pEF1α-mCherry-C1 Vector

Table of Contents
Product Information ........................................................................................................................................ 1
Description .................................................................................................................................................. 2
Location of Features .................................................................................................................................. 3
Additional Information ............................................................................................................................... 3
Quality Control Data .................................................................................................................................. 4

Catalog No.  Amount       Lot Number
631972          10 μg         Specified on product label.

Product Information
pEF1α-mCherry-C1 is a mammalian expression vector that constitutively expresses a protein of interest fused to the C-terminus of the red fluorescent protein mCherry, 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 mCherry in mammalian cells.

Package Contents
- 1 tube of pEF1α-mCherry-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)
Description

pEF1α-mCherry-C1 is designed to constitutively express a protein of interest fused to the C-terminus of mCherry, a mutant fluorescent protein derived from the tetrameric Discosoma sp. red fluorescent protein, DsRed (1). The excitation and emission maxima of the native mCherry protein are 587 nm and 610 nm, respectively. Expression of fusion proteins that retain the fluorescence properties of the unmodified mCherry protein can be monitored by flow cytometry and localized by fluorescence microscopy.

The multiple cloning site (MCS) in pEF1α-mCherry-C1 is positioned downstream of the mCherry 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 (2). A Kozak consensus sequence located immediately upstream of the mCherry gene enhances the translational efficiency of the fusion in eukaryotic systems (3), and SV40 polyadenylation signals downstream of the mCherry 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 φ1 origin for single-stranded DNA production. A neomycin-resistance cassette (Neo') allows stably transfected eukaryotic cells to be selected using G418 (4). This cassette consists of the SV40 early promoter ($P_{SV40e}$), 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.
Certificate of Analysis  
*pEF1α-mCherry-C1 Vector*  

**Location of Features**

- $P_{\text{EF1}\alpha}$ (human elongation factor 1 alpha promoter): 12–1346
- Kozak consensus sequence: 1363–1373
- mCherry: 1370–2077
- MCS (multiple cloning site): 2078–2143
- SV40 polyA signal: 2298–2332
- f1 origin of replication: 2395–2850 (complementary)
- $P_{\text{SV40}\epsilon}$ (SV40 early promoter and enhancer sequences): 3024–3292
- SV40 origin of replication: 3191–3329
- Kan'/Neo' (kanamycin/neomycin resistance gene): 3375–4169
- HSV TK polyA signals: 4405–4423
- pUC origin of replication: 4754–5397

**Additional Information**

Genes cloned into the MCS must be in-frame with the mCherry coding sequence, and do not require start or stop codons. The pEF1α-mCherry-C1 vector can be transfected into mammalian cells using any standard transfection method. pEF1α-mCherry-C1 can be used as a cotransfection marker, as the unmodified vector will express mCherry in mammalian cells. If required, stable transfectants can be selected using G418 (4).

For Western analysis, the mCherry protein can be detected with either the Living Colors® mCherry Monoclonal Antibody (Cat. No. 632543), the DsRed Polyclonal Antibody (Cat. No. 632496) or the DsRed Monoclonal Antibody (Cat. Nos. 632392 and 632393).

**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 mCherry**

- Excitation: 587 nm
- Emission: 610 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:

<table>
<thead>
<tr>
<th>Enzyme(s)</th>
<th>Fragment(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BamHI</td>
<td>5.5 kb</td>
</tr>
<tr>
<td>AgeI</td>
<td>1.1 &amp; 4.4 kb</td>
</tr>
</tbody>
</table>

- Vector identity was confirmed by sequencing.

- A260/A280: 1.8–2.0
pEF1alpha-mCherry-C1 Vector

CATALOG NO.

631972

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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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