



Description

pDsRed-Monomer-Mem Hyg 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 hygromycin resistance cassette (Hyg^r), consisting of the SV40 early promoter, the hygromycin resistance gene, and SV40 polyadenylation signals, allows stably transfected eukaryotic cells to be selected using hygromycin B. A bacterial promoter upstream of the ampicillin gene expresses ampicillin resistance in *E. coli*. The pDsRed-Monomer-Mem Hyg 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 Hyg 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 hygromycin B. The added feature of a hygromycin resistance cassette complements the neomycin resistance cassette in the currently available Living Colors vectors. pDsRed-Monomer-Mem Hyg 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)
- 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
- Hygromycin resistance gene:
Start codon (ATG): 2617–2619; stop codon: 3640–3642
- SV40 early mRNA polyadenylation signal: 3790–3795 & 3819–3824; mRNA 3' ends: 3828 & 3840
- Bacterial promoter for expression of Ampr gene:
–35 region: 3989–3994; –10 region: 4012–4017
- Ampicillin resistance gene:
Start codon (ATG): 4059–4061; stop codon: 4917–4919
- pUC plasmid replication origin: 5082–5725

Propagation in *E. coli*

- Suitable host strains: DH5 α , 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 ampicillin (100 μ g/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

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.

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