pLVX-EF1α-IRES-mCherry Vector

Table of Contents
Product Information .................................................................................................................................................. 1
Description ............................................................................................................................................................ 2
Location of Features ............................................................................................................................................... 3
Additional Information .......................................................................................................................................... 3
Quality Control Data ............................................................................................................................................. 4

Catalog No. Amount Lot Number
631987 10 μg Specified on product label.

Product Information
pLVX-EF1α-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 (EF1α) 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-EF1α-IRES-mCherry Vector (20 μl/tube)

Storage Conditions
• Store plasmid at –20°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/μl
Certificate of Analysis

pLVX-EF1α-IRES-mCherry Vector

Shipping Conditions
- Dry ice (–70°C)

![Diagram of pLVX-EF1α-IRES-mCherry vector map]

Figure 1. pLVX-EF1α-IRES-mCherry vector map.

![Diagram of pLVX-EF1α-IRES-mCherry multiple cloning site (MCS)]

Figure 2. pLVX-EF1α-IRES-mCherry multiple cloning site (MCS).

Description

pLVX-EF1α-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 (1). 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; 2) 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 EF1α promoter ($P_{EF1α}$), which continues to be constitutively active even after vector integration into the host cell genome (3).

pLVX-EF1α-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 (4), 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 (5). Finally, pLVX-EF1α-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 (6). The vector also contains a pUC origin of replication and an *E. coli* ampicillin resistance gene (Amp') for propagation and selection in bacteria.

**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*EF1α (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' (ampicillin resistance gene; β-lactamase): 7590–8586 (complementary)

**Additional Information**

*pLVX-EF1α-IRES-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 (7).

**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: 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 mCherry**

- Excitation: 587 nm
- Emission: 610 nm

**References**


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:

<table>
<thead>
<tr>
<th>Enzyme(s)</th>
<th>Fragment(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BamHI</td>
<td>8.9 kb</td>
</tr>
<tr>
<td>Acc65I</td>
<td>1.6 &amp; 7.3 kb</td>
</tr>
</tbody>
</table>

- Vector identity was confirmed by sequencing.
- $A_{260}/A_{280}$: 1.8–2.0
pLVX-EF1alpha-IRES-mCherry Vector

CATALOG NO.

631987

NOTICE TO PURCHASER:

Clontech products are to be used for research purposes only. They may not be used for any other purpose, including, but not limited to, use in drugs, in vitro diagnostic purposes, therapeutics, or in humans. Clontech products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without prior written approval of Clontech Laboratories, Inc.

Your use of this product is also subject to compliance with the licensing requirements listed below and described on the product’s web page at http://www.clontech.com. It is your responsibility to review, understand and adhere to any restrictions imposed by these statements.

STATEMENT 44

The DsRed-Monomer and the Fruit Fluorescent Proteins are covered by one or more of the following U.S. Patents: 7,005,511; 7,157,566; 7,393,923 and 7,250,298.

STATEMENT 55

cPPT Element This product and its use are the subject of U.S. Pat. No. 6,682,907. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot disclose information, sell or otherwise transfer this product, its components or materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for any commercial purposes. If the buyer is not willing to accept the limitations of this limited use statement, Clontech is willing to accept return of the product with a full refund. For information on purchasing a license to the DNA-Flap technology for purposes other than research, contact the Transfer of Technology Office, Institut Pasteur, 28 rue du Docteur Roux, 75 724 Paris Cedex 15 (www.pasteur.fr).

STATEMENT 56

Clontech has a license to sell products containing WPRE, under the terms described below. Any use of WPRE outside of Clontech’s product or the product’s intended use, requires a license as detailed below. Before using the product containing WPRE, please read the following license agreement. If you do not agree to be bound by its terms, contact Clontech within 10 days for authorization to return the unused product containing WPRE and to receive a full credit. Patents: The WPRE technology is covered by one or more of the following U.S. Patents and corresponding patent claims outside the U.S.: 6,136,597; 6,284,469; 6,312,912; 6,287,814, issued to The Salk Institute for Biological Studies. Individual License Agreement: Clontech grants you a non-exclusive license to use the enclosed product containing WPRE in its entirety for its intended use. The product is being transferred to you in
furtherance of, and reliance on, such license. Any use of WPRE outside of Clontech’s product or the product’s intended use, requires a license from the Salk Institute for Biological Studies. Termination of License: This license agreement is effective until terminated. You may terminate it at any time by destroying all products containing WPRE in your control. It will also terminate automatically if you fail to comply with the terms and conditions of the license agreement. You shall, upon termination of the license agreement, destroy all products containing WPRE in your control, and so notify Clontech in writing. This License shall be governed in its interpretation and enforcement by the laws of the State of California. Contact for WPRE Licensing: The Salk Institute for Biological Studies 10010 North Torrey Pines Road La Jolla, CA 92037 Attn.: Office of Technology Management Phone: 858.453.4100 ext. 1275 Fax: 858.546.8093

STATEMENT 72

Living Colors Fluorescent Protein Products: Not-For-Profit Entities: Orders may be placed in the normal manner by contacting your local representative or Clontech Customer Service at. Any and all uses of this product will be subject to the terms and conditions of the Non-Commercial Use License Agreement (the “Non-Commercial License”), a copy of which can be found below. As a condition of sale of this product to you, and prior to using this product, you must agree to the terms and conditions of the Non-Commercial License. Under the Non-Commercial License, Clontech grants Not-For-Profit Entities a non-exclusive, non-transferable, non-sublicensable and limited license to use this product for internal, non-commercial scientific research use only. Such license specifically excludes the right to sell or otherwise transfer this product, its components or derivatives thereof to third parties. No modifications to the product may be made without express written permission from Clontech. Any other use of this product requires a different license from Clontech. For license information, please contact a licensing representative by phone at 650.919.7320 or by e-mail at licensing@clontech.com. For-Profit Entities wishing to use this product are required to obtain a license from Clontech. For license information, please contact a licensing representative by phone at 650.919.7320 or by e-mail at licensing@clontech.com.

TRADEMARKS:

Clontech and the Clontech logo are trademarks of Clontech Laboratories, Inc.

All other marks are the property of their respective owners. Certain trademarks may not be registered in all jurisdictions. Clontech is a Takara Bio Company. ©2014 Clontech Laboratories, Inc. This document has been reviewed and approved by the Clontech Quality Assurance Department.