**Description**

pDsRed-Monomer-N1 is a mammalian expression vector that encodes DsRed-Monomer (DsRed.M1), a monomeric mutant derived from the tetrameric *Discosoma* sp. red fluorescent protein DsRed (1). DsRed-Monomer contains forty-five amino acid substitutions (listed on page 2). 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 high expression in mammalian cells (2).

DsRed-Monomer is well suited for use as a fusion tag. The multiple cloning site (MCS) in pDsRed-Monomer-N1 is positioned between the immediate early promoter of CMV (P<sub>CMV IE</sub>) and the DsRed-Monomer coding sequence. Genes cloned into the MCS are expressed as fusions to the N-terminus of DsRed-Monomer if they are in the same reading frame as DsRed-Monomer and there are no intervening stop codons. A Kozak consensus sequence is located immediately upstream of the DsRed-Monomer gene to enhance translational efficiency in eukaryotic systems (3). SV40 polyadenylation signals downstream of the DsRed-Monomer gene direct proper processing of the 3' end of the DsRed-Monomer 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 confers kanamycin resistance in E. coli.

(Pr0x3719; published October 2010)
Use

pDsRed-Monomer-N1 can be used to construct fusions to the N-terminus of DsRed-Monomer. If a fusion construct retains the fluorescent properties of the native DsRed-Monomer protein, its expression can be monitored by flow cytometry and its localization in vivo can be determined by fluorescence microscopy. The target gene must be cloned into pDsRed-Monomer-N1 so that it is in frame with the DsRed-Monomer coding sequence, with no intervening in-frame stop codons. The inserted gene must include an initiating ATG codon. Recombinant pDsRed-Monomer-N1 can be transfected into mammalian cells using any standard transfection method. If required, stable transfectants can be selected using G418 (4). pDsRed-Monomer-N1 can also be used as a cotransfection marker; the unmodified vector will express DsRed-Monomer.

The DsRed1-N Sequencing Primer (Cat. No. 632387) can be used to sequence genes cloned adjacent to the 5’ end of the DsRed-Monomer coding region.

For Western blotting, the Living Colors® DsRed Polyclonal Antibody (Cat. No. 632496) can be used to recognize the DsRed-Monomer protein. However, to generate optimal results it may be necessary to use a higher concentration of antibody than recommended on the DsRed Polyclonal Antibody Certificate of Analysis.

Location of features

- Human cytomegalovirus (CMV) immediate early promoter: 1–589
  - Enhancer region: 59–465; TATA box: 554–560
  - Transcription start point: 583
  - C→G mutation to remove Sac I site: 569
- Multiple Cloning Site: 591–671
- Human codon-optimized DsRed-Monomer gene
  - Kozak consensus translation initiation site: 672–682
  - Start codon (ATG): 679–681; Stop codon: 1354–1356
  - Amino acid substitutions (DsRed→DsRed-Monomer):
    - GCC→GAC (Ala-2 to Asp) mutation: 682–684
    - TCC→AAC (Ser-3 to Asn) mutation: 685–687
    - TCC→ACC (Ser-4 to Thr) mutation: 688–690
    - AAG→GAG (Lys-5 to Glu) mutation: 691–693
    - AAC→GAC (Asn-6 to Asp) mutation: 694–696
    - CGC→CAG (Arg-13 to Gln) mutation: 715–717
    - ACC→TCC (Thr-21 to Ser) mutation: 739–741
    - GAG→TAC (Glu-26 to Tyr) mutation: 754–756
    - CGC→AAG (Arg-36 to Lys) mutation: 784–786
    - CAC→ACC (His-41 to Thr) mutation: 799–801
    - AAC→CAG (Asn-42 to Gln) mutation: 802–804
    - GTG→GCC (Val-44 to Ala) mutation: 808–810
    - AAG→CAG (Lys-47 to Gln) mutation: 817–819
    - GTG→GCC (Val-71 to Ala) mutation: 889–891
    - AAG→ATG (Lys-83 to Met) mutation: 925–927
    - AAG→ACC (Lys-92 to Thr) mutation: 952–954
    - GTG→TCC (Val-96 to Ser) mutation: 964–966
    - ACC→GAG (Thr-106 to Glu) mutation: 994–996
    - ACC→CAG (Thr-108 to Gln) mutation: 1000–1002
    - TCC→ACC (Ser-17 to Thr) mutation: 1027–1029
    - ATC→AAG (Ile-125 to Lys) mutation: 1051–1053
    - TCC→GCC (Ser-131 to Ala) mutation: 1069–1071
    - ATG→GCC (Met-141 to Ala) mutation: 1099–1101
    - GCC→CCC (Ala-145 to Pro) mutation: 1111–1113
    - CGC→AAG (Arg-149 to Lys) mutation: 1123–1125
    - CGC→CAG (Arg-153 to Gln) mutation: 1135–1137
    - CAC→TCC (His-162 to Ser) mutation: 1162–1164
    - AAG→CAC (Lys-163 to His) mutation: 1165–1167
    - CTG→ACC (Leu-174 to Thr) mutation: 1198–1200
    - GTG→TGC (Val-175 to Cys) mutation: 1201–1203
    - GAG→GAC (Glu-176 to Asp) mutation: 1204–1206
    - TCC→ACC (Ser-179 to Thr) mutation: 1213–1215
    - ATC→GTG (Ile-180 to Val) mutation: 1216–1218
    - ATG→AAG (Met-182 to Lys) mutation: 1222–1224
    - TAC→AAC (Tyr-192 to Asn) mutation: 1252–1254
pDsRed-Monomer-N1

**Vector Information**

- **TAC→CAC** (Tyr-193 to His) mutation: 1255–1257
- **TCC→AAC** (Ile-210 to Val) mutation: 1306–1308
- **ATC→GTG** (Ile-210 to Val) mutation: 1306–1308
- **ACG→CAC** (Arg-216 to His) mutation: 1324–1326
- **ACC→GCC** (Thr-217 to Ala) mutation: 1327–1329
- **CGC→GCC** (Gly-219 to Ala) mutation: 1333–1335
- **CAC→TCC** (His-222 to Ser) mutation: 1342–1344
- **CTG→GGC** (Leu-223 to Gly) mutation: 1345–1347
- **TTC→TCC** (Phe-224 to Ser) mutation: 1348–1350
- **CTG→CAG** (Leu-225 to Gln) mutation: 1351–1353

- **SV40 early mRNA polyadenylation signal**
  - Polyadenylation signals: 1510–1515 & 1539–1544; mRNA 3' ends: 1548 & 1560
- **f1 single-strand DNA origin**: 1607–2062 (Packages the noncoding strand of DsRed-Monomer)
- **Bacterial promoter for expression of Kan’ gene**:
  - ~35 region: 2124–2129; ~10 region: 2147–2152
  - Transcription start point: 2159
- **SV40 origin of replication**: 2403–2538
- **SV40 early promoter**
  - Enhancer (72-bp tandem repeats): 2236–2307 & 2308–2379
  - 21-bp repeats: 2383–2403, 2404–2424 & 2426–2446
  - Early promoter element: 2459–2465
  - Major transcription start points: 2455, 2493, 2499 & 2504
- **Kanamycin/neomycin resistance gene**
  - Neomycin phosphotransferase coding sequences: Start codon (ATG): 2587–2589; Stop codon: 3379–3381
  - G→A mutation to remove Pst I site: 2769
  - C→A (Arg to Ser) mutation to remove BssH II site: 3115
- **Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal**
  - Polyadenylation signals: 3617–3622 & 3630–3635
- **pUC plasmid replication origin**: 3966–4609

**Sequencing primer location**
- **DsRed1-N Sequencing Primer (Cat. No. 632387; 5'-GTACTGGAACTGGGGGACAG-3')**: 879–859

**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
- Plasmid incompatibility group: pMB1/ColE1

**Excitation and emission maxima of DsRed-Monomer**
- Excitation maximum = 557 nm
- Emission maximum = 592 nm

**References**

**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 Laboratories, Inc.. This vector has not been completely sequenced.
Notice to Purchaser

Clontech products are to be used for research purposes only. They may not be used for any other purpose, including, but not limited to, use in drugs, in vitro diagnostic purposes, therapeutics, or in humans. Clontech products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without written approval of Clontech Laboratories, Inc.

Not-For-Profit Entities: Orders may be placed in the normal manner by contacting your local representative or Clontech Customer Service at 650.919.7300. At its discretion, Clontech grants Not-For-Profit Entities a non-exclusive, personal, limited license to use this product for non-commercial life science research use only. Such license specifically excludes the right to sell or otherwise transfer this product, its components or derivatives thereof to third parties. No modifications to the protein coding sequence may be made without express written permission from Clontech. Any other use of this product requires a license from Clontech. For license information, please contact a licensing representative by phone at 650.919.7320 or by e-mail at licensing@clontech.com. For-Profit Entities wishing to use this product are required to obtain a license from Clontech. For license information, please contact a licensing representative by phone at 650.919.7320 or by e-mail at licensing@clontech.com or click here for more information.

The DsRed Monomer and the Fruit Fluorescent Proteins are covered by one or more of the following U.S. Patents: 7,157,566; 7,393,923; 7,005,511 and 7,250,298.

Clontech, the Clontech logo and all other trademarks are the property of Clontech Laboratories, Inc., unless noted otherwise. Clontech is a Takara Bio Company. ©2010 Clontech Laboratories, Inc.