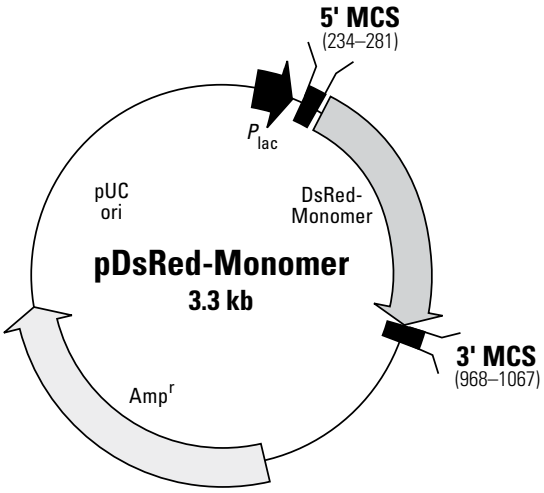
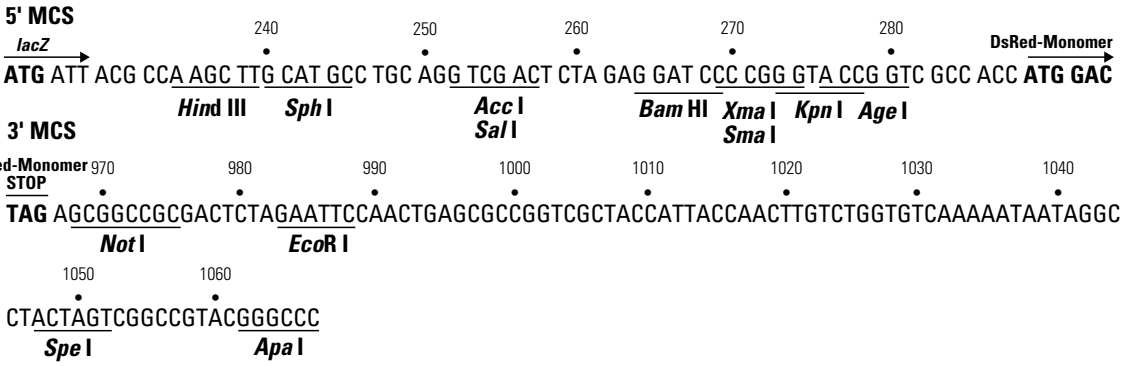
Vector Information

pDsRed-Monomer Vector Information PT3794-5

Cat. No. 632467





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 592 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-71to 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'-GTACTGGAACTGGGGGGACAG-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 = 592 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.