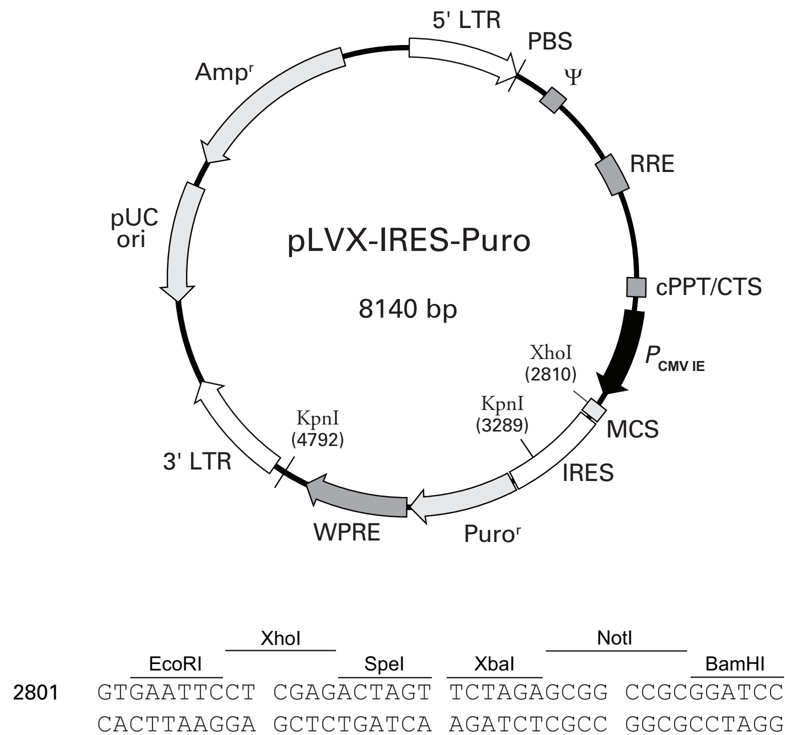
pLVX-IRES-Puro Vector Information

PT4063-5

Catalog No. 632183



pLVX-IRES-Puro Vector Map and Multiple Cloning Site (MCS).

Description

pLVX-IRES-Puro is an HIV-1-based, lentiviral expression vector that allows the simultaneous expression of your protein of interest and puromycin resistance (Puro^r) in virtually any mammalian cell type, including primary cells. The vector expresses your protein of interest and Puro^r from a bicistronic mRNA transcript, allowing puromycin resistance to be used as an indicator of transduction efficiency and a marker for selection.

Expression of the bicistronic transcript is driven by the constitutively active human cytomegalovirus immediate early promoter ($P_{CMV IE}$) located just upstream of the MCS. An encephalomyocarditis virus (EMCV) internal ribosome entry site (IRES), positioned between the MCS and Puro^r, facilitates cap-independent translation of Puro^r from an internal start site at the IRES/Puro^r junction (1).

pLVX-IRES-Puro contains all of the viral processing elements necessary for the production of replication-incompetent lentivirus, as well as elements to improve viral titer, transgene expression, and overall vector function. The woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) promotes RNA processing events and enhances nuclear export of viral RNA (2), leading to increased viral titers from packaging cells. In addition, the vector includes a Rev-response element (RRE), which further increases viral titers by enhancing the transport of unspliced viral RNA out of the nucleus (3). Finally, pLVX-IRES-Puro also contains a central polypurine tract/central termination sequence element (cPPT/CTS). During target cell infection, this element creates a central DNA flap that increases nuclear import of the viral genome, resulting in improved vector integration and more efficient transduction (4). The vector also contains a pUC origin of replication and an *E. coli* ampicillin resistance gene (Amp^r) for propagation and selection in bacteria.

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Use

pLVX-IRES-Puro is available as part of the Lenti-X™ Bicistronic Expression System (Puro; Cat. No. 632183). The vector is designed to constitutively coexpress your protein of interest and puromycin resistance from $P_{CMV\ IE}$ when transduced into mammalian cells. Before it can be transduced into target cells, the vector must be packaged into viral particles in HEK293T cells, using our Lenti-X™ HT Packaging System (Cat. Nos. 632160 and 632161). This packaging system allows the safe production of high titer, infectious, replication-incompetent, VSV-G pseudotyped lentiviral particles that can infect a wide range of cell types, including nondividing and primary cells (5).

Location of Features

- 5' LTR (5' long terminal repeat): 1–635
- PBS (primer binding site): 636–653
- Ψ (packaging signal): 685–822
- RRE (Rev-response element): 1303–1536
- cPPT/CTS (central polypurine tract/central termination sequence): 2028–2151
- $P_{CMV\ IE}$ (human cytomegalovirus immediate early promoter): 2185–2787
- MCS (multiple cloning site): 2803–2840
- IRES (encephalomyocarditis virus internal ribosome entry site): 2841–3448
- $Puro^r$ (puromycin resistance gene): 3449–4048
- WPRE (woodchuck hepatitis virus posttranscriptional regulatory element): 4062–4653
- 3' LTR (3' long terminal repeat): 4857–5493
- pUC origin of replication: 5963–6633 (complementary)
- Amp^r (ampicillin resistance gene; β-lactamase): 6778–7774 (complementary)

Selection of Stable Transductants

- Selectable marker: vector confers resistance to puromycin.

Propagation in *E. coli*

- Suitable host strains: DH5α, DH10B and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: high

Notes:

The vector sequence was compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by Clontech. This vector has not been completely sequenced.

The viral supernatants produced by this lentiviral vector could contain potentially hazardous recombinant virus. Due caution must be exercised in the production and handling of recombinant lentivirus. Appropriate NIH, regional, and institutional guidelines apply.

References

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4. Zennou, V. *et al.* (2000) *Cell* **101**(2):173–185.
5. Wu, X. *et al.* (2000) *Mol. Ther.* **2**(1):47–55.

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