pLVX-EF1α-AcGFP1-N1 Vector

Catalog No. 631983
Amount Each
Lot Number Specified on product label.

Product Information
pLVX-EF1α-AcGFP1-N1 is a 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 allows a gene-of-interest to be fused to the N-terminus of the green fluorescent protein AcGFP1. Expression of the fusion 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 fusion 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 AcGFP1 fusions, without time-consuming drug and clonal selection.

Package Contents
- 1 tube of pLVX-EF1α-AcGFP1-N1 Vector (20 μl/tube)

Storage Conditions
- Store plasmids 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

Shipping Conditions
- Dry ice (–70°C)

Product Documents
Documents for our products are available for download at takarabio.com/manuals
The following documents apply to this product:
- pLVX-EF1alpha-AcGFP1-N1 Vector Information
Certificate of Analysis

pLVX-EF1α-AcGFP1-N1 Vector

Figure 1. pLVX-EF1α-AcGFP1-N1 vector map.

Figure 2. pLVX-EF1α-AcGFP1-N1 multiple cloning site (MCS).

Description

pLVX-EF1α-AcGFP1-N1 is an HIV-1-based, lentiviral expression vector designed to constitutively express a protein of interest fused to the N-terminus of AcGFP1, a green fluorescent protein derived from *Aequorea coerulescens*. The excitation and emission maxima of native AcGFP1 are 475 nm and 505 nm, respectively. Stable, constitutive expression of the fusion protein is driven by the EF1α promoter (P_{EF1α}), which continues to be constitutively active even after the vector integrates into the host cell genome (Stem Cells Dev., 2008).

pLVX-EF1α-AcGFP1-N1 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-EF1α-AcGFP1-N1 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).

In addition to lentiviral elements, pLVX-EF1α-AcGFP-N1 contains a puromycin resistance gene (Puro') 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') 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 polyuridine tract/central termination sequence): 2028–2151
- $P_{EF1α}$ (human elongation factor 1 alpha promoter): 2186–3519
- MCS (multiple cloning site): 3528–3600
- AcGFP1 (human-codon-optimized): 3607–4326
- $P_{PGK}$ (phosphoglycerate kinase promoter): 4345–4853
- Puro’ (puromycin resistance gene): 4874–5473
- WPRE (woodchuck hepatitis virus posttranscriptional regulatory element): 5487–6078
- 3’ LTR (3’ long terminal repeat): 6281–6917
- pUC origin of replication: 7387–8060 (complementary)
- Amp’ (ampicillin resistance gene; β-lactamase): 8205–9201 (complementary)

Additional Information

Genes cloned into the MCS must: be in-frame with the AcGFP1 coding sequence; contain a start codon (ATG); and lack in-frame stop codons. pLVX-EF1α-AcGFP1-N1 will constitutively express your N-terminal AcGFP1 fusion when transduced into target cells. Before the vector can be transduced into target cells, however, 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.

Selection of Stable Transfectants

- Selectable marker: plasmid confers resistance to puromycin.

Propagation in E. coli

- Suitable host strains: DH5α, HB101 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: 475 nm
- Emission: 505 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>9.5 kb</td>
</tr>
<tr>
<td>Acc65I</td>
<td>2.6 &amp; 6.9 kb</td>
</tr>
</tbody>
</table>

- Vector identity was confirmed by sequencing.
- A_{260}/A_{280}: 1.8–2.0

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

pLVX-EF1alpha-AcGFP1-N1 Vector

CATALOG NO.

631983

NOTICE TO PURCHASER:

Our products are to be used for Research Use Only. They may not be used for any other purpose, including, but not limited to, use in humans, therapeutic or diagnostic use, or commercial use of any kind. Our 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 our prior written approval.

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

STATEMENT 39

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

STATEMENT 56

Takara Bio USA, Inc. has a license to sell products containing WPRE, under the terms described below. Any use of WPRE outside of Takara Bio USA’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 Takara Bio USA 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: Takara Bio USA 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 Takara Bio USA’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. You shall, upon termination of the license agreement, destroy all products containing WPRE in your control, and so notify Takara Bio USA 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:

Takara Bio USA, Inc.
1290 Terra Bella Avenue, Mountain View, CA 94043, USA
U.S. Technical Support: techUS@takarabio.com

<table>
<thead>
<tr>
<th>United States/Canada</th>
<th>Asia Pacific</th>
<th>Europe</th>
<th>Japan</th>
</tr>
</thead>
<tbody>
<tr>
<td>800.662.2566</td>
<td>+1.650.919.7300</td>
<td>+33.(0)1.3904.6880</td>
<td>+81.(0)77.565.6999</td>
</tr>
</tbody>
</table>

9/28/2018
Not-For-Profit Entities: Orders may be placed in the normal manner by contacting your local representative or Takara Bio USA, Inc. Customer Service. 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, Takara Bio USA, Inc. 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 Takara Bio USA, Inc. Any other use of this product requires a different license from Takara Bio USA, Inc. For license information, please contact a licensing representative by phone at 650.919.7320 or by e-mail at licensing@takarabio.com.

For-Profit Entities wishing to use this product are required to obtain a license from Takara Bio USA, Inc. For license information, please contact a licensing representative by phone at 650.919.7320 or by e-mail at licensing@takarabio.com.

Not-For-Profit Non-Commercial Use License:

**STATEMENT 55**

cPPT Element. This product and its use are the subject to one or more of the following U.S. Pat. Nos. 6,682,907, 7,981,671, 8,093,042, 8,367,068, 8,460,678, 8,512,994 and foreign equivalents. 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, Takara Bio USA, Inc. 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).

**TRADEMARKS:**

©2018 Takara Bio Inc. All Rights Reserved.

All trademarks are the property of Takara Bio Inc. or its affiliate(s) in the U.S. and/or other countries or their respective owners. Certain trademarks may not be registered in all jurisdictions.