Product Manual

ViraSafe™ shRNA Lentiviral Expression System (GFP), Ecotropic

Catalog Number

VPK-222-ECO **1 kit**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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Creating Solutions for Life Science Research

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. Also, most commercial lentivirus transfer vectors contain promoters, antibiotic selection markers and/or reporter genes which may not be optimal or even suitable for your particular expression studies.

Cell Biolabs' ViraSafe™ Lentiviral Expression System provides a much safer method to package lentivirus, while still providing high viral titers. The sequence homology with native HIV-1 has been reduced by 80-90% even compared with other commercial third-generation packaging systems. 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.

pSMPUW-U6-GFP Lentiviral Expression Vector contains MCS for cloning of your shRNA sequence of interest after the mU6 promoter (Figure 2).

Key Features of the ViraSafe™ Lentiviral Expression System:

1. Transfer Plasmid: Reduce extent of HIV sequences to increase capability up to 10 kb and reduce likelihood of recombination between vector components. Add elements to increase titer and further improve safety.
2. Packaging Plasmid: 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 VA_I element
3. Flexible: All vectors including packaging vectors are provided separately to allow end-user to optimize the vector ratio for maximal lentivirus production.

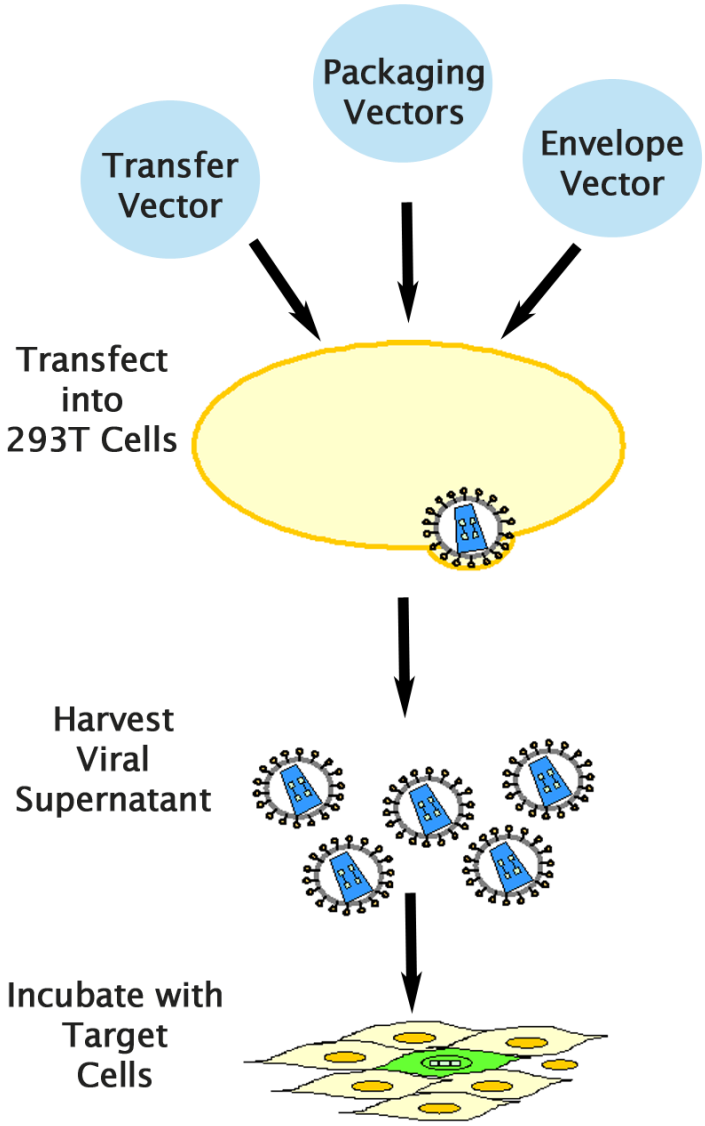
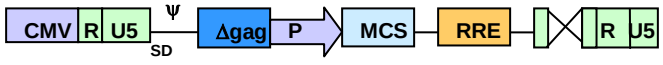
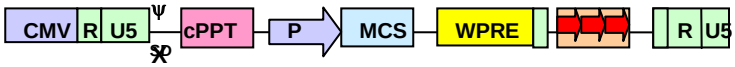
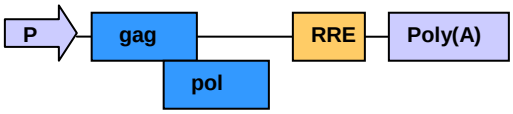
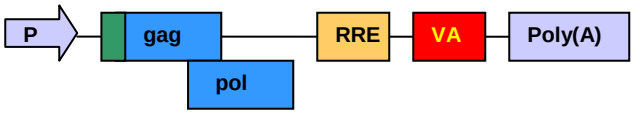
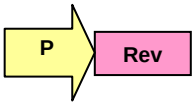
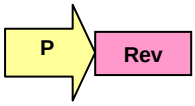
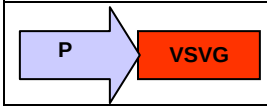
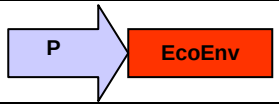


Figure 1. Lentivirus Production in 293T Cells

Related Products

1. LTV-100: 293LTV Cell Line
2. LTV-200: ViraDuctin™ Lentivirus Transduction Kit
3. LTV-300: GFP Lentivirus Control
4. VPK-104: ViraBind™ Lentivirus Purification Kit
5. VPK-107: QuickTiter™ Lentivirus Titer Kit (Lentivirus-Associated HIV p24)
6. VPK-108-H: QuickTiter™ Lentivirus Quantitation Kit (HIV p24 ELISA)
7. VPK-205: ViraSafe™ Lentivirus Packaging System, Ecotropic
8. VPK-211: pSMPUW Universal Lentiviral Expression Vector (Promoterless)

Unique Elements of the ViraSafe™ Lentivirus Expression System

Third Generation Lentivirus Expression System	ViraSafe™ Lentivirus Expression System
TRANSFER VECTOR	
	
PACKAGING VECTOR #1	
	
PACKAGING VECTOR #2	
	
ENVELOPE VECTOR	
	

Vector Name	Element	Name	Benefits compared to other 3rd Generation Systems
ELEMENTS ADDED			
Transfer Vector	cPPT	Central Polypurine Tract	• Increased gene expression levels
		Hybrid 3' LTR Poly(A)	• Increased safety: prevents read-through transcription • Increased viral titer: vector transcript more stable in packaging cells
	WPRE	WPRE	• Increased viral titer
Packaging Vector #1		Codon Wobble	• Increased safety: reduces sequence homology
	VA	Adenovirus VA	• Increased viral titer
ELEMENTS REMOVED			
Transfer Vector	Δgag	Gag sequence	• Increased safety: reduces sequence homology
	RRE	Rev-Responsive Element	• Increased safety: reduces sequence homology

Kit Components

1. pSMPUW-U6-GFP Lentiviral Expression Vector (Part No. VPK-222): One 40 µL vial at 0.25 mg/mL. The plasmid is kanamycin resistant.
   Note: Please see Figure 2 for important instructions on bacterial culture of this plasmid.
2. pRSV-Rev Packaging Vector (Part No. 320022): One 40 µL vial at 0.25 mg/mL.
3. pCMV-Eco Envelope Vector (Part No. 320026): One 40 µL vial at 0.25 mg/mL.
4. pCgpV Packaging Vector (Part No. 320024): One 40 µL vial at 0.25 mg/mL.
5. pSMPUW-LacZ Control Vector (Part No. 320025): One 40 µL vial at 0.25 mg/mL containing a nuclear localized LacZ driven by MND retroviral LTR promoter. The plasmid is kanamycin resistant.
   Note: Please see Figure 2 for important instructions on bacterial culture of this plasmid.

Materials Not Supplied

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

Storage

Upon receipt, store all other kit components at -20ºC 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™ 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.

pSMPUW-U6-GFP Vector

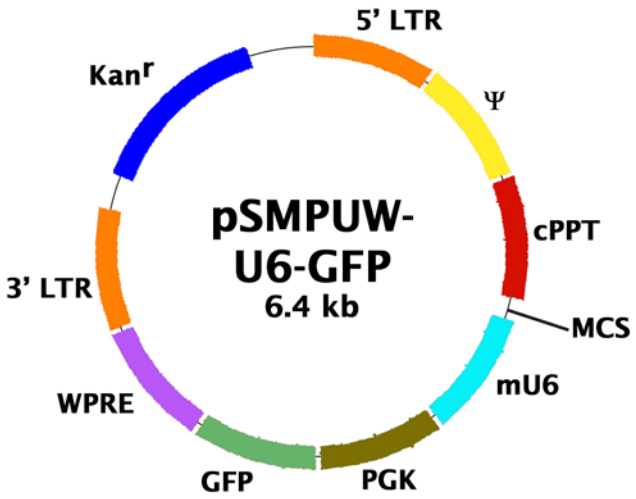


Figure 2: pSMPUW-U6-GFP Lentiviral Expression Vector (6413 bp, **Kanamycin**-resistant).

*Note: Bacterial culture of pSMPUW vectors should be done in medium containing **10 µg/mL** Kanamycin. For maximal plasmid yield and quality, we recommend Stbl3 endoA1+ competent cells (Invitrogen) and treatment with alkaline proteinase (Promega #A1441 or Sigma #P8038) for 4-5 min using 10 units of proteinase per mL of bacterial lysate before adding neutralization solution.*

MCS:

- Enzyme Sites: 5'- BamHI, EcoRI, SalI -3'
- MCS Sequence: AGCCGGATCCAACGAATTCAACGTCGACTTTT

Designing Oligonucleotides:
It is necessary to synthesize two complementary oligonucleotides (a top strand and a bottom strand) for each shRNA target site (Figure 2). The key features of the oligonucleotides include:

- A 5'-BamH I restriction site overhang on the top strand and a 5'-EcoR I restriction site overhang on the bottom strand. These restriction sites will enable directional cloning of the annealed oligonucleotides into the pSMPUW-U6-Puro vector.
- A guanine (G) residue added upstream of the 5'-end of the shRNA sense strand as Pol III transcription start site, if the target sequence does not start with a purine.
- A 19-base oligonucleotide sense sequence (target sense sequence) of the shRNA target site.
- A 9-nucleotide hairpin loop sequence (5'-TTCAAGAGA- 3').
- The 19-base oligonucleotide antisense sequence (target antisense sequence) of the shRNA target site.
- A RNA Pol III terminator sequence consisting of a 6-nucleotide poly(T) tract.

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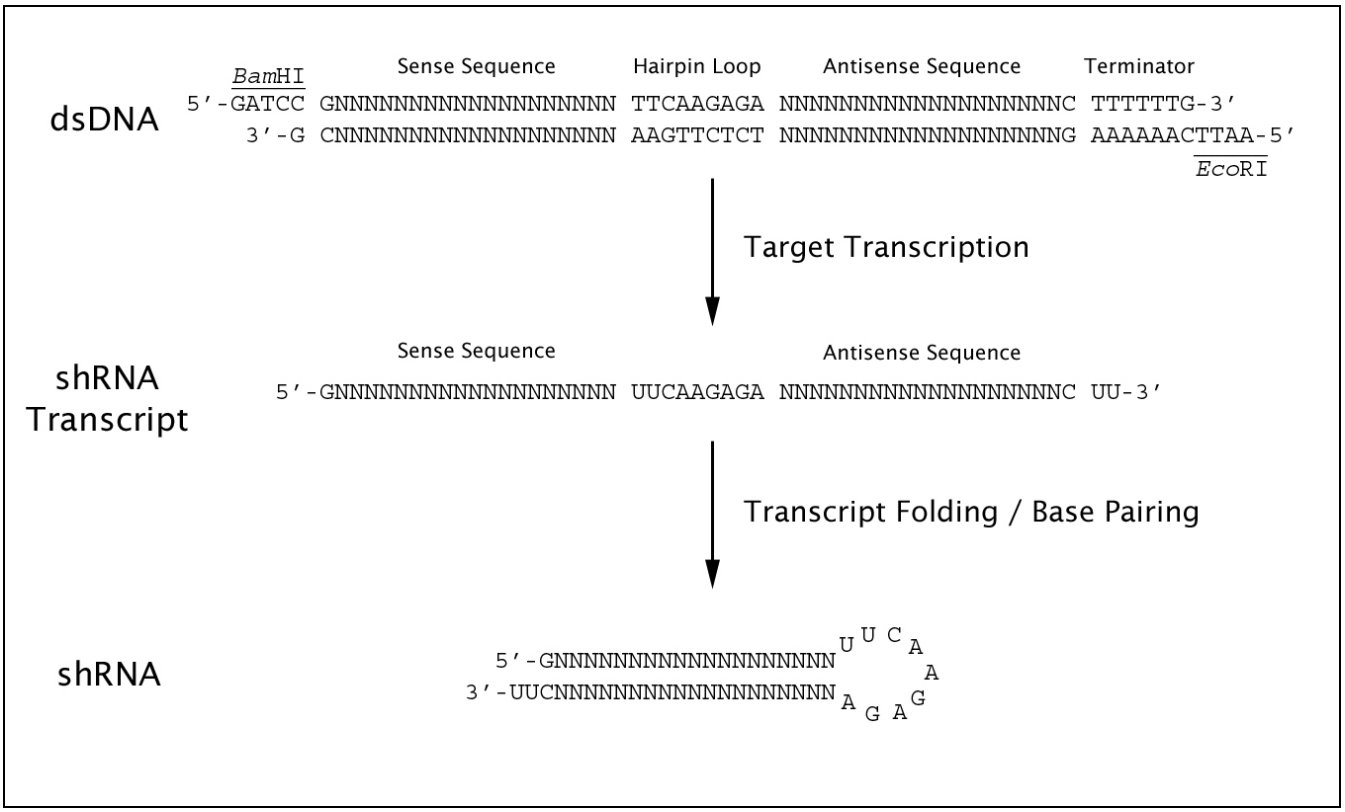


Figure 3. shRNA Features.

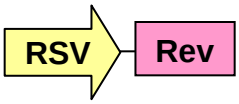


Figure 4: pRSV-Rev Packaging Vector (4180 bp, **Ampicillin**-resistant). EcoRI Digestion: 300 bp + 3880 bp

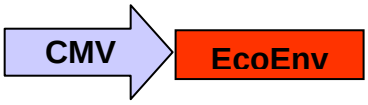


Figure 5: pCMV-Eco Envelop Vector (6763 bp, **Ampicillin**-resistant). BamHI Digestion: 777 bp + 5986 bp.

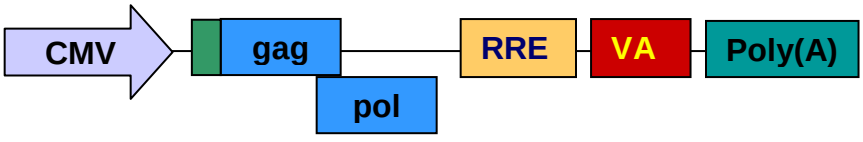


Figure 6: pCgpV Packaging Vector (9118 bp, **Ampicillin**-resistant). Pst I Digestion: 927 bp + 1424 bp + 6767 bp.

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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 (pSMPUW: pCMV-Eco: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 4ºC 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 -80ºC 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.

Appendix

pSMPUW-U6-GFP Plasmid Sequence

5' CMV/LTR, ψ, cPPT: 1-1070
MCS: 1246-1273
mU6 promoter: 1274-1582
pPGK: 1593-2101
EGFP: 2125-2844
WPRE: 2887-3475
3' LTR: 3467-3845
Kanamycin Resistance gene: 5463-6278

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