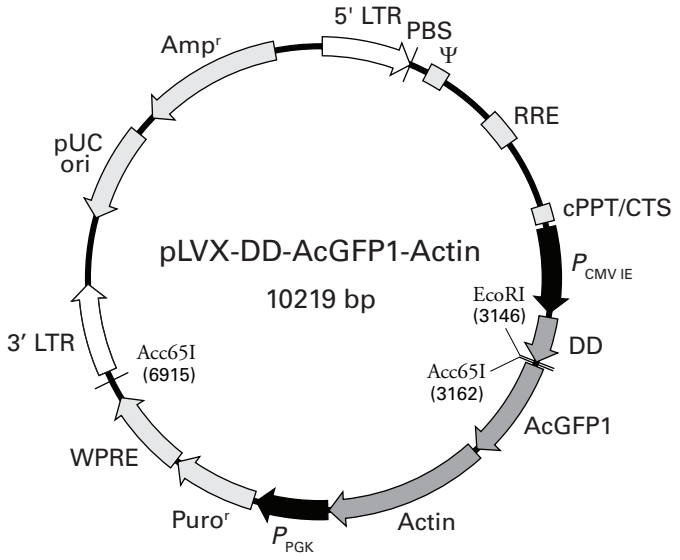
Vector Information

pLVX-DD-AcGFP1-Actin Vector Information PT5115-5

Sold as part of Cat. No. 631077



pLVX-DD-AcGFP1-Actin Vector Map

Description

pLVX-DD-AcGFP1-Actin is an HIV-1-based, lentiviral vector that expresses the ligand-dependent, destabilized fusion protein DD-AcGFP1-Actin. This protein is composed of human cytoplasmic β-actin fused to AcGFP1, a monomeric green fluorescent protein derived from *Aequorea coerulescens* (excitation and emission maxima: 475 nm and 505 nm, respectively; 1). The fusion protein also contains an N-terminal ProteoTuner™ destabilization domain (DD; 2), which—in the absence of the stabilizing ligand Shield1—causes rapid, proteasomal degradation of the fusion protein. When added to the culture medium, Shield1 binds to the destabilization domain, preventing degradation of DD-AcGFP1-Actin. The stabilized fusion protein can then be incorporated into growing actin filaments, allowing actin-containing subcellular structures to be visualized in living and fixed cells (3, 4).

pLVX-DD-AcGFP-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 (5), 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 (6). Finally, pLVX-DD-AcGFP-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 (7).

In addition to lentiviral elements, pLVX-DD-AcGFP-Actin contains a puromycin resistance gene (Puro^r) under the control of the murine phosphoglycerate kinase 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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Use

pLVX-DD-AcGFP1-Actin, available as part of the Lenti-X™ Actin Dynamics Monitoring Kit (Cat. No. 631077), allows you to monitor how newly synthesized, Shield1-stabilized DD-AcGFP1-Actin is integrated into the dynamic actin filament network. pLVX-DD-AcGFP-Actin expresses a DD-AcGFP-Actin fusion protein when transduced into target cells; the fusion protein is degraded until Shield1 is added to the culture medium. The stabilized fusion protein is incorporated into actin filaments, allowing the visualization of actin-containing subcellular structures 15–20 minutes after the addition of Shield1 to the medium.

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 (8). 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
- DD (destabilization domain): 2821–3144
- AcGFP1-Actin fusion: 3175–5037
- P_{PGK} (phosphoglycerate kinase promoter): 5048–5556
- Puro^r (puromycin resistance gene): 5577–6176
- WPRE (woodchuck hepatitis virus posttranscriptional regulatory element): 6190–6781
- 3' LTR: 6984–7620
- pUC origin of replication: 8090–8760 (complementary)
- Amp^r (ampicillin resistance gene; β-lactamase): 8905–9901 (complementary)

Propagation in *E. coli*

- Recommended host strains: DH5α™ 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

Excitation and emission maxima of AcGFP1

- Excitation maximum = 475 nm
- Emission maximum = 505 nm

References

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3. Westphal, M. *et al.* (1997) *Curr. Biol.* **7**(3):176–183.
4. de Hostos, E. L., unpublished data.
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6. Cochrane, A. W. *et al.* (1990) *Proc. Natl. Acad. Sci. USA* **87**(3):1198–1202.
7. Zennou, V. *et al.* (2000) *Cell* **101**(2):173–185.
5. Wu, X. *et al.* (2000) *Mol. Ther.* **2**(1):47–55.

Note: 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.

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AcGFP is covered by U.S. Patent No. 7,432,053.

cPPT/CTS Element:
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