Certificate of Analysis

pEF1α-AcGFP1-C1 Vector

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Catalog No.	Amount	Lot Number
631974	10 µg	Specified on product label.

Product Information

pEF1α-AcGFP1-C1 is a mammalian expression vector that constitutively expresses a protein of interest fused to the C-terminus of the green fluorescent protein AcGFP1, even after stable integration of the vector into the host cell genome. Stable, constitutive expression of the bicistronic transcript is driven by the human elongation factor 1 alpha (EF1α) promoter, allowing the monitoring of a variety of cellular processes (such as differentiation in primary or stem cells) without the transgene silencing associated with CMV promoters. The unmodified vector can be used to express modified AcGFP1 in mammalian cells.

Package Contents

- 1 tube of pEF1α-AcGFP1-C1 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)

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(PA124332)

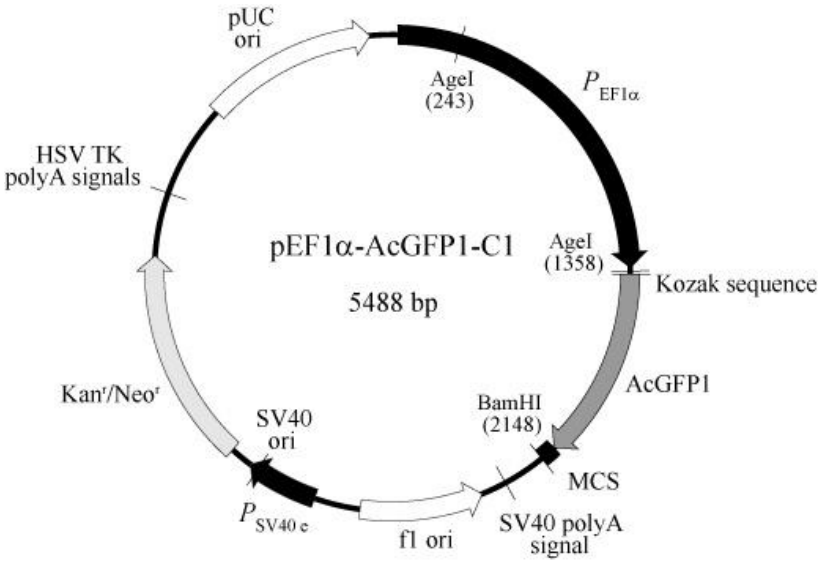

Figure 1. pEF1α-AcGFP1-C1 vector map. Please note that the vector DNA provided by Clontech is methylated. If you wish to digest the vector with methylation-sensitive enzymes, you will first need to transform the vector into a dam$^-$ host strain and purify fresh plasmid DNA.

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                End of AcGFP1  BspEI                                  HindIII   EcoRI
2078  CTG TAC AAG TCC GGA CTC AGA TCT CGA GCT CAA GCT TCG AAT TCT GCA

                                                    BamHI
          AccI    Acc65I          XmaI
          SalI    KpnI            SmaI                          Stop  Stop  Stop
2126  GTC GAC GGT ACC GCG GGC CCG GGA TCC ACC GGA TCT AGA TAA CTG AT
```

Figure 2. pEF1α-AcGFP1-C1 multiple cloning site (MCS).

Description

pEF1α-AcGFP1-C1 is designed to express a protein of interest fused to the C-terminus of AcGFP1, a human-codon-optimized, monomeric green fluorescent protein derived from *Aequorea coerulescens*. The excitation and emission maxima of the native AcGFP1 protein are 475 nm and 505 nm, respectively. Expression of fusion proteins that retain the fluorescence properties of the unmodified AcGFP1 protein can be monitored by flow cytometry and localized by fluorescence microscopy.

The multiple cloning site (MCS) in pEF1α-AcGFP1-C1 is positioned downstream of the AcGFP1 coding sequence. Expression of the fusion protein is driven by the EF1α promoter ($P_{EF1\alpha}$), which remains constitutively active even after stable integration of the vector into the host cell genome (1). A Kozak consensus sequence located immediately upstream of the AcGFP1 gene enhances the translational efficiency of the fusion in eukaryotic systems (2), and SV40 polyadenylation signals downstream of the AcGFP1 gene direct proper processing of the 3' end of the mRNA.

The vector backbone 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. A neomycin-

resistance cassette (Neo^r) allows stably transfected eukaryotic cells to be selected using G418 (3). This cassette consists of the SV40 early promoter ($P_{SV40\ e}$), the Tn5 neomycin/kanamycin resistance gene, and polyadenylation signals from the herpes simplex virus thymidine kinase (HSV TK) gene. 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
- Kozak consensus sequence: 1363–1373
- AcGFP1(human-codon-optimized): 1370–2086
- MCS (multiple cloning site): 2087–2152
- SV40 polyA signal: 2307–2341
- f1 origin of replication: 2404–2859 (complementary)
- $P_{SV40\ e}$ (SV40 early promoter and enhancer sequences): 3033–3301
- SV40 origin of replication: 3200–3338
- Kan^r/Neo^r (kanamycin/neomycin resistance gene): 3384–4178
- HSV TK polyA signals: 4414–4432
- pUC origin of replication: 4763–5406

Additional Information

Genes cloned into the MCS must be in-frame with the AcGFP1 coding sequence, and do not require start or stop codons. The pEF1α-AcGFP1-C1 vector can be transfected into mammalian cells using any standard transfection method. pEF1α-AcGFP1-C1 can be used as a cotransfection marker, as the unmodified vector will express AcGFP1 in mammalian cells. If required, stable transfectants can be selected using G418 (3).

Propagation in *E. coli*

- Suitable host strains: DH5α, HB101 and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid, such as the JM109 or XL1-Blue strains.
- 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

1. Wang, R. *et al.* (2008) *Stem Cells Dev.* **17**(2):279−289.
2. Kozak, M. (1987) *Nucleic Acids Res*. **15**(20):8125–8148.
3. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II*. Ed. D. M. Glover (IRL Press, Oxford, U.K.) pp. 143–190.

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(s)	Fragment(s)
BamHI	5.5 kb
AgeI	1.1 & 4.4 kb

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

Notice to Purchaser

pEF1alpha-AcGFP1-C1 Vector

CATALOG NO.

631974

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STATEMENT 39

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

STATEMENT 72

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