Certificate of Analysis

pEF1α-DsRed-Monomer-N1 Vector

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Catalog No.	Amount	Lot Number
631978	10 µg	Specified on product label.

Product Information

pEF1α-DsRed-Monomer-N1 is a mammalian expression vector that constitutively expresses a protein of interest fused to the N-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 DsRed-Monomer in mammalian cells.

Package Contents

- 1 tube of pEF1α-DsRed-Monomer-N1 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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(PA124336)

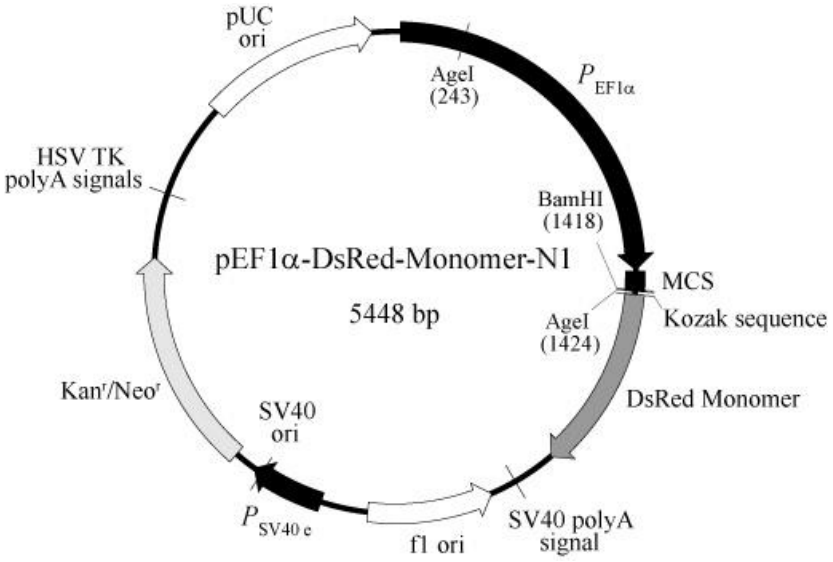

Figure 1. pEF1α-DsRed-Monomer-N1 vector map. Please note that the vector DNA provided by Clontech is methylated. If you wish to digest the vector with methylation-sensitive enzymes, you will first need to transform the vector into a dam⁻ host strain and purify fresh plasmid DNA.

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          NheI                                                  HindIII    EcoRI
1346  ACG CTA GCG CTA CCG GAC TCA GAT CTC GAG CTC AAG CTT CGA ATT CTG

                                          BamHI
          AccI     KpnI          XmaI                                  Start of
          SalI     Acc65I        SmaI                                  DsRed-Monomer
1394  CAG TCG ACG GTA CCG CGG GCC CGG GAT CCA CCG GTC GCC ACC ATG GAC AAC
```

Figure 2. pEF1α-DsRed-Monomer-N1 multiple cloning site (MCS).

Description

pEF1α-DsRed-Monomer-N1 is designed to express a protein of interest fused to the N-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-N1 is positioned between the EF1α promoter ($P_{EF1\alpha}$) and the DsRed-Monomer coding sequence. Expression of the fusion protein is driven by the EF1α promoter, 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 DsRed-Monomer in eukaryotic systems (4), and SV40 polyadenylation signals direct proper processing of the 3' end of the DsRed-Monomer 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
- MCS (multiple cloning site): 1348–1422
- Kozak consensus sequence: 1429–1439
- DsRed-Monomer (human-codon-optimized): 1436–2110
- SV40 polyA signal: 2267–2301
- f1 origin of replication: 2364–2819 (complementary)
- $P_{SV40\ e}$ (SV40 early promoter and enhancer sequences): 2993–3261
- SV40 origin of replication: 3160–3295
- Kan^r/Neo^r (kanamycin/neomycin resistance gene): 3344–4138
- HSV TK polyA signals: 4374–4392
- pUC origin of replication: 4723–5366

Additional Information

The gene of interest must be cloned into pEF1α-DsRed-Monomer-N1 so that it is in-frame with the DsRed-Monomer coding sequence. The gene must contain a start codon (ATG), and lack in-frame stop codons.

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-N1 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	1.2 & 4.3 kb

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

Notice to Purchaser

pEF1alpha-DsRed-Monomer-N1 Vector

CATALOG NO.

631978

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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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