

## pLenti-p53 (human) shRNA Lentiviral Vector

CATALOG NUMBER: LTV-451

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*.

The p53 is a tumor suppressor; mutations in p53 are found in most tumor types. p53 tumor suppressor is normally found at low levels, but when DNA damage is sensed, p53 levels rise and initiate protective measures. p53 binds to many regulatory sites in the genome and begins production of proteins that halt cell division until the damage is repaired. If the damage is too severe, p53 initiates the process of programmed cell death, or apoptosis, which directs the cell to commit suicide, permanently removing the damage. Besides tumor suppressor, p53 forms a barrier to the generation of induced pluripotent stem cells (iPS), and knockdown of p53 by siRNA can significantly improve the reprogramming efficiency during iPS generation.

The lentiviral vector contains an shRNA to human p53 and puromycin resistance gene (Figure 1).

Lentiviral supernatant can be produced by cotransfecting 293T cells (Cat.# LTV-100) with pLenti-p53 shRNA 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.

### **Related Products**

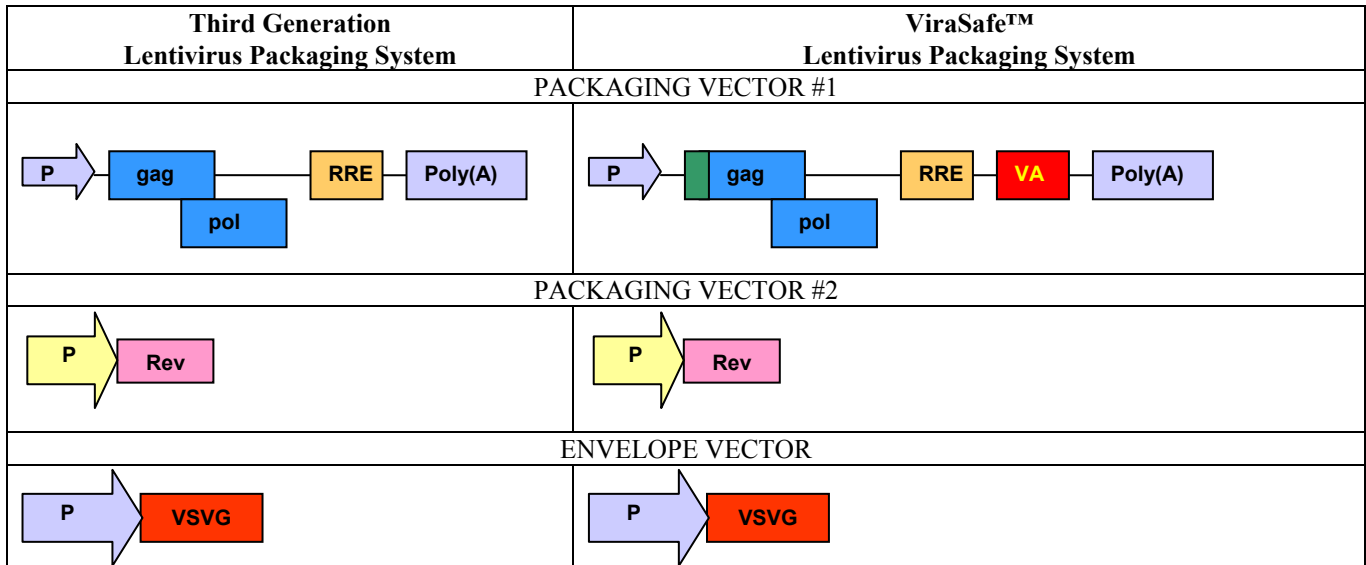
1. VPK-205: ViraSafe™ Lentiviral Packaging System, Ecotropic
2. VPK-206: ViraSafe™ Lentiviral Packaging System, Pantropic
3. VPK-107: QuickTiter™ Lentivirus Titer Kit (Lentivirus-Associated HIV p24)
4. VPK-090: ViraBind™ Lentivirus Concentration and Purification Kit
5. LTV-200: ViraDuctin™ Lentivirus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

### **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

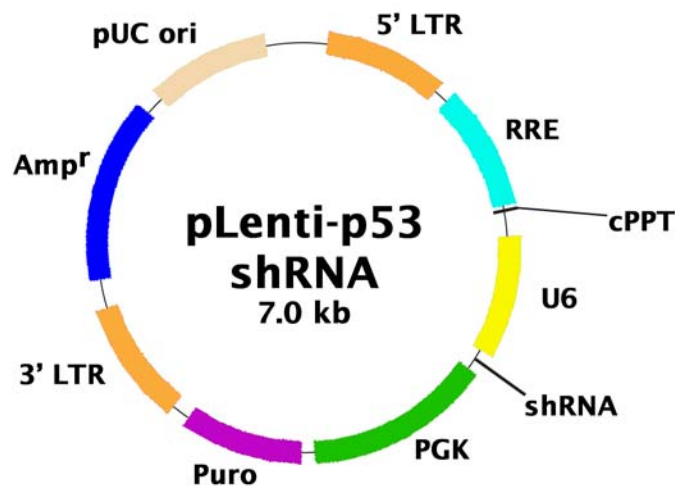
Expression System is designed to minimize the chance of generating replication-competent lentivirus, but precautions should still be taken to avoid direct contact with viral supernatants.

**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>

**pLenti-p53 shRNA Vector**



**Figure 1.** Schematic representation of human p53 shRNA Lentiviral Vector.

**Human p53 shRNA Hairpin Structure:**

GACTCCAGTGGTAATCTACTTCAAGAGAGTAGATTACACTGGAGTCTTTTT  
(Human p53 sense) (Hairpin) (Human p53 antisense)

**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-shRNA: 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.

## **References**

1. Chen, M. et al. (2002). *Nature Genetics* **32**(4): 670-675.
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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.
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7. Hong, H. et al. (2009) *Nature* **460**, 1132-1135.

## **Warranty**

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***This product is for RESEARCH USE ONLY; not for use in diagnostic procedures.***

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