



Product Manual

ViraSafe™ Lentiviral Packaging System, Pantropic

Catalog Number

VPK-206

1 kit

**FOR RESEARCH USE ONLY
Not for use in diagnostic procedures**

Introduction

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 (Figure 1). 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.

Cell Biolabs' ViraSafe™ Lentiviral Packaging System provides a much safer method to package lentivirus, while still providing high viral titers. In addition, each plasmid is provided separately rather than in a packaging mixture. This allows you the flexibility to amplify individual plasmids and optimize the ratio of plasmids for your experiment.

Key Features of ViraSafe™ Lentiviral Packaging System

1. Packaging Plasmids: Improve the packaging plasmid to increase performance and reduce the likelihood of recombination between vector components.
 - a. Minimize HIV sequences – no accessory proteins, Tat or Rev, or LTRs
 - b. Prevent overlap with vector SM by codon wobbling Gag sequences
 - c. Boost particle production by incorporating adenovirus VA₁ element
2. Flexible: All vectors including packaging vectors are provided separately to allow end-user to optimize the vector ratio for maximal lentivirus production.

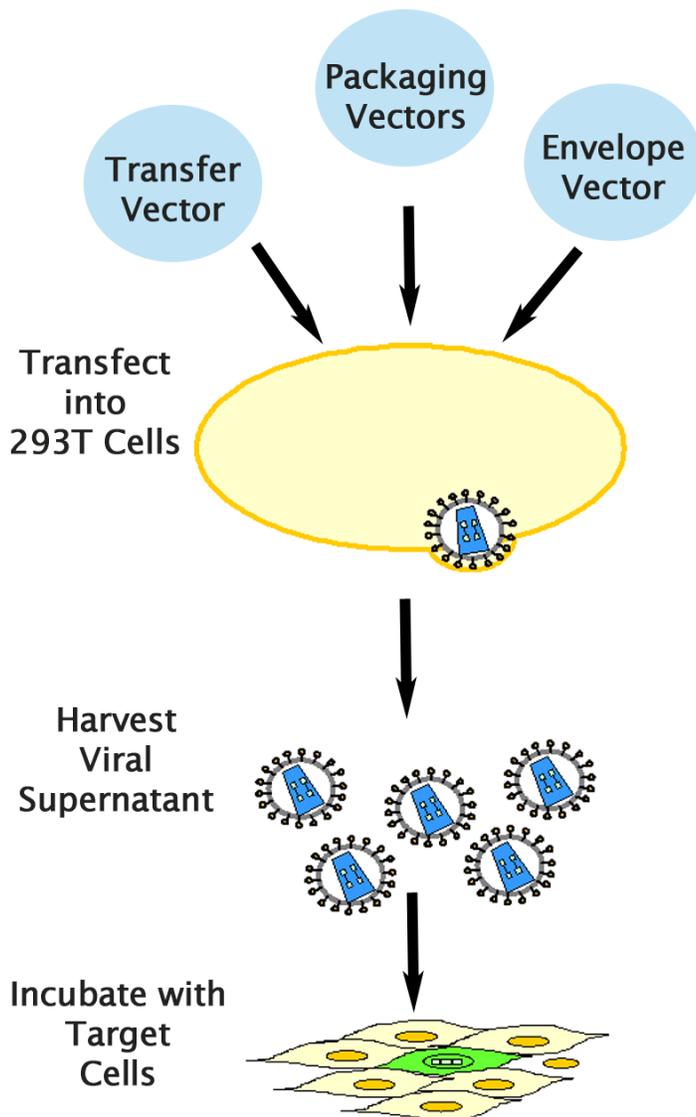


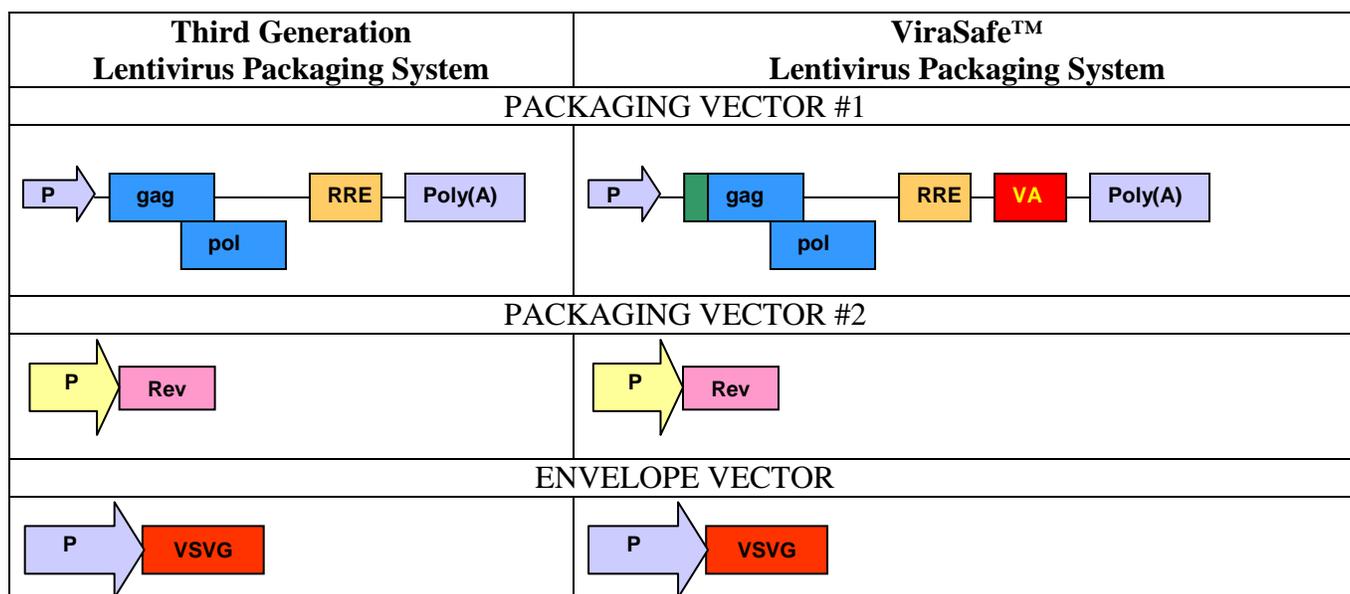
Figure 1. Lentivirus Production in 293T Cells

Related Products

1. LTV-100: 293LTV Cell Line
2. LTV-200: ViraDuctin™ Lentivirus Transduction Kit
3. LTV-300: GFP Lentivirus Control
4. VPK-090: ViraBind™ Lentivirus Concentration and Purification Kit
5. VPK-104: ViraBind™ Lentivirus Purification Kit
6. VPK-107: QuickTiter™ Lentivirus Titer Kit (Lentivirus-Associated HIV p24)
7. VPK-108-F: QuickTiter™ Lentivirus Quantitation Kit (FIV p24 ELISA)

8. VPK-108-H: QuickTiter™ Lentivirus Quantitation Kit (HIV p24 ELISA)
9. VPK-200: ViraSafe™ Universal Lentivirus Expression System
10. VPK-205: ViraSafe™ Lentivirus Packaging System, Ecotropic
11. VPK-211: pSMPUW Universal Lentiviral Expression Vector (Promoterless)
12. VPK-220: pSMPUW miR-GFP/Puro Lentiviral Expression Vector

Unique Elements of the ViraSafe™ Lentivirus Packaging System



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

Kit Components

1. pRSV-Rev Packaging Vector (Part No. 320022): One 40 µL vial at 0.25 mg/mL.
2. pCMV-VSV-G Envelope Vector (Part No. 320023): One 40 µL vial at 0.25 mg/mL.
3. pCgpV Packaging Vector (Part No. 320024): One 40 µL vial at 0.25 mg/mL.

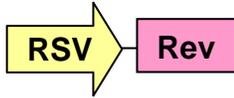


Figure 2: pRSV-Rev Packaging Vector (4180 bp, **Ampicillin**-resistant). EcoRI Digestion: 300 bp + 3880 bp

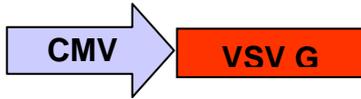


Figure 3: pCMV-VSV-G Envelop Vector (6051 bp, **Ampicillin**-resistant). EcoRI Digestion: 787 bp + 1668 bp + 3596 bp.

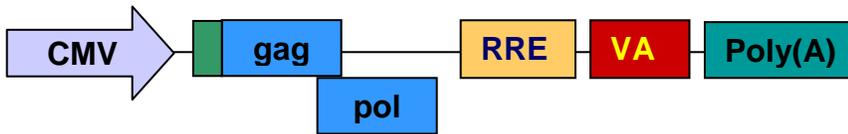


Figure 4: pCgpV Packaging Vector (9118 bp, **Ampicillin**-resistant). Pst I Digestion: 927 bp + 1424 bp + 6767 bp.

Materials Not Supplied

1. Lentiviral Transfer Vector
2. 293T cells: we recommend 293LTV Cell Line (Cat. # LTV-100) for high titer production of lentivirus.
3. Cell Culture Medium
4. Transfection Reagents

Storage

Upon receipt, store all other kit components at -20°C until their expiration dates.

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.

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 (transfer vector: 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

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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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Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com

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Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium