



Restriction Map and Multiple Cloning Site (MCS) of pDsRed-Monomer. All sites shown are unique. NOTE: The *Xba* I and *Bcl* I sites are methylated in the DNA provided by Clontech Laboratories, Inc. If you wish to digest the vector with these enzymes, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

Description

pDsRed-Monomer is a prokaryotic 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). The excitation and emission spectra is comparable to that of DsRed-Express (DsRed-Monomer excitation and emission maxima = 557 nm and 585 nm, respectively). The DsRed-Monomer coding sequence has been human codon-optimized for high expression in mammalian cells (2).

In pDsRed-Monomer, the DsRed-Monomer coding sequence is flanked at the 5' and 3' ends by separate and distinct multiple cloning sites (MCS), making it easy to excise the gene for use in other cloning applications. Alternatively, the DsRed-Monomer coding sequence can be amplified by PCR. In *E. coli*, DsRed-Monomer is expressed from the *lac* promoter as a fusion with several amino acids, including the first five amino acids of the *LacZ* protein. Note, however, that if you excise the DsRed-Monomer coding sequence using a restriction site in the 5' MCS, the resulting fragment will encode solely the DsRed-Monomer protein (i.e., without the additional amino acids that are expressed using the *lac* promoter). A Kozak consensus sequence is located immediately upstream of the DsRed-Monomer gene to enhance translational efficiency in eukaryotic systems (3). The entire DsRed-Monomer expression cassette in pDsRed-Monomer is supported by a pUC19 backbone, which contains a high-copy number origin of replication and an ampicillin resistance gene for propagation and selection in *E. coli*.

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Use

pDsRed-Monomer is primarily intended to serve as a source of DsRed-Monomer cDNA. The flanking MCS regions make it possible to excise the DsRed-Monomer coding sequence and insert it into other vector systems of choice. The vector can also be used in bacteria to produce DsRed-Monomer protein.

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

- *lac* Promoter: 95–178
 - CAP binding site: 111–124
 - 35 region: 143–148; –10 region: 167–172
 - Transcription start point: 179
 - lac* operator: 179–199
- *lacZ*-DsRed-Monomer fusion protein expressed in *E. coli*
 - Ribosome binding site: 206–209
 - Start codon (ATG): 217–219; Stop codon 964–966
 - Amino acid substitutions (DsRed→DsRed-Monomer)
 - GCC→GAC (Ala-2 to Asp) mutation: 292–294
 - TCC→AAC (Ser-3 to Asn) mutation: 295–297
 - TCC→ACC (Ser-4 to Thr) mutation: 298–300
 - AAG→GAG (Lys-5 to Glu) mutation: 301–303
 - AAC→GAC (Asn-6 to Asp) mutation: 304–306
 - CGC→CAG (Arg-13 to Gln) mutation: 325–327
 - ACC→TCC (Thr-21 to Ser) mutation: 349–351
 - GAG→TAC (Glu-26 to Tyr) mutation: 364–366
 - CGC→AAG (Arg-36 to Lys) mutation: 394–396
 - CAC→ACC (His-41 to Thr) mutation: 409–411
 - AAC→CAG (Asn-42 to Gln) mutation: 412–414
 - GTG→GCC (Val-44 to Ala) mutation: 418–420
 - AAG→CAG (Lys-47 to Gln) mutation: 427–429
 - GTG→GCC (Val-71 to Ala) mutation: 499–501
 - AAG→ATG (Lys-83 to Met) mutation: 535–537
 - AAG→ACC (Lys-92 to Thr) mutation: 562–564
 - GTG→TCC (Val-96 to Ser) mutation: 574–576
 - ACC→GAG (Thr-106 to Glu) mutation: 604–606
 - ACC→CAG (Thr-108 to Gln) mutation: 610–612
 - TCC→ACC (Ser-117 to Thr) mutation: 637–639
 - ATC→AAG (Ile-125 to Lys) mutation: 661–663
 - TCC→GCC (Ser-131 to Ala) mutation: 679–681
 - ATG→GCC (Met-141 to Ala) mutation: 709–711
 - GCC→CCC (Ala-145 to Pro) mutation: 721–723
 - CGC→AAG (Arg-149 to Lys) mutation: 733–735
 - CGC→CAG (Arg-153 to Gln) mutation: 745–747
 - CAC→TCC (His-162 to Ser) mutation: 772–774
 - AAG→CAC (Lys-163 to His) mutation: 775–777
 - CTG→ACC (Leu-174 to Thr) mutation: 808–810
 - GTG→TGC (Val-175 to Cys) mutation: 811–813
 - GAG→GAC (Glu-176 to Asp) mutation: 814–816
 - TCC→ACC (Ser-179 to Thr) mutation: 823–825
 - ATC→GTG (Ile-180 to Val) mutation: 826–828
 - ATG→AAG (Met-182 to Lys) mutation: 832–834
 - TAC→AAC (Tyr-192 to Asn) mutation: 862–864
 - TAC→CAC (Tyr-193 to His) mutation: 865–867
 - TCC→AAC (Ser-203 to Asn) mutation: 895–897
 - ATC→GTG (Ile-210 to Val) mutation: 916–918
 - CGC→CAC (Arg-216 to His) mutation: 934–936
 - ACC→GCC (Thr-217 to Ala) mutation: 937–939
 - GGC→GCC (Gly-219 to Ala) mutation: 943–945

- CAC→TCC (His-222 to Ser) mutation: 952–954
- CTG→GGC (Leu-223 to Gly) mutation: 955–957
- TTC→TCC (Phe-224 to Ser) mutation: 958–960
- CTG→CAG (Leu-225 to Gln) mutation: 961–963
- 5' Multiple Cloning Site: 234–281
- Human codon-optimized DsRed-Monomer gene
 - Kozak consensus translation initiation site: 282–292
 - Start codon (ATG): 289–291; Stop codon: 964–966
- 3' Multiple cloning site: 968–1067
- Ampicillin resistance gene
 - Promoter
 - 35 region: 1441–1446; –10 region: 1464–1469
 - Transcription start point: 1476
 - Ribosome binding site: 1499–1503
 - β-lactamase coding sequences
 - Start codon (ATG): 1513–1515; Stop codon: 2371–2373
 - β-lactamase signal peptide: 1513–1581
 - β-lactamase mature protein: 1582–2370
- pUC plasmid replication origin: 2521–3163

Sequencing primer locations

- **Recommended:** DsRed-Monomer-C sequencing primer (5'-AGCTGGACATCACCAACCACAACG-3'): 881–904
- DsRed1-N Sequencing Primer (Cat. No. 632387; 5'-GTACTGGAAGTGGGGGGACAG-3'): 489–469
 - Note:** The DsRed1-C Sequencing Primer (Cat. No. 632388) **cannot** be used as a sequencing primer for pDsRed-Monomer.

Propagation in *E. Coli*

- Recommended host strain: DH5α
- Selectable marker: plasmid confers resistance to ampicillin (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 = 585 nm

References

1. Matz, M. V., et al. (1999) *Nature Biotech.* **17**(10):969–973.
2. Haas, J., et al. (1996) *Curr. Biol.* **6**(3):315–324.
3. Kozak, M. (1987) *Nucleic Acids Res.* **15**(20):8125–8148.

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.

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