pEF1α-E2-Crimson Vector

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Catalog No.  Amount Lot Number
631981  10 μg Specified on product label.

Product Information
pEF1α-E2-Crimson is a mammalian expression vector that constitutively expresses the far-red fluorescent protein E2-Crimson, even after stable integration of the vector into the host cell genome. Stable, constitutive expression of E2-Crimson 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-E2-Crimson 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)
pEF1α-E2-Crimson Vector

Figure 1. pEF1α-E2-Crimson vector map.

Description

pEF1α-E2-Crimson is designed to constitutively express E2-Crimson in mammalian cells. E2-Crimson is a far-red fluorescent protein derived from the tetrameric red fluorescent protein DsRed-Express2 (1, 2). E2-Crimson retains the reduced cyto- and phototoxicity, increased solubility, fast maturation, and high photostability characteristic of DsRed-Express2. Unlike other far-red fluorescent proteins, E2-Crimson is not cytotoxic in bacterial and mammalian cells, making it well-suited for in vivo applications involving sensitive cells, such as primary or stem cells. E2-Crimson has an emission maximum at 646 nm, and absorbance and excitation maxima at 611 nm, giving it the furthest red-shifted excitation spectrum of any available fluorescent protein (1).

The E2-Crimson 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 E2-Crimson, even after stable integration of the vector into the host cell genome (3). A Kozak consensus sequence located immediately upstream of the E2-Crimson coding sequence enhances translational efficiency of E2-Crimson in eukaryotic cells (4), and SV40 polyadenylation signals downstream of the E2-Crimson 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') 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: 1378–1388
- E2-Crimson: 1385–2062
- SV40 polyA signal: 2214–2248
Certificate of Analysis

pEF1α-E2-Crimson Vector

- f1 origin of replication: 2311–2766 (complementary)
- \( P_{SV40} \alpha \) (SV40 early promoter and enhancer sequences): 2940–3208
- SV40 origin of replication: 3107–3245
- Kan'/Neo' (kanamycin/neomycin resistance gene): 3291–4085
- HSV TK polyA signals: 4321–4339
- pUC origin of replication: 4670–5313

Additional Information

pEF1α-E2-Crimson is designed for use as a marker for cotransfection or for cell labeling. The vector can be transfected into mammalian cells using any standard transfection method. E2-Crimson matures faster than any previously described far-red fluorescent protein (the half-time for fluorophore maturation is 26 minutes at 37°C; 1). Cells expressing E2-Crimson can be detected by fluorescence microscopy or flow cytometry 8–12 hours after transfection. The protein can be efficiently excited with a standard 633 nm laser, which is useful in multi-color labeling experiments with orange and green fluorescent proteins. If required, stable transfectants can be selected using G418 (5).

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 E2-Crimson

- Excitation: 611 nm
- Emission: 646 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>NotI</td>
<td>5.4 kb</td>
</tr>
<tr>
<td>AgeI</td>
<td>1.1 &amp; 4.3 kb</td>
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</table>

- Vector identity was confirmed by sequencing.
- \( A_{260}/A_{280} \): 1.8–2.0
Notice to Purchaser

pEF1alpha-E2-Crimson Vector

CATALOG NO.
631981

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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 69


STATEMENT 72

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