Certificate of Analysis



pDsRed-Express Vector

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Catalog No. Amount Lot Number 632412 Specified on product label. $20 \mu g$

Product Information

pDsRed-Express is a prokaryotic expression vector that encodes DsRed-Express, a variant of *Discosoma* sp. red fluorescent protein (DsRed; 1). pDsRed-Express is primarily intended to serve as a source of DsRed-Express cDNA. The flanking MCS regions make it possible to excise the DsRed-Express coding sequence and insert it into other expression vectors of choice. The vector can also be used in bacteria to produce DsRed-Express protein.

DsRed-Express contains nine amino acid substitutions which improve the solubility of the protein, reduce the time from transfection to detection of red fluorescence, and decrease the level of residual green emission (2). When DsRed-Express is expressed in mammalian cell cultures, red-emitting cells can be detected by either fluorescence microscopy or flow cytometry 8–12 hours after transfection. Although DsRed-Express most likely forms the same tetrameric structure as wild-type DsRed, DsRed-Express displays a reduced tendency to aggregate (2). The DsRed-Express coding sequence is human codon-optimized for high expression in mammalian cells (3).

In pDsRed-Express, the DsRed-Express 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-Express coding sequence can be amplified by PCR. In E. coli, DsRed-Express 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-Express coding sequence using a restriction site in the 5' MCS, the resulting fragment will encode the native (i.e., non-fusion) DsRed-Express protein. A Kozak consensus sequence is located immediately upstream of the DsRed-Express gene to enhance translational efficiency in eukaryotic systems (4). The entire DsRed-Express expression cassette in pDsRed-Express 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.

Package Contents

20 µg pDsRed-Express Vector

Storage Conditions

- Store at -20° C.
- Spin briefly to recover contents.
- Avoid repeated freeze/thaw cycles.

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Shelf Life

• 1 year from date of receipt under proper storage conditions.

Storage Buffer

• 10 mM Tris-HCl (pH 8.0), 1 mM EDTA (pH 8.0)

Concentration

• 500 ng/μl

Shipping Conditions

• Dry ice (-70°C)

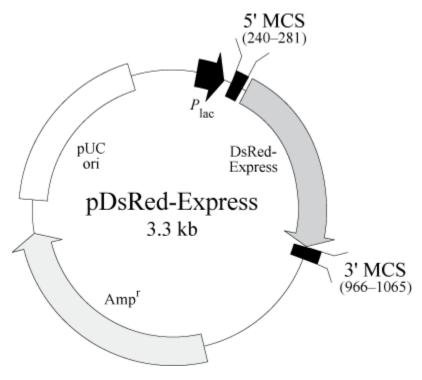


Figure 1. pDsRed-Express vector map.

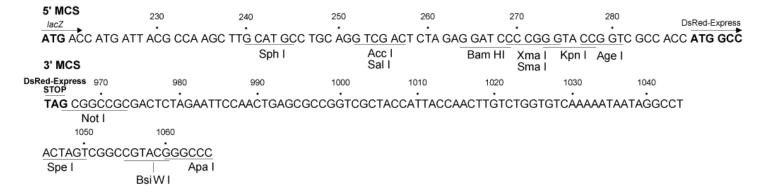


Figure 2. pDsRed-Express multiple cloning sites.

Location of Features

• P_{lac} (lac promoter): 95–178

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pDsRed-Express Vector

- lacZ-DsRed-Express fusion protein expressed in E. coli: 217–966
- 5' MCS (5' multiple cloning site): 240–281
- Kozak consensus sequence: 282–292
- DsRed-Express: 289–966
- 3' MCS (3' multiple cloning site): 966–1065
- Amp^r (ampicillin resistance gene: β-lactamase):1511–2371
- pUC origin of replication: 2519–3162

Additional Information

Recommended Primer Location:

• 489–469

Propagation in E. coli

- Recommended host strain: DH5α
- Selectable marker: plasmid confers resistance to ampicillin (50 μg/ml) to *E. coli* hosts.
- E. coli replication origin: pUC
- Copy number: High

Excitation and Emission Maxima of DsRed-Express

Excitation: 557 nmEmission: 579 nm

References

- 1. Matz, M. V., et al. (1999) Nat. Biotechnol. 17:969-973.
- 2. Bevis, B. J. & Glick, B. S. (2002) *Nature Biotechnol.* **20**:83–87.
- 3. Haas, J., et al. (1996) Curr. Biol. 6:315-324.
- 4. Kozak, M. (1987) Nucleic Acids Res. 15:8125-8148.

Quality Control Data

Plasmid Identity & Purity

• Digestion with the indicated restriction enzymes produced fragments of the indicated sizes on a 0.8% agarose/EtBr gel:

Enzyme Fragment(s) (kb)

BamHI 3.3 PstI 2.9 & 0.4

- Vector identity was confirmed by sequencing.
- A_{260}/A_{280} : 1.8–2.0

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pDsRed-Express Vector

CATALOG NO. 632412

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LICENSING STATEMENTS:

The RCFP's (including DsRedExpress and DsRedExpress2) are covered by one or more of the following U.S. Patent Nos. 7,166,444; 7,157,565; 7,217,789; 7,338,784; 7,338,783; 7,537,915 6,969,597, 7,150,979 and 7,442,522.

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