

## pLVX-EF1 $\alpha$ -IRES-mCherry Vector

**Catalog No.**  
631987

**Amount**  
10  $\mu$ g

**Lot Number**  
Specified on product label.

### Product Information

pLVX-EF1 $\alpha$ -IRES-mCherry is a bicistronic lentiviral expression vector that can be used to generate high-titer lentivirus for transducing virtually any dividing or nondividing mammalian cell type, including primary and stem cells. The vector contains an internal ribosomal entry site (IRES) that allows a gene-of-interest and the red fluorescent protein mCherry to be simultaneously coexpressed from a single mRNA transcript. Expression of the transcript is driven by the human elongation factor 1 alpha (EF1 $\alpha$ ) promoter, which continues to be constitutively active even after stable integration of the vector into the host cell genome. Stable expression of the transcript allows the monitoring of a variety of cellular processes (such as differentiation in primary or stem cells) without the transgene silencing associated with CMV promoters. In addition, the vector allows efficient flow cytometric detection of stably or transiently transfected mammalian cells expressing mCherry and a protein of interest, without time-consuming drug and clonal selection.

### Package Contents

- 1 tube of pLVX-EF1 $\alpha$ -IRES-mCherry Vector (20  $\mu$ l/tube)

### Storage Conditions

- Store plasmids at  $-20^{\circ}\text{C}$ .
- Spin briefly to recover contents.
- Avoid repeated freeze/thaw cycles.

### Shelf Life

- 1 year from date of receipt under proper storage conditions.

### Storage Buffer

- 10 mM Tris-HCl (pH 8.0), 1 mM EDTA (pH 8.0)

### Concentration

- 500 ng/ $\mu$ l

### Shipping Conditions

- Dry ice ( $-70^{\circ}\text{C}$ )

### Product Documents

Documents for our products are available for download at [takarabio.com/manuals](http://takarabio.com/manuals)

The following documents apply to this product:

- Lenti-X Lentiviral Expression Systems User Manual
- pLVX-EF1 $\alpha$ -IRES-mCherry Vector Information

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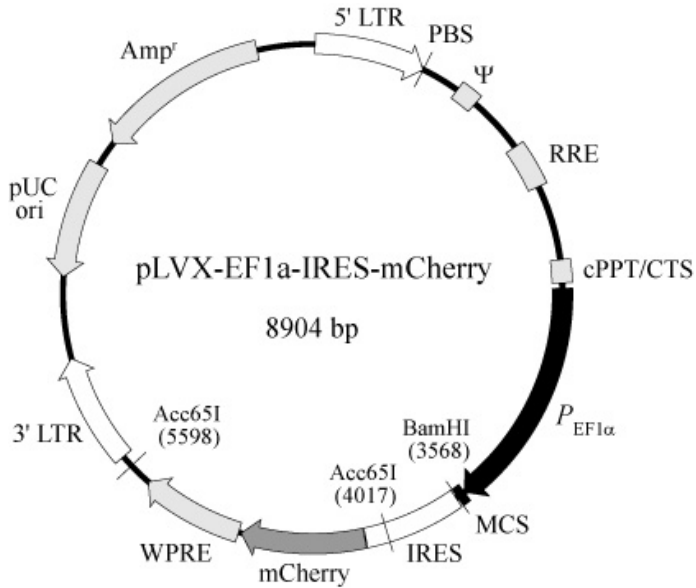


Figure 1. pLVX-EF1 $\alpha$ -IRES-mCherry vector map.

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                EcoRI      SpeI      XbaI      NotI      BamHI
3531  CCGTGAATTC  CTCGAGACTA  GTTCTAGAGC  GGCCGCGGAT  CCCGCCCTC
      GCCACTTAAG  GAGCTCTGAT  CAAGATCTCG  CCGGCGCCTA  GGGCGGGGAG
    
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Figure 2. pLVX-EF1 $\alpha$ -IRES-mCherry multiple cloning site (MCS).

## Description

pLVX-EF1 $\alpha$ -IRES-mCherry is an HIV-1-based, lentiviral expression vector designed to simultaneously and constitutively express a protein of interest and the green fluorescent protein mCherry from a bicistronic transcript in mammalian cells. mCherry is a mutant fluorescent protein derived from the tetrameric *Discosoma* sp. red fluorescent protein, DsRed (Nat. Biotechnol., 2004). The excitation and emission maxima of the native mCherry protein are 587 nm and 610 nm, respectively.

Simultaneous expression of a protein of interest and mCherry is made possible by the presence of an encephalomyocarditis virus internal ribosome entry site (IRES; J. Virol, 1988) positioned between the multiple cloning site (MCS) and the mCherry gene. The IRES allows a protein of interest and mCherry to be translated from a single bicistronic mRNA. Stable, constitutive expression of the bicistronic transcript is driven by the EF1 $\alpha$  promoter ( $P_{EF1\alpha}$ ), which continues to be constitutively active even after vector integration into the host cell genome (Stem Cells Dev., 2008).

pLVX-EF1 $\alpha$ -IRES-mCherry 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 (J. Virol., 1999), 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 (Proc. Natl. Acad. Sci. USA, 1990). Finally, pLVX-EF1 $\alpha$ -IRES-mCherry 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 (Cell, 2000). The vector also contains a pUC origin of replication and an *E. coli* ampicillin resistance gene (Amp<sup>r</sup>) for propagation and selection in bacteria.

### Location of Features

- 5' LTR (5' long terminal repeat): 1–635
- PBS (primer binding site): 636–653
- $\Psi$  (packaging signal): 685–822
- RRE (Rev-response element): 1303–1536
- cPPT/CTS (central polypurine tract/central termination sequence): 2028–2151
- *P*<sub>EF1 $\alpha$</sub>  (human elongation factor 1 alpha promoter): 2185–3519
- MCS (multiple cloning site): 3535–3572
- IRES (internal ribosome entry site): 3574–4148
- mCherry: 4149–4859
- WPRE (woodchuck hepatitis virus posttranscriptional regulatory element): 4873–5464
- 3' LTR (3' long terminal repeat): 5667–6303
- pUC origin of replication: 6772–7445 (complementary)
- Amp<sup>r</sup> (ampicillin resistance gene;  $\beta$ -lactamase): 7590–8586 (complementary)

### Additional Information

pLVX-EF1 $\alpha$ -IRES2-mCherry can be used to quickly identify cells expressing a gene of interest by screening for mCherry fluorescence. Genes inserted into the MCS must contain a start codon (ATG) and a stop codon.

Before the vector can be transduced into target cells, it must be packaged into viral particles in HEK293T cells, using our Lenti-X™ HTX Packaging System (Cat. Nos. 631247 and 631249). 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 (Mol. Ther., 2000).

#### Caution!

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.

### Propagation in *E. coli*

- Suitable host strains: DH5 $\alpha$  and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100  $\mu$ g/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: high

### Excitation and Emission Maxima of mCherry

- Excitation: 587 nm
- Emission: 610 nm

## References

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- Jang, S. K. *et al.* A segment of the 5' nontranslated region of encephalomyocarditis virus RNA directs internal entry of ribosomes during in vitro translation. *J. Virol.* **62**, 2636–43 (1988).
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- Zennou, V. *et al.* HIV-1 genome nuclear import is mediated by a central DNA flap. *Cell* **101**, 173–85 (2000).
- Zufferey, R., Donello, J. E., Trono, D. & Hope, T. J. Woodchuck hepatitis virus posttranscriptional regulatory element enhances expression of transgenes delivered by retroviral vectors. *J. Virol.* **73**, 2886–92 (1999).

## Quality Control Data

### Plasmid Identity & Purity

- Digestion with the indicated restriction enzymes produced fragments of the indicated sizes on a 0.8% agarose/EtBr gel:

Enzyme(s)	Fragment(s)
BamHI	8.9 kb
Acc65I	1.6 & 7.3 kb

- Vector identity was confirmed by sequencing.
- A<sub>260</sub>/A<sub>280</sub>: 1.8–2.0

It is certified that this product meets the above specifications, as reviewed and approved by the Quality Department.

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