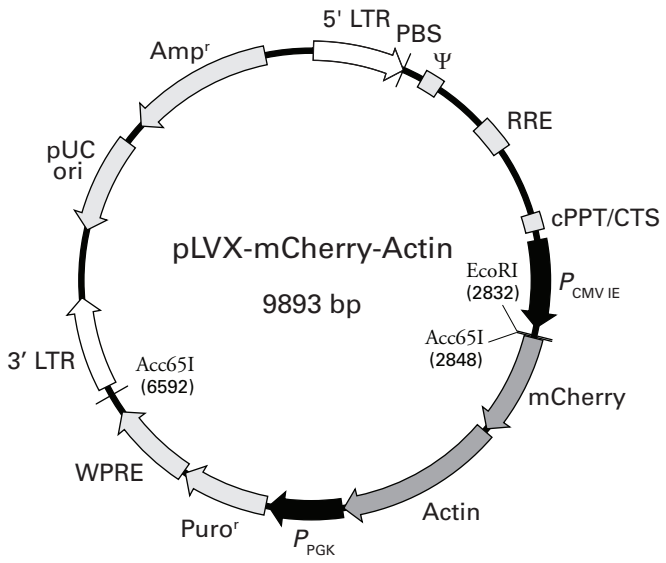
Vector Information

pLVX-mCherry-Actin Vector Information PT5114-5

Catalog No. 631078



pLVX-mCherry-Actin Vector Map

Description

pLVX-mCherry-Actin is an HIV-1-based, lentiviral expression vector that expresses human β-actin fused to mCherry, a mutant fluorescent protein derived from the tetrameric *Discosoma sp.* red fluorescent protein, DsRed (1). The excitation and emission maxima of the native mCherry protein are 587 nm and 610 nm, respectively. Expression of the mCherry-actin fusion protein is driven by the constitutively active human cytomegalovirus immediate early promoter ($P_{CMV\ IE}$), located just upstream of the mCherry coding sequence. Lentiviral particles derived from the vector allow the expression of the mCherry-actin fusion protein in virtually any cell type, including primary cells.

pLVX-mCherry-Actin 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 and transgene RNA (2), leading to increased viral titers from packaging cells, and enhanced expression of your gene of interest in target 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-mCherry-Actin 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).

In addition to lentiviral elements, pLVX-mCherry-Actin contains a puromycin resistance gene (Puro^r) under the control of the murine phosphoglycerate kinase (PGK) promoter (P_{PGK}) for the selection of stable transductants. 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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(PR9Z3415; published 8 January 2010)

Use

pLVX-mCherry-Actin constitutively expresses an mCherry-actin fusion protein when transduced into target cells, where the fusion protein is incorporated into actin filaments, allowing the visualization of actin-containing subcellular structures in both live and fixed cells (5, 6). Note: pLVX-mCherry-Actin is not designed to be used as a cloning vector.

Before the vector can be transduced, it must be transfected into 293T packaging cells with our Lenti-X™ HT Packaging System (Cat. Nos. 632160 and 632161). This packaging system allows you to safely produce high titer, infectious, replication-incompetent, VSV-G pseudotyped lentiviral particles that can infect a wide range of cell types, including non-dividing and primary cells (7). If required, stable transfectants can be selected using puromycin.

Location of Features

- 5' LTR: 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
- mCherry-human β-Actin fusion: 2861–4714
- P_{PGK} (phosphoglycerate kinase promoter): 4725–5233
- Puro^r (puromycin resistance gene): 5254–5853
- WPRE (woodchuck posttranscriptional regulatory element): 5867–6458
- 3' LTR: 6661–7297
- pUC origin of replication: 7767–8434 (complementary)
- Amp^r (ampicillin resistance gene; β-lactamase): 8579–9575 (complementary)

Selection of Stable Transfectants

- Selectable marker: plasmid 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: ColE1
- Copy number: high

Excitation and emission maxima of mCherry

- Excitation maximum = 587 nm
- Emission maximum = 610 nm

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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6. de Hostos, E. L., unpublished data.
7. Wu, X. *et al.* (2000) *Mol. Ther.* **2**(1):47–55.

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