Certificate of Analysis

pEF1α-DsRed-Monomer-C1 Vector

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Catalog No.	Amount	Lot Number
631977	10 µg	Specified on product label.

Product Information

pEF1α-DsRed-Monomer-C1 is a mammalian expression vector that constitutively expresses a protein of interest fused to the C-terminus of the red fluorescent protein DsRed-Monomer, even after stable integration of the vector into the host cell genome. Stable, constitutive expression of the fusion protein 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 DsRed-Monomer in mammalian cells.

Package Contents

- 1 tube of pEF1α-DsRed-Monomer-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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(PA124335)

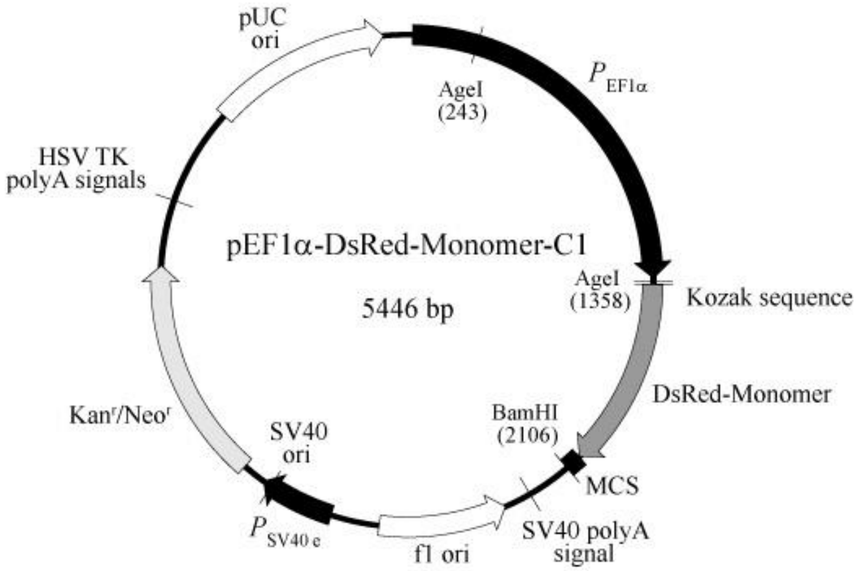

Figure 1. pEF1α-DsRed-Monomer-C1 vector map. Please note that the vector DNA provided by Clontech is methylated. If you wish to digest the vector with methylation-sensitive enzymes, you will first need to transform the vector into a dam$^-$ host strain and purify fresh plasmid DNA.

End of DsRed-Monomer; BspEI; HindIII; EcoRI

2036 GGC TCC CAG' TCC GGA CTC AGA TCT CGA GCT CAA GCT TCG AAT TCT GCA

SalI/AccI; Acc65I/KpnI; XmaI/SmaI; BamHI; Stop; Stop; Stop

2084 GTC GAC GGT ACC GCG GGC CCG GGA TCC ACC GGA TCT AGA TAA CTG ATC

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

Description

pEF1α-DsRed-Monomer-C1 is designed to express a protein of interest fused to the C-terminus of DsRed-Monomer, a monomeric mutant of the *Discosoma sp.* red fluorescent protein DsRed (1). The DsRed-Monomer coding sequence has been human-codon-optimized for high expression in mammalian cells (2). The excitation and emission maxima of native DsRed-Monomer are 557 nm and 585 nm, respectively. Expression of fusion proteins that retain the fluorescence properties of unmodified DsRed-Monomer can be monitored by flow cytometry and localized by fluorescence microscopy.

The multiple cloning site (MCS) in pEF1α-DsRed-Monomer-C1 is positioned downstream of the DsRed-Monomer coding sequence. Expression of the fusion protein is driven by the EF1α promoter ($P_{EF1α}$), which remains constitutively active even after stable integration of the vector into the host cell genome (3). A Kozak consensus sequence located immediately upstream of the DsRed-Monomer gene enhances the translational efficiency of the fusion in eukaryotic systems (4), and SV40 polyadenylation signals downstream of the DsRed-Monomer 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 (5). 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
- DsRed-Monomer (human-codon-optimized): 1370–2044
- MCS (multiple cloning site): 2045–2110
- SV40 polyA signal: 2265–2299
- f1 origin of replication: 2362–2817 (complementary)
- $P_{SV40\ e}$ (SV40 early promoter and enhancer sequences): 2991–3259
- SV40 origin of replication: 3158–3296
- Kan^r/Neo^r (kanamycin/neomycin resistance gene): 3342–4136
- HSV TK polyA signals: 4372–4390
- pUC origin of replication: 4721–5364

Additional Information

Genes cloned into the MCS must be in-frame with the DsRed-Monomer coding sequence, and do not require start or stop codons. The pEF1α-DsRed-Monomer-C1 vector can be transfected into mammalian cells using any standard transfection method. Cells expressing DsRed-Monomer fusions can be detected by flow cytometry or fluorescence microscopy 12–16 hr after transfection. If required, stable transfectants can be selected using G418 (5). pEF1α-DsRed-Monomer-C1 can also be used as a cotransfection marker, as the unmodified vector will express DsRed-Monomer in mammalian cells.

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 DsRed-Monomer

- Excitation: 557 nm
- Emission: 585 nm

References

1. Matz, M. V., *et al*. (1999) *Nat. Biotechnol*. **17**(10):969–973.
2. Haas, J., *et al*. (1996) *Curr. Biol*. **6**(3):315–324.
3. Wang, R. *et al.* (2008) *Stem Cells Dev.* **17**(2):279−289.
4. Kozak, M. (1987) *Nucleic Acids Res*. **15**(20):8125–8148.
5. 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	5.4 kb
AgeI	1.1 & 4.3 kb

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

Notice to Purchaser

pEF1alpha-DsRed-Monomer-C1 Vector

CATALOG NO.

631977

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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 72

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