RFP-Puro and ViraSafe™ Lentiviral Packaging System (Cat. # VPK-206). 293AD (Cat.# AD-100) cells are seeded at 100,000 cells/well in a 6-well plate overnight. Cells were infected with GFP lentivirus in the presence of 8 µg/mL Polybrene for 72 hrs.

### **References**

1. Chen, M. et al. (2002). *Nature Genetics* **32(4)**: 670-675.
2. Naldini, L., U. Blomer, P. Gally, D. Ory, R. Mulligan, F. H. Gage, I. M. Verma, and D. Trono (1996) *Science* **272**:263-267.

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4. Kahl C. A., Marsh J., Fyffe J., Sanders D. A., and K. Cornetta (2004) *J Virol.* **78**:1421-30.
5. White S. M., Renda M., Nam N. Y., Klimatcheva E., Zhu Y., Fisk J., Halterman M., Rimel B. J., Federoff H., Pandya S., Rosenblatt J. D., and V. Planelles (1999) *J Virol.* **73**:2832-40.
6. Kafri T., van Praag H., Ouyang L., Gage F. H., and I. M. Verma (1999) *J Virol.* **73**:576-84.

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