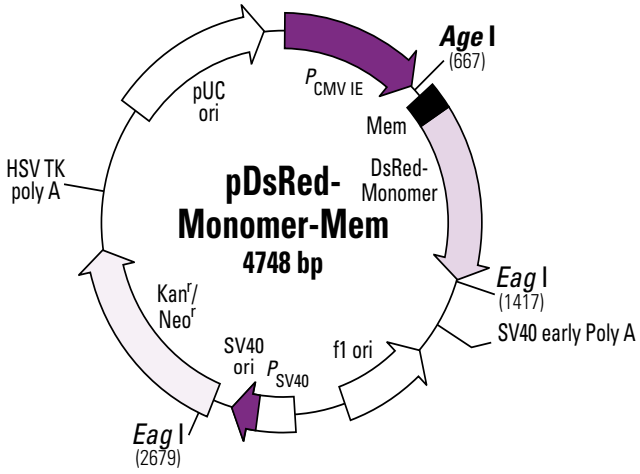
Vector Information

pDsRed-Monomer-Mem Vector Information PT3892-5

Catalog No. 632512



Description

pDsRed-Monomer-Mem encodes a fusion protein consisting of the N-terminal 20 amino acids of neuromodulin, also called GAP-43, and a monomeric red fluorescent protein. The neuromodulin fragment contains a signal for posttranslational palmitoylation of cysteines 3 and 4 that targets DsRed-Monomer to cellular membranes, and the plasma membrane in particular.

DsRed-Monomer (DsRed.M1) is a monomeric mutant derived from the tetrameric *Discosoma* sp. red fluorescent protein DsRed (1). DsRed-Monomer contains a total of forty-five amino acid substitutions. When DsRed-Monomer is expressed in mammalian cell cultures, red fluorescent cells can be detected by either fluorescence microscopy or flow cytometry 12–16 hr after transfection (DsRed-Monomer excitation and emission maxima = 557 nm and 592 nm, respectively). The DsRed-Monomer coding sequence is human-codon-optimized for increased translation efficiency in mammalian cells (2). SV40 polyadenylation signals downstream of the DsRed-Monomer-Mem gene direct proper processing of the 3′ end of the DsRed-Monomer-Mem mRNA. The vector backbone also contains an SV40 origin for replication in mammalian cells expressing the SV40 T-antigen. A neomycin resistance cassette (Neo^r), consisting 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, allows stably transfected eukaryotic cells to be selected using G418. A bacterial promoter upstream of this cassette expresses kanamycin resistance in *E. coli.* The pDsRed-Monomer-Mem backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

Use

DsRed-Monomer-Mem is designed for use as a plasma membrane marker, as well as a cotransfection marker. Because it remains attached to the plasma membrane, it can be detected by fluorescence microscopy in permeabilized cells after ethanol fixation (3). The vector can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (4). pDsRed-Monomer-Mem cannot be used as an exclusive plasma membrane marker because it also partially labels intracellular membranes.

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Location of features

- Human cytomegalovirus (CMV) immediate early promoter: 1–589
  Enhancer region: 59–465; TATA box: 554–560
- DsRed-Monomer-Mem fusion gene
  Start codon (ATG): 679–681
  Neuromodulin N-terminal sequence: 679–738
  Discosoma sp. monomeric red fluorescent protein (DsRed-Monomer) gene: 739–1410
  Stop codon: 1411–1413
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1567–1572 & 1596-1601; mRNA 3′ ends: 1605 & 1617
- f1 single-strand DNA origin: 1664–2119 (packages the noncoding strand of DsRed-Monomer)
- Bacterial promoter for expression of Kan^r gene
  –35 region: 2181-2186; –10 region: 2204–2209
- SV40 origin of replication: 2460–2598
- SV40 early promoter
  Enhancer (72 bp tandem repeats): 2293–2364 & 2365–2436
  21 bp repeats: 2440–2460, 2461–2481 & 2483–2503
  Early promoter element: 2516–2522
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 2644–2646; stop codon: 3436–3438
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3674–3679 & 3687–3692
- pUC plasmid replication origin: 4023–4666

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) in *E. coli* hosts.
- *E. coli* replication origin: pUC; copy number: high

References

1. Matz, M. V., et al. (1999) *Nature Biotech.* **17(10)**:969–973.
2. Haas, J., et al. (1996) *Curr. Biol.* **6**:315–324.
3. Jiang, W. & Hunter, T. (1998) *BioTechniques* **24**:348–354.
4. Gorman, C. (1985) In *DNA Cloning: A Practical Approach,* Vol. II, Ed. Glover, D. M. (IRL Press, Oxford, UK) pp. 143–190.

Note: The attached sequence file has been 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.