pEF1α-IRES-AcGFP1 Vector

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Catalog No. 631971
Amount 10 μg
Lot Number Specified on product label.

Product Information
pEF1α-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 (EF1α) 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 of pEF1α-IRES-AcGFP1 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

Shipping Conditions
- Dry ice (–70°C)
Figure 1. pEF1α-IRES-AcGFP1 vector map.

Figure 2. pEF1α-IRES-AcGFP1 multiple cloning site (MCS).

Description

pEF1α-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; 1, 2) 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 EF1α promoter (P_{EF1α}), which continues to be constitutively active even after vector integration into the host cell genome (3).

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 f1 origin for single-stranded DNA production. This vector also has a neomycin-resistance cassette (Neo') that allows G418 selection of stably transfected eukaryotic cells (4). 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**

- \( P_{\text{EF1}\alpha} \) (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
- \( P_{\text{SV40e}} \) (SV40 early promoter and enhancer sequences): 3609–3877
- Kan'/Neo' (kanamycin/neomycin resistance gene): 3960–4754
- HSV TK polyA signals: 4990–5008
- pUC origin of replication: 5339–5982

**Additional Information**

*pEF1α-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. *pEF1α-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: DH5α 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 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:

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<tr>
<th>Enzyme(s)</th>
<th>Fragment(s)</th>
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<tbody>
<tr>
<td>BamHI</td>
<td>6.1 kb</td>
</tr>
<tr>
<td>AgeI/NheI</td>
<td>1.1 &amp; 5.0 kb</td>
</tr>
</tbody>
</table>

- Vector identity was confirmed by sequencing.

- A$_{260}$/A$_{280}$: 1.8–2.0
pEF1alpha-IRES-AcGFP1 Vector

CATALOG NO.
631971

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STATEMENT 39
AcGFP is covered by U.S. Patent No. 7,432,053.

STATEMENT 72
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