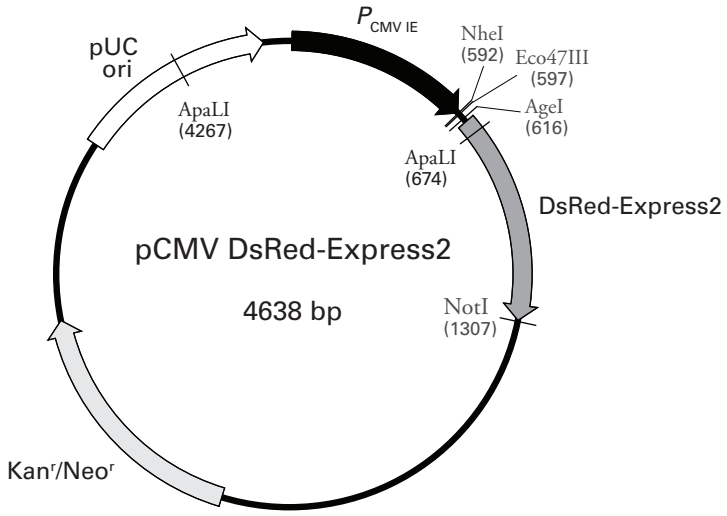
Vector Information

pCMV DsRed-Express2 Vector Information PT4078-5

Catalog No. 632539



pCMV DsRed-Express2 Vector Map.

Description

pCMV DsRed-Express2 is a mammalian expression vector designed to be used for whole cell labeling or as a marker of cotransfection. In mammalian cells, the vector constitutively expresses DsRed-Express2, a variant of the *Discosoma sp.* red fluorescent protein, DsRed (1). DsRed-Express2 retains the fast maturation and high photostability characteristic of its predecessor, DsRed-Express (2), and has been engineered (through additional amino acid substitutions) for increased solubility (3). Although it most likely forms the same tetrameric structure as wild-type DsRed, DsRed-Express2 displays a greatly reduced tendency to aggregate, resulting in reduced cyto- and phototoxicity, and making DsRed-Express2 much better suited for *in vivo* applications involving sensitive cells, such as primary or stem cells. In fact, DsRed-Express2 has been shown to be the best red fluorescent protein for whole cell labeling applications (3). DsRed-Express2 also exhibits extremely low residual green fluorescence, which allows cells expressing the protein to be effectively separated from other fluorescently labeled cell populations by flow cytometry.

The DsRed-Express2 gene is positioned just downstream of the constitutively active human cytomegalovirus immediate early promoter ($P_{CMV\ IE}$). As a result, mammalian cells transfected with this vector will constitutively express the red fluorescent protein. A Kozak consensus sequence (4) has been placed immediately upstream of the DsRed-Express2 coding sequence to enhance translational efficiency in eukaryotic cells. SV40 polyadenylation signals downstream of the DsRed-Express2 gene direct proper processing of the 3' end of the DsRed-Express2 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

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Clontech

United States/Canada
800.662.2566
Asia Pacific
+1.650.919.7300
Europe
+33.(0)1.3904.6880
Japan
+81.(0)77.543.6116

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

resistance cassette (Neo^r) allows stably transfected eukaryotic cells to be selected using G418 (5). This cassette consists of the SV40 early promoter, a Tn5 kanamycin/neomycin resistance gene, and herpes simplex virus thymidine kinase (HSVTK) polyadenylation signals. A bacterial promoter upstream of the cassette expresses kanamycin resistance in *E. coli*.

Use

pCMV DsRed-Express2 can be used for whole cell labeling or as a cotransfection marker. The vector can be transfected into mammalian cells using any standard transfection method. Cells expressing DsRed-Express2 (excitation and emission maxima: 554 nm and 591 nm, respectively) can be detected by fluorescence microscopy or flow cytometry 8-12 hours after transfection. If required, stable transfectants can be selected using G418. After cotransfection with pCMV DsRed-Express2 and an expression construct of interest, cells can also be sorted by flow cytometry to enrich for transfected cells.

Location of features

- $P_{CMV\ IE}$ (human cytomegalovirus immediate early promoter): 1–589
- DsRed-Express2 (*Discosoma sp.* red fluorescent protein variant)
  Kozak consensus translation initiation site: 621–631
  Start codon (ATG): 628–630; Stop codon: 1303–1305
- Kan^r/Neo^r (kanamycin/neomycin resistance gene)
  Neomycin phosphotransferase coding sequences: 2534–3328
- pUC origin of replication: 3913–4556

Propagation in *E. coli*

- Recommended host strain: 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) in *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: high
- Plasmid incompatibility group: pMB1/ColE1

Excitation and emission maxima of DsRed-Express2

- Excitation maximum = 554 nm
- Emission maximum = 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. 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.

Note: The vector sequence was compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by Clontech. This vector has not been completely sequenced.

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CMV Sequence:

DsRed-Express & DsRed-Express2:

Living Colors® Products AcGFP1, DsRed, HcRed, AsRed, AmCyan, ZsGreen, ZsYellow and their variants:

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