

pEF1 α -IRES-AcGFP1 Vector

Catalog No.
631971

Amount
10 μ g

Lot Number
Specified on product label.

Description

pEF1 α -IRES-AcGFP1 is a bicistronic mammalian expression vector that allows the simultaneous, constitutive expression of a protein of interest and the green fluorescent protein AcGFP1. Expression of the bicistronic transcript is driven by the human elongation factor 1 alpha (EF1 α) promoter, which continues to be constitutively active even after stable integration of the vector into the host cell genome. Stable expression of the bicistronic 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 AcGFP1 and a protein of interest, without time-consuming drug and clonal selection.

Package Contents

- 1 tube pEF1 α -IRES-AcGFP1 Vector (20 μ l/tube)

Storage Conditions

- Store plasmid at -20°C .
- Spin briefly to recover contents.
- Avoid repeated freeze/thaw cycles.

Expiration Date

- Specified on product label.

Storage Buffer

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

Concentration

- 500 ng/ μ l

Shipping Conditions

- Dry ice

Product Documents

Documents for our products are available for download at takarabio.com/manuals

The following documents apply to this product:

- pEF1 α -IRES-AcGFP1 Vector Information
- pEF1 α -IRES-AcGFP1 vector sequence in GenBank format

Takara Bio USA, Inc.

2560 Orchard Parkway, San Jose, CA 95131, USA

U.S. Technical Support: technical_support@takarabio.com

United States/Canada
800.662.2566
(032423)

Asia Pacific
+1.650.919.7300

Europe
+33.(0)1.3904.6880

Japan
+81.(0)77.565.6999

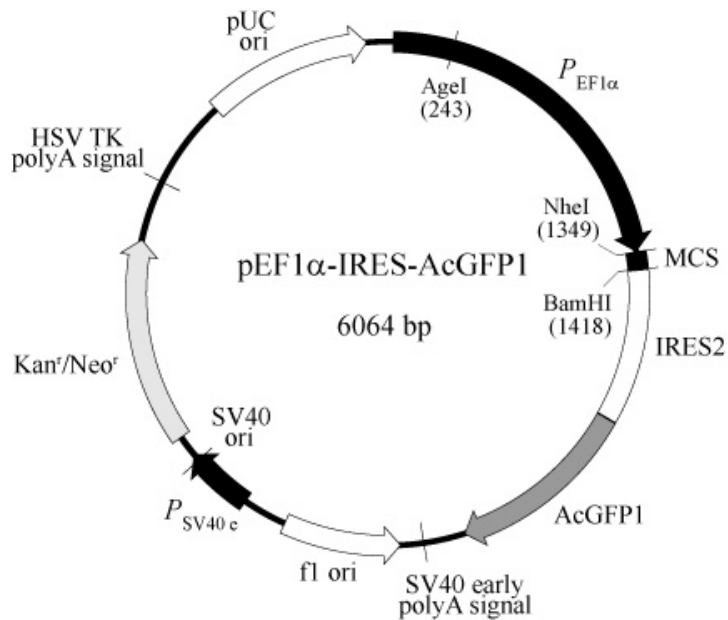


Figure 1. pEF1 α -IRES-AcGFP1 vector map.

	NheI			
	AfeI			
1341	TCGTGACGCT	AGCGCTACCG	GACTCAGATC	TCGAGCTCAA
	AGCACTGCGA	TCGCGATGGC	CTGAGTCTAG	AGCTCGAGTT
			SmaI	
			XmaI	
	EcoRI	Sall		BamHI
		AccI		
1381	GCTTCGAATT	CTGCAGTCGA	CGGTACCGCG	GGCCCCGGGAT
	CGAAGCTTAA	GACGTCAGCT	GCCATGGCGC	CCGGGCCCTA
				GCGGGGAGA

Figure 2. pEF1 α -IRES-AcGFP1 multiple cloning site (MCS).

Description

pEF1 α -IRES-AcGFP1 is designed to simultaneously and constitutively express a protein of interest and AcGFP1 in mammalian cells. AcGFP1 is a human-codon-optimized, monomeric green fluorescent protein derived from *Aequorea coerulea* (the excitation and emission maxima of native AcGFP1 are 475 nm and 505 nm, respectively). Simultaneous expression of a protein of interest and AcGFP1 is made possible by the presence of an encephalomyocarditis virus (EMCV) internal ribosome entry site (IRES; Jackson, Howell, and Kaminski 1990, Jang et al. 1988) positioned between the multiple cloning site (MCS) and the AcGFP1 gene. The IRES allows a protein of interest and AcGFP1 to be translated from a single bicistronic mRNA. Stable, constitutive expression of the bicistronic transcript is driven by the EF1 α promoter ($P_{EF1\alpha}$), which continues to be constitutively active even after vector integration into the host cell genome (Wang et al. 2008).

The vector backbone also contains an SV40 origin for replication in mammalian cells expressing the SV40 large T antigen, a pUC origin of replication for propagation in *E. coli*, and an fl origin for single-stranded DNA production. This

pEF1 α -IRES-AcGFP1 Vector

vector also has a neomycin-resistance cassette (Neo^r) that allows G418 selection of stably transfected eukaryotic cells (Gorman 1985). This cassette consists of the SV40 early promoter (P_{SV40e}), a Tn5 kanamycin/neomycin resistance gene, and herpes simplex virus thymidine kinase (HSV TK) polyadenylation signals. A bacterial promoter upstream of the cassette drives expression of the kanamycin resistance gene in *E. coli*.

Location of Features

- $P_{EF1\alpha}$ (human elongation factor 1 alpha promoter): 12–1346
- MCS (multiple cloning site): 1348–1422
- IRES2 (internal ribosome entry site): 1423–2007
- AcGFP1 (human-codon-optimized): 2011–2727
- SV40 early polyA signal: 2883–2933
- f1 origin of replication: 2980–3435 (complementary)
- SV40 origin of replication: 3776–3911
- P_{SV40e} (SV40 early promoter and enhancer sequences): 3609–3877
- Kan^r/Neo^r (kanamycin/neomycin resistance gene): 3960–4754
- HSV TK polyA signals: 4990–5008
- pUC origin of replication: 5339–5982

Additional Information

pEF1 α -IRES2-AcGFP1 can be used to quickly identify cells expressing a gene of interest by screening for AcGFP1 fluorescence. Genes inserted into the MCS must contain a start codon (ATG) and a stop codon. pEF1 α -IRES2-AcGFP1 can be introduced into mammalian cells using any standard transfection method. Cells expressing AcGFP1 can be detected by flow cytometry or fluorescence microscopy 24 hours after transfection. However, in some cases, up to 48 hours may be required for detection of green-emitting cells. If required, stable transformants can be selected using G418 (4).

Propagation in *E. coli*

- Suitable host strains: DH5 α and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM101 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (50 μ g/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

- Gorman, C. In DNA cloning: A practical approach, vol. II. Ed. DM Glover. (1985).
- Jackson, R. J., Howell, M. T. & Kaminski, A. The novel mechanism of initiation of picornavirus RNA translation. *Trends Biochem. Sci.* **15**, 477–483 (1990).
- 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**, 2636–2643 (1988).
- Wang, R., Liang, J., Jiang, H., Qin, L.-J. & Yang, H.-T. Promoter-dependent EGFP expression during embryonic stem cell propagation and differentiation. *Stem Cells Dev.* **17**, 279–290 (2008).

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	Fragments
BamHI	6.1 kb
AgeI/NheI	1.1 & 5.0 kb

- 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.

pEF1alpha-IRES-AcGFP1 Vector

CATALOG NO.

631971

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 72

Living Colors Fluorescent Protein Products:

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 e-mail at licensing@takarabio.com.

Not-For-Profit Non-Commercial Use License:

TRADEMARKS:

Takara Bio USA, Inc.

2560 Orchard Parkway, San Jose, CA 95131, USA

U.S. Technical Support: technical_support@takarabio.com

United States/Canada

Asia Pacific

Europe

Japan

800.662.2566

+1.650.919.7300

+33.(0)1.3904.6880

+81.(0)77.565.6999

3/24/2023

Notice to Purchaser



©2023 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.

Takara Bio USA, Inc.

2560 Orchard Parkway, San Jose, CA 95131, USA

U.S. Technical Support: technical_support@takarabio.com

United States/Canada

Asia Pacific

Europe

Japan

800.662.2566

+1.650.919.7300

+33.(0)1.3904.6880

+81.(0)77.565.6999

3/24/2023