pCMV-DsRed-Express Vector

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Catalog No. Amount Lot Number
632416 20 μg Specified on product label.

Product Information

pCMV-DsRed-Express is a mammalian expression vector designed for use as a cotransfection marker, together with an expression construct of interest. It constitutively expresses the red fluorescent protein DsRed-Express, which can be detected by fluorescence microscopy to provide direct visual evidence of transfection. Cells can also be sorted by flow cytometry to enrich for transfected cells.

pCMV-DsRed-Express encodes DsRed-Express, a variant of Discosoma sp. red fluorescent protein (DsRed; 1). DsRed-Express contains nine amino acid substitutions which improve the solubility of the protein, reduce the time from transfection to detection of red fluorescence, and decrease the level of residual green emission (2). When DsRed-Express is expressed in mammalian cell cultures, red-emitting cells can be detected by either fluorescence microscopy or flow cytometry 8–12 hours after transfection. Although DsRed-Express most likely forms the same tetrameric structure as wild-type DsRed, DsRed-Express displays a reduced tendency to aggregate (2). The DsRed-Express coding sequence is human codon-optimized for high expression in mammalian cells (3).

The DsRed-Express gene is positioned just downstream of the immediate early promoter of cytomegalovirus (P_{CMV IE}). As a result, cells transfected with this vector will express the red fluorescent protein constitutively. The sequence upstream of the DsRed-Express gene has been converted to a Kozak consensus sequence (4) to enhance translation efficiency in eukaryotic cells. SV40 polyadenylation signals downstream of the DsRed-Express gene direct proper processing of the 3' end of the DsRed-Express mRNA. The vector backbone contains an SV40 origin for replication in mammalian cells expressing the SV40 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. This cassette consists of the SV40 early promoter, the neomycin/kanamycin resistance gene of Tn5, and polyadenylation signals from the Herpes simplex virus thymidine kinase (HSV TK) gene. A bacterial promoter upstream of the cassette expresses kanamycin resistance in E. coli.

pCMV-DsRed-Express Vector can be transfected into mammalian cells using any standard transfection method. If required, stable transfectants can be selected using G418 (5).

Package Contents

- 20 μg pCMV-DsRed-Express Vector
Certificate of Analysis
pCMV-DsRed-Express Vector

Storage Conditions
- Store 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)

Figure 1. pCMV-DsRed-Express vector map. Unique restriction sites are in bold. The NotI site follows the DsRed-Express stop codon.

Location of Features
- $P_{CMV\text{ IE}}$ (human cytomegalovirus immediate early promoter): 1–589
- Kozak consensus sequence: 621–631
- DsRed-Express (human-codon-optimized): 628–1305
- SV40 early polyA signals: 1457–1462 & 1486–1491
- f1 origin of replication: 1554–2009
Certificate of Analysis

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- $P$ (promoter for Kan'): 2071–2099
- SV40 origin of replication: 2350–2485
- $P_{SV40+}$ (SV40 early promoter and enhancer): 2183-24121
- Kan’/Neo’ (kanamycin/neomycin resistance gene): 2534–3328
- HSV TK polyA signals (herpes simplex virus thymidine kinase polyadenylation signals): 3564–3582
- pUC origin of replication: 3913–4556

Additional Information

Recommended Sequencing Primer Location:
- 828–808

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 JM109 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (50 μg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: High

Excitation and Emission Maxima of DsRed-Express
- Excitation: 557 nm
- Emission: 579 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</th>
<th>Fragment(s) (kb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PstI</td>
<td>4.6</td>
</tr>
<tr>
<td>StuI</td>
<td>3.2 &amp; 1.4</td>
</tr>
</tbody>
</table>
- Vector identity was confirmed by sequencing.
- $A_{260}/A_{280}$: 1.8–2.0
Notice to Purchaser

pCMV-DsRed-Express Vector

CATALOG NO.
632416

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LICENSING STATEMENTS:
The RCFP’s (including DsRedExpress and DsRedExpress2) 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 and 7,442,522.

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This document has been reviewed and approved by the Clontech Quality Assurance Department.