pSMPUW-IRES-Puro Lentiviral Expression Vector

CATALOG NUMBER: VPK-215 **STORAGE:** -20ºC

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*.

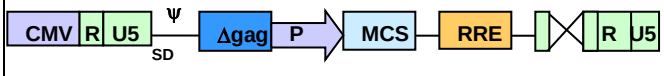
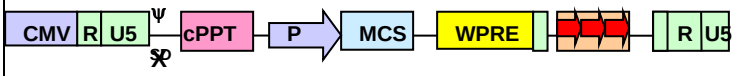
Lentivirus particles are produced from 293T cells through transient transfection of plasmids that encode for the components of the virion. Due to safety concerns regarding the infectious nature of HIV-1, recent lentiviral packaging systems have separated the viral components into 3 or 4 plasmids. However,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

3rd Generation Lentivirus Expression Vector	pSMPUW Lentivirus Expression Vector
	

Element	Name	Benefits compared to other 3rd Generation Systems
	ELEMENTS ADDED	
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
	ELEMENTS DELETED	
Δgag	Gag sequence	• Increased safety: reduces sequence homology
RRE	Rev-Responsive Element	• 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-IRES-Puro Vector

MCS: AACGTTCGGCCGGCCAGATATCTCCCTTCGGACCAAGGGTCATTAATTAAGAATTCCGCCCCTCT
FseI EcoRV AhdI PacI EcoRI

Figure 1: pSMPUW-IRES-Puro Lentiviral Expression Vector (6237 bp, **Kanamycin**-resistant). EcoRI and XhoI Digestion: 1687 bp + 4550 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 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.

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-Puro Plasmid Sequence

Pink: 5' CMV/LTR, ψ, cPPT
Blue: EF-1
Purple: MCS
Red: IRES
Green: Puro
Brown: WPRE
Orange: 3' LTR
Blue: Kanamycin Resistance gene

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References

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Recent Product Citations

1. Zhao, G. et al. (2020). NLRX1 knockout aggravates lipopolysaccharide (LPS)-induced heart injury and attenuates the anti-LPS cardioprotective effect of CYP2J2/11,12-EET by enhancing activation of NF-κB and NLRP3 inflammasome. *Eur J Pharmacol*. doi: 10.1016/j.ejphar.2020.173276.
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Warranty

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

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