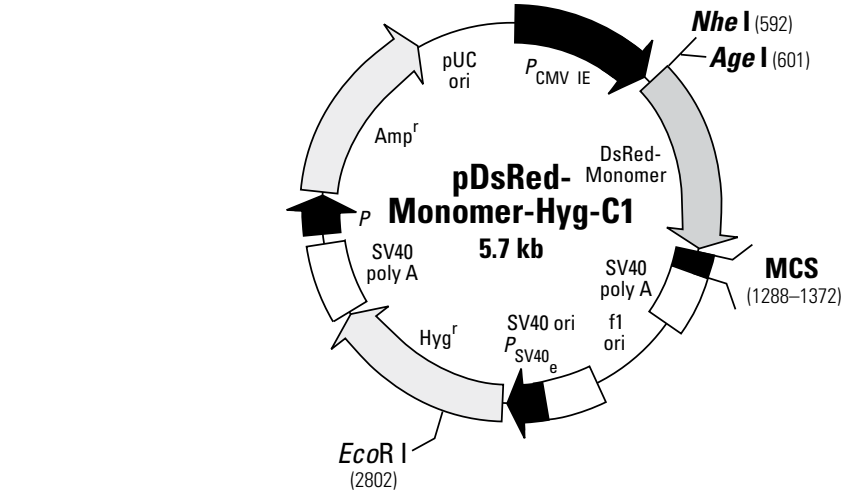
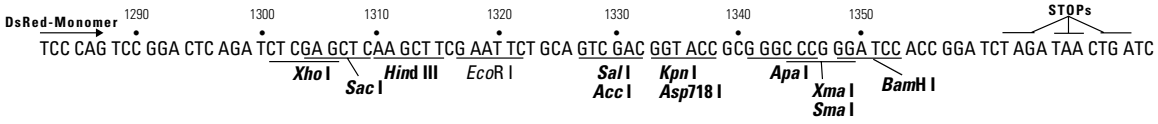
Vector Information

pDsRed-Monomer-Hyg-C1 Vector Information PT3842-5

Cat. No. 632495





Restriction Map and Multiple Cloning Site (MCS) of pDsRed-Monomer-Hyg-C1. Unique restriction sites are in bold. 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-Hyg-C1 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 levels in mammalian cells (2).

DsRed-Monomer is well suited for use as a fusion tag. The multiple cloning site (MCS) in pDsRed-Monomer-Hyg-C1 is positioned between the DsRed-Monomer coding sequence and the SV40 polyadenylation signal (SV40 poly A). Genes cloned into the MCS are expressed as fusions to the C-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 DsRed-Monomer gene to enhance the translational efficiency in eukaryotic systems (3). SV40 polyadenylation signals downstream of the MCS direct proper processing of the 3' end of mRNA transcripts. The vector backbone contains an SV40 origin for replication in mammalian cells expressing the SV40T antigen, a pUC origin of replication for propagation in *E. coli*, and an f1 origin for single-stranded DNA production. 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. A bacterial promoter-resistance gene cassette confers ampicillin resistance in *E. coli*.

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Use

pDsRed-Monomer-Hyg-C1 can be used to construct fusions to the C-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-Hyg-C1 so that it is in frame with the DsRed-Monomer coding sequences, with no intervening in-frame stop codons. pDsRed-Monomer-Hyg-C1 can be transfected into mammalian cells using any standard transfection method. If required, stable transfectants can be selected using hygromycin. pDsRed-Monomer-Hyg-C1 can also be used as a cotransfection marker; the unmodified vector will express DsRed-Monomer.

This vector can also be cotransfected with pAcGFP1-N1 (Cat. No. 632469) or pAcGFP1-C1 (Cat. No. 632470) to establish stable cell lines expressing two different fluorescent proteins. Different selection markers (hygromycin for pDsRed-Monomer-Hyg-C1, neomycin for pAcGFP1-N1 and pAcGFP1-C1) allow for the generation of cell lines that simultaneously express red and green fluorescent proteins.

We recommend using the DsRed-Monomer-C sequencing primer (see Sequencing primer location information below) to sequence genes cloned adjacent to the 3' 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, for optimal results, it may be necessary to use a higher concentration of antibody than recommended on the DsRed Polyclonal Antibody Product Analysis Certificate.

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
- Human codon-optimized DsRed-Monomer gene
  - Kozak consensus translation initiation site: 606–616
  - Start codon (ATG): 613–615; Stop codons: 1362–1364, 1366–1368 & 1370–1372
  - Amino acid substitutions (DsRed→DsRed-Monomer)
  - GCC→GAC (Ala-2 to Asp) mutation: 616–618
  - TCC→AAC (Ser-3 to Asn) mutation: 619–621
  - TCC→ACC (Ser-4 to Thr) mutation: 622–624
  - AAG→GAG (Lys-5 to Glu) mutation: 625–627
  - AAC→GAC (Asn-6 to Asp) mutation: 628–630
  - CGC→CAG (Arg-13 to Gln) mutation: 649–651
  - ACC→TCC (Thr-21 to Ser) mutation: 673–675
  - GAG→TAC (Glu-26 to Tyr) mutation: 688–690
  - CGC→AAG (Arg-36 to Lys) mutation: 718–720
  - CAC→ACC (His-41 to Thr) mutation: 733–735
  - AAC→CAG (Asn-42 to Gln) mutation: 736–738
  - GTG→GCC (Val-44 to Ala) mutation: 742–744
  - AAG→CAG (Lys-47 to Gln) mutation: 751–753
  - GTG→GCC (Val-71to Ala) mutation: 823–825
  - AAG→ATG (Lys-83 to Met) mutation: 859–861
  - AAG→ACC (Lys-92 to Thr) mutation: 886–888
  - GTG→TCC (Val-96 to Ser) mutation: 898–900
  - ACC→GAG (Thr-106 to Glu) mutation: 928–930
  - ACC→CAG (Thr-108 to Gln) mutation: 934–936
  - TCC→ACC (Ser-117 to Thr) mutation: 961–963
  - ATC→AAG (Ile-125 to Lys) mutation: 985–987
  - TCC→GCC (Ser-131 to Ala) mutation: 1003–1005
  - ATG→GCC (Met-141 to Ala) mutation: 1033–1035
  - GCC→CCC (Ala-145 to Pro) mutation: 1045–1047
  - CGC→AAG (Arg-149 to Lys) mutation: 1057–1059
  - CGC→CAG (Arg-153 to Gln) mutation: 1069–1071
  - CAC→TCC (His-162 to Ser) mutation: 1096–1098
  - AAG→CAC (Lys-163 to His) mutation: 1099–1101
  - CTG→ACC (Leu-174 to Thr) mutation: 1132–1134
  - GTG→TGC (Val-175 to Cys) mutation: 1135–1137

GAG→GAC (Glu-176 to Asp) mutation: 1138–1140
TCC→ACC (Ser-179 to Thr) mutation: 1147–1149
ATC→GTG (Ile-180 to Val) mutation: 1150–1152
ATG→AAG (Met-182 to Lys) mutation: 1156–1158
TAC→AAC (Tyr-192 to Asn) mutation: 1186–1188
TAC→CAC (Tyr-193 to His) mutation: 1189–1191
TCC→AAC (Ser-203 to Asn) mutation: 1219–1221
ATC→GTG (Ile-210 to Val) mutation: 1240–1242
CGC→CAC (Arg-216 to His) mutation: 1258–1260
ACC→GCC (Thr-217 to Ala) mutation: 1261–1263
GGC→GCC (Gly-219 to Ala) mutation: 1267–1269
CAC→TCC (His-222 to Ser) mutation: 1276–1278
CTG→GGC (Leu-223 to Gly) mutation: 1279–1281
TTC→TCC (Phe-224 to Ser) mutation: 1282–1284
CTG→CAG (Leu-225 to Gln) mutation: 1285–1287

- Multiple Cloning Site: 1288–1372
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1507–1512 & 1536–1541; mRNA 3' ends: 1545 & 1657
- f1 single-strand DNA origin: 1604–2059 (Packages the noncoding strand of DsRed-Monomer)
- SV40 origin of replication: 2401–2536
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2234–2305 & 2306–2377
  21-bp repeats: 2381–2401, 2402–2422 & 2424–2441
  Early promoter element: 2457–2463
  Major transcription start points: 2453, 2491, 2497 & 2502
- Hygromycin resistance gene:
  Start codon (ATG): 2558–2560; stop codon: 3581–3583
- SV40 early mRNA polyadenylation signal: 3730–3735 & 3759–3764; mRNA 3' ends: 3768 & 3780
- Bacterial promoter for expression of Amp^r gene:
  –35 region: 3930–3935; –10 region: 3953–3958
- Ampicillin resistance gene:
  Start codon (ATG): 4000–4002; stop codon: 4858–4860
- pUC plasmid replication origin: 5043–5666

Sequencing primer location

- DsRed-Monomer-C sequencing primer (5'-AGCTGGACATCACCAACCACAACG-3'): 1205–1228
  Note: The DsRed1-C Sequencing Primer (Cat. No. 632388) **cannot** be used as a sequencing primer for pDsRed-Monomer-Hyg-C1.

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 ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: high

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**:315–324.
3. Kozak, M. (1987) *Nucleic Acids Res.* **15**: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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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.