

pSMPUW-Neo Lentiviral Expression Vector

CATALOG NUMBER: VPK-213

STORAGE: -20°C

QUANTITY AND CONCENTRATION: 10 µg at 0.25 µg/µL in TE

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*.

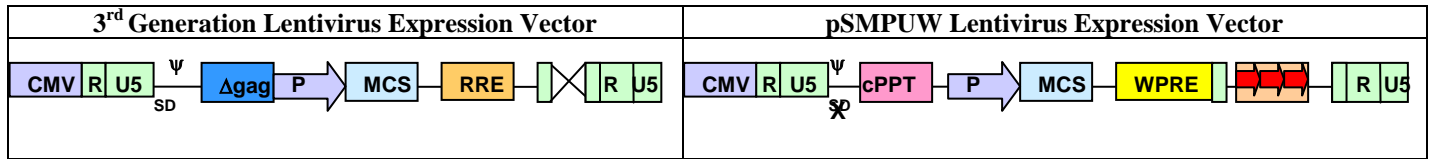
Lentivirus particles are produced from 293T cells through transient transfection of plasmids that encode for the components of the virion. Due to safety concerns regarding the infectious nature of HIV-1, recent lentiviral packaging systems have separated the viral components into 3 or 4 plasmids. However, 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.

pSMPUW-Neo Lentiviral Expression Vector contains EF-1α promoter ahead of the multiple cloning sites, followed by PGK promoter and neomycin resistant gene (Figure 1).

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 pSMPUW Universal Lentivirus Expression Vector

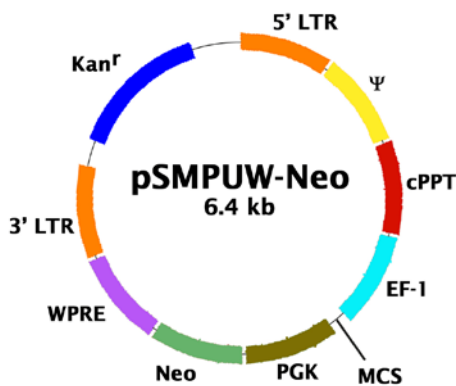


Element	Name	Benefits compared to other 3 rd Generation Systems
ELEMENTS ADDED		
	Central Polypurine Tract	<ul style="list-style-type: none"> Increased gene expression levels
	Hybrid 3' LTR Poly(A)	<ul style="list-style-type: none"> Increased safety: prevents read-through transcription Increased viral titer: vector transcript more stable in packaging cells
	WPRE	<ul style="list-style-type: none"> Increased viral titer
ELEMENTS DELETED		
	Gag sequence	<ul style="list-style-type: none"> Increased safety: reduces sequence homology
	Rev-Responsive Element	<ul style="list-style-type: none"> Increased safety: reduces sequence homology

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.

pSMPUW-Neo Vector



MCS: AGTCGCCGTGAACGTTTCGGCCGGCCAGATATCTCCCTTCGGACCAAGGGTCATTAATTAAGTACCGGGTAGGGGA
 FseI EcoRV AhdI PacI

Figure 1: pSMPUW-Neo Lentiviral Expression Vector (6352 bp, **Kanamycin**-resistant). Hind III Digestion: 1331 bp + 1982 bp + 3039 bp.

Note: Bacterial culture of pSMPUW vectors should be done in medium containing 10 µg/mL Kanamycin. For maximal plasmid yield and quality, we recommend Stb13 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.

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-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.

pSMPUW-Neo Plasmid Sequence

Pink: 5' CMV/LTR, ψ , cPPT

Blue: EF-1

Purple: MCS

Green: PGK

Red: Neo

Brown: WPRE

Orange: 3' LTR

Blue: Kanamycin Resistance gene

ACTAGTCGGGGTCATTAGTTCATAGCCCATATATGGAGTTCGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCATTGAC
GTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTCCATTGACGTCAATGGTGGAGTATTTACGGTAACTGCCCACTTGGCAGTACATCAAGTG
TATCATATGCCAAGTACGCCCTTATTGACGTCAATGACGGTAAATGGCCCGCTGGCATATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACA
TCTACGTATTAGTTCATCGCTATTACCATGGTGTATGCGGTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTGACTCACGGGATTTCCAAGTCTCCACCCCAT
TGACGTCAATGGGAGTTTGGTTTGGCACCAAAATCAACGGGACTTTCAAAATGTCGTAACAACCTCCGCCCATGACGCAAAATGGGCGGTAGGCGTGTACGGTGG
GAGGCTATATAAGCAGAGCTGGTTAGTGAACCGGCTCTCTGGTTAGACCAGATTGAGCCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCT
CAATAAGCTTGCCTTGGTGTCTCAAGTAGTGTGTGCCGTCTGTTGTGTGACTCTGGTAACTAGAGATCCCTCAGACCTTTTGTAGTCAAGTGTGAAAAATCTCTA
GCAGTGGCGCCGCAACAGGACCTGAAAGCGAAAGGGAACCCAGAGGAGCTCTCTCGACGAGGACTCGGCTTGTGAAGCGCGCACGGCAAGAGGCGAGGGGCGG
CGACTGCAGAGTACGCCAAAATTTGACTAGCGGAGGCTAGAAGGAGAGAGATGGGTGCGAGAGCGTCAATTAAGCGGGGAAAATAGCGGCCCAAAATTTT
AAAAGAAAAGGGGGATTTGGGGGTACAGTGCAGGGGAAGAATAGTAGACATAATGCAACAGACATACAAAATAAAGAATTACAAAAACAAATTCAAAAATTC
AAATTTTCGGGGATCCGCTCCCCGTACCACCCCCCAACCCGCCCCGACCGGAGCTGAGAGTAATTCATACAAAAGGACTCGCCCTGCCCTGGGGAAATCCC
AGGGACCGTCTTAACTCCCACTAACGTAGAACCAGAGATCGTTCGCTCCCGCCCTCACCAGCCCGCTCTCGTCACTACTGAGGTGGAGAAGAGCATGCGT
GAGGCTCCGGTCCCGTCACTGGGCGAGCGCACATCGCCACAGTCCCGAGAAAGTGGGGGAGGGGTGCGCAATTGAACCGGTGCCAGAGAAGGTGGCGCGG
GGTAACTGGGAAAGTGTCTGTACTGGCTCCGCTTTTCCCGAGGGTGGGGAGAACCCTATATAAGTGCAGTAGTCCCGTGAACGTTCCGCGGCCAGAA
TATCTCCCTTCGGACCAAGGTCATTAATTAAGTACCGGGTAGGGGAGCGCTTTTCCAAAGGCAGTCTGGAGCATGCGCTTTAGCAGCCCGCTGGGCACTTGGC
GCTACACAAGTGGCTCTGGCTCGCACACATCCACATCCACCGGTAGGCGCAACCGGCTCCGTTCTTTGGTGGCCCTTCGCGCCACCTTCTACTCTCCCT
AGTCAGGAAGTTCGCGCCGCGCCGACGCTCGCGTGTGAGGAGCTGACAAATGGAAGTAGCAGTCTCACTAGTCTCGTGCAGATGGACAGCACCGCTGAGCAA
TGGAAAGCGGTAGGCTTTGGGGCAGCGGCAATAGCAGCTTTGCTCTTCGCTTTTGGGCTCAGGGGCGGGCGCGCCGAAGTCTCCGAGGCGCCGGCA
TCTGCACGCTTCAAAGCGCAGCTGTCGCGCTGTTCTCTCTCTCCTCATCTCCGGGCTTTGCACTTAGACACGTTGACAATTAATGTACACACCATGGCC
ACAACCATGGTTATTGAACAAGATGGATTGCACGAGGTTCTCCGCGCTTGGGTGGAGAGGCTATTCCGCTATGACTGGGCACAACAGACAATCGGCTGCTCTG
ATGCCGCGTGTCCGGCTGTGAGCGAGGGGCGCCGCTCTTTTGTCAAGACCGACTGTCCGCTGCCCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATC
GTGGTGGCCACGAGCGGGCTTCTTGGCGAGCTGTCTCGACGTTGTCACTGAAGCGGGAAGGACTGGCTGCTATTGGGCGAAGTGCAGGGGAGGATCTCCCTG
TCATCTCACCTTGTCTGCGGAGAAATATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTGATCCGGCTACTGCCCATTCGACCCCAAGCGAAAC
ATCGATCGAGCGAGCAGTACTCGGATGGAAGCCGCTTGTGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCGAAGTCTCGCCAGGCT
CAAGGCGCGCATGCCGACGCGGAGGATCTCGTGTGACCCATGGCGATGCTGCTTGGCAATATCATGGTGGAAAATGGCCGCTTTCTGATTATCGACTGT
GGCCGGCTGGGTGTGGCGGACCGTATCAGGACATAGCGTTGGCTACCGTGATATTGCTGAAGAGCTTGGCGGCAATGGGCTGACCGTCTCTCGTGTATTAG
GTATCGCCGCTCCCGATTCCGAGCGCATCGCTTCTATCGCTTCTTGAAGTTCGACAATCAACCTCTGGATTACAAAATTTGTGAAAGATTGAC
TGGTATTCTAACTATGTTGCTCTTTTACGCTATGTGGATACGCTGCTTTAATGCTTTGTATCATGCTATTGCTTCCGATGGCTTTCAATTTCTCTCTCTG
TATAAATCCTGGTTGCTGTCTTTATGAGGAGTTGTGGCCGTTGTGAGCAACGTTGGGCTGGTGTGCACTGTTTGTGACGCAACCCCACTGGTGGGGCA
TTGCCACCACCTGTGAGCTCTTTCCGGGACTTTGCTTTCCCTTCTTATGCAACGCGGAACTCATCGCCGCTGCTTGGCGCTGTGGACAGGGGCTCG
GCTGTTGGGCACTGACAATTCGTTGGTGTGTGCGGGAAATCATGTCCTTCTTGGTGTGCTGCGCTGTTGGCACCTGGATTCTGCGCGGGAGCTCTTCTGC
TACGTCCTTCGGCCCTCAATCCAGCGGACCTTCTTCCGCGGCTGTGCGGCTCTGCGGCTCTTCCGCTTTCGCTTCCGCTCAGACGAGTCCGATCT
CCCTTGGGCGCCCTCCCGCTTAGTACTGGTACCTTTAAGACCAATGACTTACAAGGCACTGTAGATCTTAGCCACTTTTAAAAGAAAAGGGGGACTGGAAG
GGCTAATTCCTCCCAACGAAGACAAGATCCCGAATTTATTTGTGAAATTTGTGATGCTATTGGCTTTATTTGTAAACCGGTGCACTGCTTTTTCCTGTACTGG
GTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAAGCTCAATAAAGCTTGCCTTGGTGTCTCAAGTAGTGTGT
GCCGCTGCTGTGTGACTCTGGTAACTAGAGATCCCTCAGACCTTTTATGTCAGTGTGGAAAATCTCTAGCATCTAGAGTATGCAAAGCATGCATCTCAATTAGT
CAGCAACAGGTGTGAAAGTCCCAAGGCTCCCAAGCAGGAGAGTATGCAAAGCATGCATCTCAATTAGTCAAGCAACCATAGTCCCGCCCTAACTCCGCCAT
CCCGCCCTAACTCCGCCAGTTCGCGCCATTCTCCGCCATGGCTGACTAATTTTTTTTATTTATGCAAGGCGGAGCCGCTCGGCTCTGAGCTATTCCAG
AAGTAGTGAGGAGGCTTTTTTGGAGGCTTAGGCTAGAGATCATAATCAGCCATACCACATTTGTAGAGGTTTTACTTGTCTTAAAAAACCTCCCAACCTCCCT
GAACCTGAAACATAAAATGAATGCAATTTGTTGTTTAACTGTTTATTGCACTTATAATGGTTACAAATAAAGCAATAGCATCACAATTTCAAAAATAAAGCA
TTTTTTTCACTGCATCTAGTGTGGTTGTCAAACCTCATCAATGTATCTTATCATGCTGCTAGCCGGCTTTTTTTTCTTAGGCTTCTTCCGCTTCTCGCT
CACTGACTCGCTGCGCTCGTCTGCTTCCGCTGCGGCGAGCGGATCAGCTCACTCAAAGGCGGTAATACGGTATCCACAGAATCAGGGGATAACGAGGAAAGAAC
ATGTGAGCAAAAAGGCGAGCAAAAAGGCGAGGAAACCGTAAAAAGGCGGCTTGTGGGCTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAATAATCGACGCT
CAAGTCAAGGTTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCTGGAAGCTCCCTCGTGGCTCTCTGTTCCGACCTGCGGCTTACCGGATA
CCTGTCCGCTTTCTCCCTTCCGGAAGCGTGGCGTTTCTCATAGCTCAGCTGTAGGTATCTCAGTTCCGTTGAGGTCGTTCTGCTCAGGCTGGGCTGTGTGCAC
GAACCCCGCTTCCAGCCGACCGCTGCGCCTTATCCGTAATCTCGTCTTGAAGTCAACCCCGTAAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACA
GGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTGAAGTGGTGGCTAACTACGGTCACTAGAAGAAGCAGTATTTGGTATCTGCGCTCTGTGAA
GCCAGTTACCTTCGAAAAAAGAGTTGGTAGCTTTGATCCGGCAAAACAAACCCAGCTGGTAGCGGTTTGTGTTTGAAGCAGCAGATTACGCGCAGAAAA
AAAGGATCTCAAGAAGATCTTTGATCTTTTCTACGGGCTGACGCTCAGTGGAAACGAAACTCAGTTAAGGATTTTGGTCAAGATTATCAAAAAGGATCT
TCACCTAGATCTTTTAAATTAATAAAGTAAATCAATCTAAAGTATATAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGGGACCTAT
CTCAGCGATCTGTCTATTTCTGTTTCAATAGTTGCTGACTCTGCGCAGTCCAAAAAAAAGGCTCCAAAAGGAGCCTTAATTTGATCGTGGGCCCTTAGAAA
AAACTCATCGAGCATCAAAATGAACTGCAATTTATTCATATCAGGATTATCAATACCATATTTTGAAGGAGCGGTTCTGTAATGAAGGAGAAACTCACCGAGG

CAGTTCCATAGGATGGCAAGATCCTGGTATCGGTCTGCGATTCCGACTCGTCCAACATCAATACAACCTATTAATTTCCCTCGTCAAAAATAAGGTTATCAAGTG
AGAAATCACCATGAGTGACGACTGAATCCGGTGAGAATGGCAAAAGCTTATGCATTTCTTTCCAGACTTGTTCACAGGCCAGCCATTACGCTCGTCATCAAAATC
ACTCGCATCAACCAAACCGTTATTCATTTCGTGATTGCGCCTGAGCGAGACGAAATACGCGATCGTGTAAAAGGACAATTACAAACAGGAATCGAATGCAACCCG
CGCAGGAACACTGCCAGCGCATCAACAATATTTTACCTGAATCAGGATATTTCTTAATACCTGGAATGCTGTTTTCCCGGGGATCGCAGTGGTGAGTAACCATG
CATCATCAGGAGTACGGATAAAAATGCTTGTATGGTTCGGAAGAGGCATAAATTCCTCAGCCAGTTTAGTCTGACCATCTCATCTGTAACATCATTGGCAACGCTACC
TTTTGCCATGTTTTAGAAAACAACCTCTGGCGCATCGGGCTTCCCATACAATCGATAGATTGTGCGACCTGATTGCCCGACATTATCGCGAGCCCATTTATACCCATAT
AAATCAGCATCCATGTTGGAATTTAATCGCGGCCCTCGAGCAAGACGTTTTCCCGTTGAATATGGCTCAATAACACCCTTGTATTACTGTTTATGTAAGCAGACAGTT
TTATTGTTTCATGATGATATATTTTTATCTTGTGCAATGTAACATCAGAGATTTTGAGACACAACGTTTAAACAAATAGTCAAAGCCTCCGGCG

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.
3. Verma, I. M., and N. Somia (1997) *Nature* **389**:239-242
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.

Notice to Purchaser

This product is sold for research and development purposes only and is not to be incorporated into products for resale without written permission from Cell Biolabs. The patented technology is covered by a license from CHLA and University of Southern California. By the use of this product you accept the terms and conditions of all applicable Limited Use Label Licenses. You may contact our Business Development department at busdev@cellbiolabs.com for information on sublicensing this technology.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

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

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

©2009-2011: Cell Biolabs, Inc. - All rights reserved. No part of these works may be reproduced in any form without permissions in writing.

