

pSMPUW-Hygro Lentiviral Expression Vector

CATALOG NUMBER: VPK-214

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*.

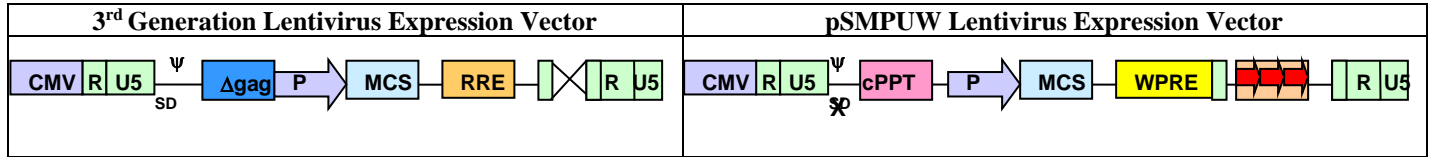
Lentivirus particles are produced from 293T cells through transient transfection of plasmids that encode for the components of the virion. Due to safety concerns regarding the infectious nature of HIV-1, recent lentiviral packaging systems have separated the viral components into 3 or 4 plasmids. However, 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-Hygro Lentiviral Expression Vector contains EF-1α promoter ahead of the multiple cloning sites, followed by PGK promoter and hygromycin 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. LTV-200: ViraDuctin™ Lentivirus Transduction Kit

Unique Elements of the pSMPUW Universal Lentivirus Expression Vector

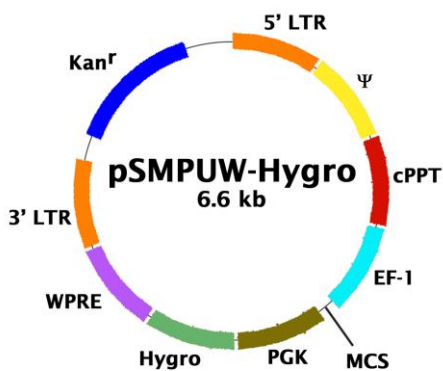


Element	Name	Benefits compared to other 3 rd Generation Systems
ELEMENTS ADDED		
	Central Polypurine Tract	<ul style="list-style-type: none"> Increased gene expression levels
	Hybrid 3' LTR Poly(A)	<ul style="list-style-type: none"> Increased safety: prevents read-through transcription Increased viral 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-Hygro Vector



MCS: AGTCGCCGTGAACGTTTCGGCCGGCCAGATATCTCCCTTCGGACCAAGGGTCATTAATTAAGTACCGGGTAGGGGA
 FseI EcoRV AhdI PacI

Figure 1: pSMPUW-Hygro Lentiviral Expression Vector (6567 bp, **Kanamycin**-resistant). Hind III Digestion: 1331 bp + 1982 bp + 3254 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 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.

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.
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-Hygro Plasmid Sequence

Pink: 5' CMV/LTR, ψ , cPPT

Blue: EF-1

Purple: MCS

Green: PGK

Red: Hygro

Brown: WPRE

Orange: 3' LTR

Blue: Kanamycin Resistance gene

ACTAGTCGGGGTCATTAGTTCATAGCCCATATATGGAGTTCGCGCTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCGCCCATTTGAC
GTCATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTCCATTGACGTCATGGGTGGAGTATTTACGGTAACTGCCACTTGGCAGTACATCAAGTG
TATCATATGCCAAGTACGCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACA
TCTACGTATTAGTTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCAT
TGACGTCAATGGGAGTGTGTTTTGGCACAAAATCAACGGGACTTCCAAAATGTCGTAACAACCTCCGCCCATTTGACGCAAAATGGGCGGTAGGCGGTGACGGTGG
GAGGCTATATAAGCAGAGCTGGTTTAGTGAACCGGGTCTCTCTGGTTAGACCAGATTTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCT
CAATAAAGCTTGCCTTGAAGTCTCAAGTAGTGTGTGCTGCTGTTGTTGCTGTAAGTAACTAGAGATCCCTCAGACCTTTTAGTCAAGTGGGAAATCTCTA
GCAGTGGCGCCGAACAGGGACCTGAAAGCGAAAGGGAAACCAGAGGAGCTCTCTCGACGAGGACTCGGCTTGTGAAGCGCGCACGGCAAGAGGGGAGGGGCGG
CGACTGCAGAGTACGCCAAAATTTGACTAGCGGAGGCTAGAAGGAGAGAGATGGGTGCGAGAGCGTCACTATTAAAGCGGGGAAAATAGCGGCCGCCAATTTT
AAAAGAAAAGGGGGGATGGGGGGTACAGTGCAGGGGAAAGAATAGTAGACATAATGCAACAGACATACAACTAAAGAATTAACAAAACAATTAACAAAATTC
AAATTTCCGGGGATCCGCTCCCGTACCACCCCGCCCAACCCCGCCCGCAGCTGAGAGTAATTCATACAAAAGGACTCGCCCTGCCTTGGGGAATCCC
AGGGACCGTCTTAACTCCCACTAACGTAGAACCCAGAGATCGTGCCTTCCGCCCTCACCAGCCCGCTCTCGTCACTACTGAGGTGGAGAAGAGCATGCGT
GAGGCTCCGCTGCCGTCAGTGGGCGAGCGCACATCGCCACAGTCCCGGAGAAGTTGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCG
GGTAAACTGGGAAGTGTGCTGTACTGGCTCCGCTTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCCGCTGAACGTTCCGCGCCGCGA
TATCTCCCTTCGACCAAGGGTCATTAATAAGTACCCGGTAGGGGAGGGGCTTTCCAAAGGCACTGGAGCATGCGCTTTAGCAGCCCGCTGGGCACTTGGC
GCTACACAAGTGGCTTGGCCTCGCACACATCCACATCCACCGGTAGGCGCAACCGGCTCCGCTTCTTTGGTGGCCCTTCGCGCACCTTCTACTCCTCCCT
AGTCAGGAAGTTCGCCCGCCCGCCGAGCTCGCGTCTGTCAGGAGCTGACAAATGGAAGTAGCAGTCTCACTAGTCTCGTGCAGATGGACAGCACCGCTGAGCAA
TGGAAAGCGGTAGGCTTTGGGGCAGCGGCAATAGCAGCTTTGCTCTTCGCTTTCTGGGCTCAGGGCGGGCGGGCCCGAAGGTTCTCCGGAGGCCCGGCA
TTCTGACCGCTTCAAAGCGCACGCTGTCGCGGCTGTTCTCTCTTCCTGATCTCGGGGCTTCGACTCTAGACACGTTGACAATTAATGACACACCCATGAAA
AAGCCTGAACCTACCGCAGCTCTGTCGAGAAGTTCGATCGAAAAGTTCGACAGCCTCCGACCTGATGCAGCTCTCGGAGGGCGAAGAATCTGCTGCTTTCA
GCTTCGATGTAGGAGGGCGTGATATGCTTCGCGGTAATAGCTGCGCGATGGTTCACAAAGATCGTTATGTTATCGGCCTTTGCATCGGCCGCGCTCCC
GATTCGGAAGTGTGACATTTGGGGAATTCAGCGAGAGCGCTCACTATTGCATCCTCCCGCTGCACAGGGTGTCACTGTGAAGACCTGCCTGAAACCGAATCG
CCCGTCTTCTGCAGCCGTCGCGGAGGCCATGGATCGATCGTGCGCCGATCTTAGCCAGAGCGAGCGGTTCCGCCATTCGGCCCAAGGAATCCGTCAT
ACACTACATGGCGTGAATTTTCATATCCCTGATGATCCCATGCTATCACTGGCAATGATGGACGACACCTCAGTGCCTCGCTCGCGCAGGCTTCGA
TGAGTGTATGCTTTGGCCGAGGACTGCCCGAAGTCCGGACCTCGTGCACGGGATTTCCGCTCCAACAATGCTCTGACGGACAATGGCCGATACACGCGGTC
ATTTGATGGAGCGAGGCGATGTTCCGGGATTCCAATACAGAGTCCGCAACATCTTCTTCCGAGGGCGTGGTGGCTTGTATGGAGCAGCAGACCGCTACTTCG
AGCGGAGGCATCCGAGTTCAGAGTCCCGCGGCTCCGGCGTATATGCTCCGATTTGGTCTGACCACTCTATCAGAGCTTGGTGGCAGGCAATTCGATGA
TGACGCTTGGGCGCAGGGTGCAGTGCAGCAGCAATCGTCCGATCCGAGCGGGACTGTCGGCGTACACAATCCGCCCGCAGAAGCGCGGCTGCTGGACATGGC
TGTGTAGAAGTACTCGCGATAGTGAACCCGACGCCAGCCTCGTCCGAGGGCAAAGGAATAGTTCGACAATCAACCTCTGGATTACAAAATTTGTGAAAGAT
TGACTGGTATTTAACTATGTTGCTCTTTTACGCTATGTGGATACGCTGCTTAAATGCTTTGTATCATGCTATTGCTCCCGTATGGCTTTTCAATTTCTCCTC
CTTGATAAACTCTGGTGTCTCTTTATGAGGAGTGTGGCCGTTGTGAGCAACGTTGGCTGTGCTGACTGTGTTGCTGACGCAACCCCACTGGTGG
GGCATTTGCCACTGCTCAGCTCTCCGATTTCCGGGACTTTCCGCTTTCCCTTCCCTATTGGCACGGGGAACCTATCGCCGCTGCTTCCCGCTGCTGGACAGGGG
CTCGGCTGTTGGGCACTGACAATCCGTTGTTGTCGGGAAATCATCGTCTTTCTTGGCTGCTCGCTGTGTTGCCACCTGGATTCTGCGGGGACGCTCCTT
CTGCTACGTCCTTCGCGCTCAATCCAGCGGACCTCCTTCCCGCGGCTGCTCCCGCTCTCGGCGCTTCCGCGCTCTCGCCTTCGCCCTCAGACGAGTCCG
ATCTCCTTTGGGCGCCTCCCGCTTAGTACTGGTACCTTAAAGACCAATGACTTCAAGGCAGCTGTAGATCTTAGCCACTTTTAAAGAAAAGGGGGGACTG
GAAGGCTAATTTCACTCCAACGAAGCAGATTCGGAAATTTATTTGTGAATTTGTGATGCTATTGGCTTATTTGTAACCGGTGACCTGCTTTTGGCCTGTA
CTGGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGTAACTAGGGAACCCACTGCTTAAAGCCTCAATAAAGCTTGCCTTGAAGTCTCAAGTAGT
GTGTGCCGCTGTTGTGTGACTCTGGTAACTAGAGATCGCTCAGACCCCTTTAGTCACTGTGGAAAATCTCTAGCATCTAGAGTATGCAAGCATGCATCTCAAT
TAGTCAGCAACAGGTGTGGAAGTCCCAAGGCTCCCAAGCAGCAGAAGTATGCAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCTAAGTCCCG
CCATCCCGCCCTAAGTCCGCCCAGTTCGCCCCTTCTCCGCCCATGCTGCTCAATTTTTTTTATTTATGACAGAGCCGAGCCCGCTCGGCTCAGGCTATT
CCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCTAGGCTAGAGATCATAATCAGCCATACCACATTTGTAGAGGTTTTACTTGTCTTAAAAAACCCTCCACACCTCC
CCCTGAACCTGAAACATAAAATGAATGCAATTTGTTGTTAACTGTTTATGTCAGCTTATAATGGTTACAAAATAAAGCAATAGCATACAAAATTTACAAAATAA
AGCATTTTTTCACTGCATTTAGTGTGGTTTTGCCAACTCAATGATATCTTATCATGCTGCTAGCCGGGCTTTTTTTTCTTAGGCTTCTTCCGCTTCTC
CGCTCACTGACTCGTCCGCTCGTTCGCTGCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACCGCAGGAAA
GAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAGGCCCGGTTGCTGGCGTTTTTCCATAGGCTCCGCCCGCTGACGAGCATCAAAAATCGA
CGCTCAAGTCAAGGTTGGCGAAACCGACAGGACTATAAAGATACCAGGCGTTTTCCCGTGAAGCTCCCTCGTGCCTCTCCTGTTCCGACCCCTGCCGCTTACCG
GATACCTGTCGCGCTTCTCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCAGCTGATAGTGTAGGTTCTCAGTTCGGTGTAGGTCGTTCCGCTCAAGCTGGGCTGTGT
GCAGCAACCCCGTTCAGCCCGACGCTGCGCTTCTCCGTTACTGCTGTTAGTAAACCCGTTAAGACACGACTTATGCCACTTATGCCAGCAGGACTGGT
AACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGTACAGAGTCTTGAAGTGGTGGCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGC
TGAAGCCAGTACCTTCGGAAGAGGTTGGTAGCTCTTATCCGGCAACAAACCACCGCTGGTAGCGGTGGTTTTTTGTTTTGCAAGCAGCAGATTACGCGCAG
AAAAAAGGATCTCAAGAAGATCTTTGATCTTTTCTACGGGCTGACGCTCAGTGGAAACGAAAACCTCAGTTAAGGGATTTTGGTCAATGAGATTATCAAAAAGG
ATCTTCACTTACGATCTTTTAAATTAATAAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTACCAGTGTAACTGATTAATCAGTGGCCAC
CTATCTCAGCGATCTGTCTATTTGCTTCAATAGTGTGCTGACTCTGCGCAGTCCAAAAAAGGCTCCAAAAGGAGCCTTAAATGTATCGGTGGGCCCTT
AGAAAACCTCATCGAGCATCAAAGAACTGCAATTTATTCATATCAGGATTTATCAATACCATATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAAAACCTCACC
GAGGCAGTTCCATAGGATGGCAAGATCTGGTATCGGTCGCGACTCGTCCCAACTCAATCAACCTATTAATTTCCCTCTGCAAAAATAGGTTATCA
AGTGAGAAATCACCAATGAGTGCAGCTGAATCCGCTGAGAAATGGCAAAAGCTTATGCAATTTCTTTCCAGACTTGTTCACAGCCGCGCTTACGCTCGTCATCAA
AATCACTCGCATCAACCAACCCTTATTCACTCGTATTGCGCTGAGCGAAGCAATACGCGATCGCTGTAAAAGGACAATTAACAACAGGAATCGAATGCAA

CCGGCGCAGGAACACTGCCAGCGCATCAACAATATTTTCACCTGAATCAGGATATTTCTTCTAATACCTGGAATGCTGTTTTCCCGGGGATCGCAGTGGTGAGTAAC
CATGCATCATCAGGAGTACGGATAAAATGCTTGATGGTCGGAAGAGGCATAAAATCCCGTCAGCCAGTTTAGTCTGACCATCTCATCTGTAACATCATTTGGCAACGC
TACCTTTGCCATGTTTCAGAAACAACCTCTGGCGCATCGGGCTTCCCATACAATCGATAGATTGTGCGCACCTGATTGCCCGACATTATCGCGAGCCATTTATACCC
ATATAAATCAGCATCCATGTTGGAATTTAATCGCGGCCTCGAGCAAGACGTTTCCCGTTGAATATGGCTCATAAACCCCTGTATTACTGTTTTATGTAAGCAGAC
AGTTTTATTGTTTCATGATGATATATTTTTATCTTGTGCAATGTAACATCAGAGATTTTGAGACACAACGTGGTTTAAACAATAGTCAAAAGCCTCCGGCG

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.

Recent Product Citations

1. Park, Y. et al. (2022). Oleuropein suppresses endometriosis progression and improves the fertility of mice with endometriosis. *J Biomed Sci.* **29(1)**:100. doi: 10.1186/s12929-022-00883-2.
2. Han, S.J. et al. (2022). Steroid receptor coactivator-3 inhibition generates breast cancer antitumor immune microenvironment. *Breast Cancer Res.* **24(1)**:73. doi: 10.1186/s13058-022-01568-2.
3. Davis, M. et al. (2013). RAC1P29S is a Spontaneously Activating Cancer-Associated GTPase. *PNAS.* **110**:912-917.
4. Arora, R. et al. (2012). Survivin is a therapeutic target in merkel cell carcinoma. *Science Translational Medicine.* **4**: 133ra56.

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

©2009-2022: Cell Biolabs, Inc. - All rights reserved. No part of these works may be reproduced in any form without permissions in writing.