Certificate of Analysis

pEF1α-DsRed-Express2 Vector

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Catalog No.	Amount	Lot Number
631979	10 µg	Specified on product label.

Product Information

pEF1α-DsRed-Express2 is a mammalian expression vector that constitutively expresses the red fluorescent protein DsRed-Express2, even after stable integration of the vector into the host cell genome. Stable, constitutive expression of DsRed-Express2 is driven by the human elongation factor 1 alpha (EF1α) promoter, which allows the protein to be expressed without the transgene silencing associated with CMV promoters. The vector, which lacks an MCS, is designed to be used for cell labeling or as a marker of transfection efficiency.

Package Contents

- 1 tube of pEF1α-DsRed-Express2 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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(PA124337)

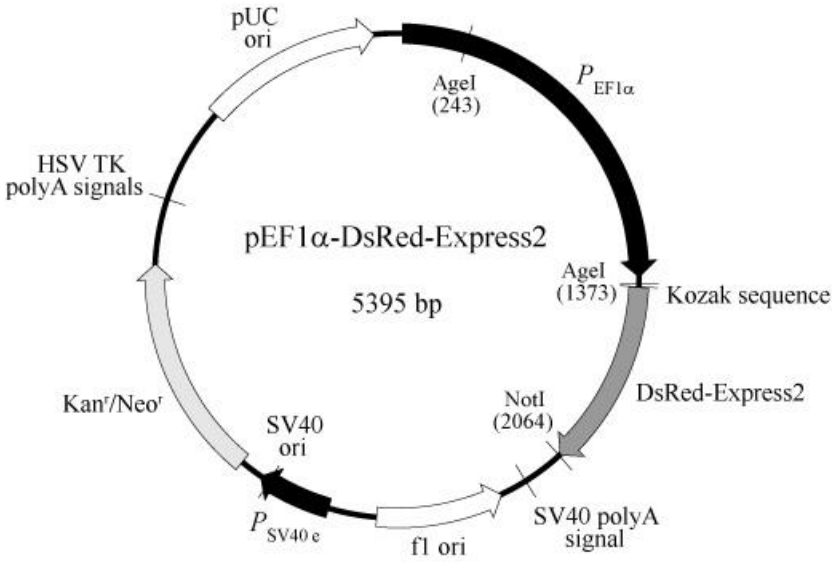

Figure 1. pEF1α-DsRed-Express2 vector map.

Description

pEF1α-DsRed-Express2 is designed to constitutively express DsRed-Express2 in mammalian cells. DsRed-Express2 is a mutant fluorescent protein derived from the *Discosoma sp*. red fluorescent protein, DsRed (1). This mutant retains the fast maturation and high photostability characteristic of its predecessor, DsRed-Express (2), and has been engineered for increased solubility (3). The excitation and emission maxima of DsRed-Express2 are 554 nm and 591 nm, respectively.

The DsRed-Express2 coding sequence is positioned just downstream of the constitutively active EF1α promoter ($P_{EF1\alpha}$). As a result, mammalian cells transfected with this vector will constitutively express DsRed-Express2, even after stable integration of the vector into the host cell genome (4). A Kozak consensus sequence located immediately upstream of the DsRed-Express2 coding sequence enhances translational efficiency of DsRed-Express2 in eukaryotic cells (5), and SV40 polyadenylation signals downstream of the DsRed-Express2 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 (6). 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: 1378–1388
- DsRed-Express2: 1385–2059
- SV40 polyA signal: 2214–2248
- f1 origin of replication: 2311–2766 (complementary)
- $P_{SV40\ e}$ (SV40 early promoter and enhancer sequences): 2940–3208
- SV40 origin of replication: 3107–3245

- Kan^r/Neo^r (kanamycin/neomycin resistance gene): 3291–4085
- HSV TK polyA signals: 4321–4339
- pUC origin of replication: 4670–5313

Additional Information

pEF1α-DsRed-Express2 can be used for whole-cell labeling or as a marker for cotransfection. pEF1α-DsRed-Express2 can be transfected into mammalian cells using any standard transfection method. Cells expressing DsRed-Express2 can be detected by fluorescence microscopy or flow cytometry 8–12 hours after transfection. If required, stable transfectants can be selected using G418 (6).

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-Express2

- Excitation: 554 nm
- Emission: 591 nm

References

1. Matz, M. V. *et al*. (1999) *Nat. Biotechnol*. **17**(10):969-973.
2. Bevis, B. J. & Glick, B. S. (2002) *Nat. Biotechnol*. **20**(1):83–87. Erratum in *Nat. Biotechnol*. (2002) **20**(11):1159.
3. Strack, R. L. *et al*. (2008) *Nat. Methods* **5**(11):955–957.
4. Wang, R. *et al.* (2008) *Stem Cells Dev.* **17**(2):279−289.
5. Kozak, M. (1987) *Nucleic Acids Res*. **15**(20):8125–8148.
6. 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)
NotI	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-Express2 Vector

CATALOG NO.

631979

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STATEMENT 24

The RCFPs (including DsRedExpress, DsRedExpress2, and E2-Crimson) are covered by one or more of the following U.S. Patent Nos. 7,166,444; 7,157,565; 7,217,789; 7,338,784; 7,338,783; 7,537,915 6,969,597, 7,150,979, 7,442,522 and 8,012,682.

STATEMENT 72

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