

## pSMPUW-IRES-Blasticidin Lentiviral Expression Vector

---

**CATALOG NUMBER:** VPK-219

**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*.

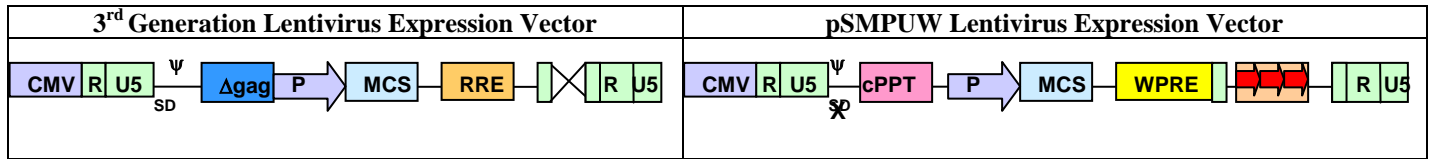
Lentivirus particles are produced from 293T cells through transient transfection of plasmids that encode for the components of the virion. Due to safety concerns regarding the infectious nature of HIV-1, recent lentiviral packaging systems have separated the viral components into 3 or 4 plasmids. However,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

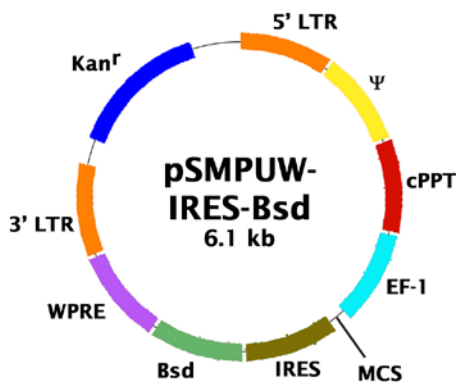


Element	Name	Benefits compared to other 3 <sup>rd</sup> Generation Systems
<b>ELEMENTS ADDED</b>		
	Central Polypurine Tract	<ul style="list-style-type: none"> <li>Increased gene expression levels</li> </ul>
	Hybrid 3' LTR Poly(A)	<ul style="list-style-type: none"> <li>Increased safety: prevents read-through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

### 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-IRES-Bsd Vector



MCS: TTCGGCCGGCCAGATATCTCCCTTCGGACCAAGGGTCAATTAATTAAGAATTCCTGCAGGCCTCGA  
 FseI EcoRV AhdI PacI EcoRI SbfI

**Figure 1:** pSMPUW-IRES-Bsd Lentiviral Expression Vector (6075 bp, **Kanamycin**-resistant). XhoI Digestion: 1701 bp + 4374 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-IRES-Blasticidin Plasmid Sequence

**Pink:** 5' CMV/LTR,  $\psi$ , cPPT

**Blue:** EF-1

**Purple:** MCS

**Red:** IRES

**Green:** Bsd

**Brown:** WPRE

**Orange:** 3' LTR

**Blue:** Kanamycin Resistance gene

ACTAGTCGGGGTCATTAGTTCATAGCCCATATATGGAGTTCGCGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCATTGAC  
GTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCATTGACGTCGAATGGGTGGAGTATTTACGGTAACTGCCACTTGGCAGTACATCAAGTG  
TATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACA  
TCTACGTATTAGTTCATCGCTATTACCATTGGTATGCGGTTTTGGCAGTACATCAATGGCGTGGATAGCGGTTTGACTCACGGGATTTCCAAGTCTCCACCCCAT  
TGACGTCAATGGGAGTGTGTTTTGGCACAAAATCAACGGGACTTTCAAAATGTCGTAACAACCTCCGCCCATTTGACGCAAAATGGGCGGTAGGCGTGTACGGTGG  
GAGGTCTATATAAGCAGAGCTGGTTTGTAGTAAACGGGTCTCTCTGGTTAGACCAGATTTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCT  
CAATAAAGCTTGCCTTGAGTGTCTCAAGTGTGTGTGCCGTCTGTTGTGTGACTCTGTTAACTAGAGATCCCTCAGACCTTTTAGTCAAGTGTGAAAAATCTCTA  
GCAGTGGCGCCGAACAGGACCTGAAAGCGAAAGGGAACACAGAGGAGCTCTCTCGACGAGGACTCGGCTTGTGAAGCGCGCACGGCAAGAGCGGAGGGCGG  
CGACTGCAGATACGCCAAAATTTGACTAGCGGAGGCTAGAAGGAGAGATGGGTGGAGAGCGTCAATTAAGCGGGGAAAAATAGCGGCCGCCAATTTT  
AAAAGAAAAGGGGGATTGGGGGTACAGTGCAGGGGAAAGAATAGTAGACATAATAGCAACAGACATACAACTAAAGAATTACAAAAACAATTAACAAAAATTC  
AAATTTTCGGGGATCCGCCTCCCGTCCACACCCCCCAACCCGCCCGACCGGAGCTGAGAGTAATTCATACAAAAGGACTCGCCCTGCTTGGGGAAATCCC  
AGGACCGTCTGTTAACTCCCATTAACGTAGAACCAGAGATCGCTGCGTTCGCCCCCTCAACCCGCGCTCTCGTCACTAGGATGGAGAAGAGCATCGCT  
GAGGCTCCGTTCCCGTCACTGGGCGAGCGCACATCGCCACATCGCCGAGAAGTTGGGGGAGGGGTCCGCAATTGAACCGTGTCTAGAGAAGTGGCGCGG  
GGTAACTGGGAAAGTGTGTGTACTGGCTCCGCCTTTTCCCGAGGGTGGGGAGAACCCTATATAAGTGCAGTAGTCCGCTGAACTGTCGGCCGCGCAG  
TATCTCCCTTCGGACCAAGGGTCATTAATTAAGAAATTCCTGCAGGCTCGAGGGCCGCGCGCCGCGCCGCTACGTAATTCGCCCTCTCCCTAACGTTACTG  
GCCAAGCCGCTTGAATAAGGCCGGTGTGCGTGTGTCTATATGTTATTTCCACCATATGCGCTCTTTGGCAATGTGAGGGCCCGAAACCTGGCCCTGTCTT  
CTTGACGAGCATTCCTAGGGTCTTCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTGCTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACA  
ACGCTGTAGGACCCCTTTGCAGGCAGCGAACCCCCACCTGGCGACAGGTGCCCTCTGGCGCCAAAAGCCACGTGTATAAGATACACCTGCAAAAGCGGCACAA  
CCCAGTGCACGTTGTGAGTTGGATAGTTGTGAAAGAGTCAAATGGCTCTCTCAAGCGTATTCAACAGGGGCTGAAGGATGCCAGAAGGTACCCCATTTGTAT  
GGGATCTGATCTGGGCGCTCGGTGCACATGCTTTACATGTGTTTGTAGTCGAGGTTAAAAAACGCTTAGGCCCCCGAACACGGGACGTGGTTTTCTTTGAAA  
ACACGATGATAATATGGCCACAACCATGGTTCCCTTGTCTCAAGAAGAATCCACCTCATTTGAAAGAGCAACGGTACAATCAACAGCATCCCATCTCTGAAGAC  
TACAGCGTCGCCAGCGCAGCTCTCTTAGCGACGGCCGATCTTCACTGGTGTCAATGTATATCATTTTACTGGGGACCTTGTGCAACTCGTGGTCTGGGCA  
CTGCTGTCTGCGGCAGCTGGCAACCTGACTTGTATCGTFCGCGATCGGAAATGAGAACAGGGGATCTTGGACCCCTGGGACCGGTGCCGACAGGTGCTTCTCGA  
TCTGCATCTGGGATCAAAGCCATAGTGAAGGACAGTGTGACAGCCGACGGCAGTTGGGATTCGTGAATTGCTGCCCTCTGGTTATGTGTGGGAGGGCTAAGTC  
GACAAACAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACATATGTTGCTCTTTTACGCTATGTGGATACGCTGCTTAAATGCCTTTGTATC  
ATGCTATTGCTTCCCGTATGGCTTTCATTTCTCTCTTGTATAAATCCTGGTGTCTCTTTATAGGAGTGTGGCCCGTGTGACGGCAACGTGGCGTGTG  
GTGCACTGTGTTGCTGACGCAACCCCACTGGTTGGGCAATGCCACCACCTGTGAGCTCTTTCCGGACTTTCGCTTCCCCCTCCCTATTGCCACGGCGGAA  
CTCATCGCCGCTGCTTGCCTGTGTCAGAGGGCTCGGCTGTGGGCACTGACAATTCGTTGGTGTGTGTCGGGAAATCATGCTCCTTTCCCTGGCTGCTCG  
CCTGTGTTGCCACCTGGATTCTGCGCGGGACGCTCTTCTGCTACGTCCTTCCGCCCTCAATCCAGCGGACCTTCCCTCCCGCGCTGCTGCCGCTCTGCGGCC  
TCTTCCGCTCTTCCGCTTCCGCTCAGACGAGTCCGATCTCCCTTTGGCCCGCTCCCGCTTGTAGTACTGGTACTTTAAGACCAATGACTTACAAGGAGCTGT  
AGATCTTAGCCACTTTTAAAGAAAAGGGGGACTGGAAGGGCTAAATCACTCCCAACGAAGCAAGATTCGGAAATTTATTTGTGAAATTTGTGATGCTATTGC  
TTTATTTGAAACCGGTGAGCTGCTTTTGCCTGTACTGGTCTCTCTGGTTAGACAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGAAACCCACTGCTTA  
AGCTCAATAAGACTGCTTCAAGTGTGCTCAAGTGTGCTGCGGCTGCTGTAATCTGGTAACTAGAGATCCCTCAGACCTTTTAGTCAAGTGTGAAAT  
CTCTAGCATCTAGATATGCAAAGCATGCACTCAATTAGTCAGCAACAGGTTGGAAAGTCCCGAGCTCCCGCAGGACAGGATGCAAAGCATGCATCTC  
AATTAGTCAGCAACCATAGTCCCGCCCTAACTCCGCCATCCCGCCCTAACTCCGCCAGTTCGCCCATTTCCGCCCATGGCTGACTAATTTTTTTTATTT  
ATGACAGAGCCGAGGCGCTCGGCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTGGAGGCTAGGCTAGAGATCATAATCAGCCATACCACATTTGTA  
GAGGTTTTACTTGTCTTAAAAAACCTCCACACCTCCCTGAACTGAAACATAAAATGAATGCAATTTGTTGTTAACTGTTTATTGCACTTATAATGGTT  
ACAAATAAGCAATAGCATCAAAATTTCAAAATAAAGCATTTTTTTCACTGCATCTAGTTGTGTTTTGTCCAAACCTCATCAATGTATCTTATCATGTCTGCTA  
GCCGGCTTTTTTTTCTTAGGCTTCTTCCGCTTCTCGCTCACTGACTCGTGCCTCGGTGTTGCTGCGGCGAGCGGTATCAGCTCACTCAAAGCGGTAA  
TACGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGAAAAGGCCAGGAACCGTAAAAGGCCGCTGTGGCGTTTTTCCA  
TAGGCTCCGCCCCCTGACGAGCATCAAAAAATCGACGCTCAAGTCAAGGTTGGGAAACCCGACAGGACTATAAAGATAACAGGCTTTCCCCCTGGAAGCTCC  
CTCGTGCCTCTCCTGTTCCGACCTGCGCTTACCAGTACCTGTCCGCTTTCTCCCTTCGGGAAAGCGTGGCGCTTTCTCATAGCTACGCTGTAGGTATCTCA  
GTTCCGGTGTAGTCTGTTCCGCTCAAGCTGGGCTGTGTGACGAACCCCGCTTCAAGCCGACCGCTGCGCTTATCCGGTAACTATGCTCTGAGTCCAAACCGGT  
AAGACACGACTTATCGCCACTGGCAGCAGCCTGTTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTGAAGTGGTGGCTAACTACGGC  
TACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAACAAACCCGCTGGTAGGC  
GTGTTTTTTTTTTGTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTCTACGGGTGCTGACGCTCAGTGGAAACGAAAACCTC  
ACGTTAAGGATTTTGGTCAAGATGATTAATAAAGGATCTTCACTAGATCTTTTAAATTAATAAAGTAAAGTTTAAATCAATCTAAAGTATATATGATGATAA  
TGGTCTGACAGTTACCAATGCTTAATCAGTGAAGCACCTATCTCAGCGATCTGTCTATTTCTGTTCACTAGTTGCTGACTCTGCGCAGTCCAAAAAAGG  
CTCCAAAAGGAGCTTAAATGTATCGGTGGGCCCTTAGAAAACTCATCGAGCATCAAAATGAACTGCAATTTTATTCATATCAGGATTAATCAATACCATATTTT  
GAAAAGCCGTTTTCTGTAATGAAGGAGAAAACCTACCCGAGGCAGTTCCATAGGATGGCAAGATCCTGGTATCGGTTCTGCGATTCCGACTCGTCCAACTCAATACA  
ACCTATTAATTTCCCTCGTCAAAAAAAGGTTTCAAGTGAAGAAATCACCATGAGTGAAGTAACTCCGGTGAAGATGGCAAAAGCTTATGCAATTTCTTCCAG  
ACTTGTCAACAGCCAGCCATTACGCTCTCATCAAAATCACTCGCATCAACCAACCCGTTATTCATTCGTGATTGCGCTGAGCGAGACGAAATACCGCATCGC  
TGTTAAAAGGACAATTAACAAACAGGAATCGAATGCAACCCGGCGCAGGAACACTGCCAGCGCATCAACAAATTTTTCACTGAATCAGGATATTTCTTAATACCTG  
GAATGCTGTTTTCCCGGGATCGCAGTGGTGAATACCATGCATCATCAGGAGTACGGATAAAATGCTTGTGTTGGTGGAAAGGACATAAATCCGCTCAGCCAGTTT

AGTCTGACCATCTCATCTGTAAACATCATTTGGCAACGCTACCTTTTGCCATGTTTCAGAAACAACCTCTGGCGCATCGGGCTTCCCATAACAATCGATAGATTGTGCGCAC  
CTGATTGCCCGACATTATCGCGAGCCCAATTATACCCATATAAATCAGCATCCATGTTGGAAATTTAATCGCGGCCCTCGAGCAAGACGTTTCCCGTTGAATATGGCT  
CATAACACCCCTTGTATTACTGTTTATGTAAGCAGACAGTTTTATGTTTCATGATGATATATTTTATCTTGTGCAATGTAACATCAGAGATTTGAGACACAACG  
TGGTTTAAACAAATAGTCAAAGCCTCCGGCG

## **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](mailto: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](mailto:tech@cellbiolabs.com)  
[www.cellbiolabs.com](http://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.

