

pDsRed-Express-1 Vector

Table of Contents

Product Information	1
Location of Features	2
Additional Information	3
Quality Control Data.....	3

Catalog No.	Amount	Lot Number
632413	20 µg	Specified on product label.

Product Information

pDsRed-Express-1 is a promoterless mammalian expression vector that can be used to monitor transcription from different promoters and promoter/enhancer combinations inserted into the multiple cloning site (MCS). It encodes DsRed-Express, a variant of *Discosoma* sp. red fluorescent protein (DsRed; 1). 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).

The sequence upstream of the DsRed-Express gene has been converted to a Kozak consensus sequence (4) to enhance translation efficiency in eukaryotic cells. SV40 polyadenylation signals downstream of the DsRed-Express gene direct proper processing of the 3' end of the DsRed-Express 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^r) 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 expresses kanamycin resistance in *E. coli*.

Promoters should be cloned into the pDsRed-Express-1 MCS upstream from the DsRed-Express coding sequence. Without addition of a functional promoter, this vector will not express DsRed-Express. The recombinant DsRed-Express vector can be transfected into mammalian cells using any standard transfection method. If required, stable transfectants can be selected using G418 (5).

Package Contents

- 20 µg pDsRed-Express-1 Vector

Storage Conditions

- Store at –20°C.

Clontech Laboratories, Inc.

A Takara Bio Company
1290 Terra Bella Avenue, Mountain View, CA 94043, USA
U.S. Technical Support: tech@clontech.com

United States/Canada	Asia Pacific	Europe	Japan
800.662.2566 (PA124372)	+1.650.919.7300	+33.(0)1.3904.6880	+81.(0)77.543.6116

Certificate of Analysis

Cat. No. 632413

pDsRed-Express-1 Vector

- Spin briefly to recover contents.
- Avoid repeated freeze/thaw cycles.

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)

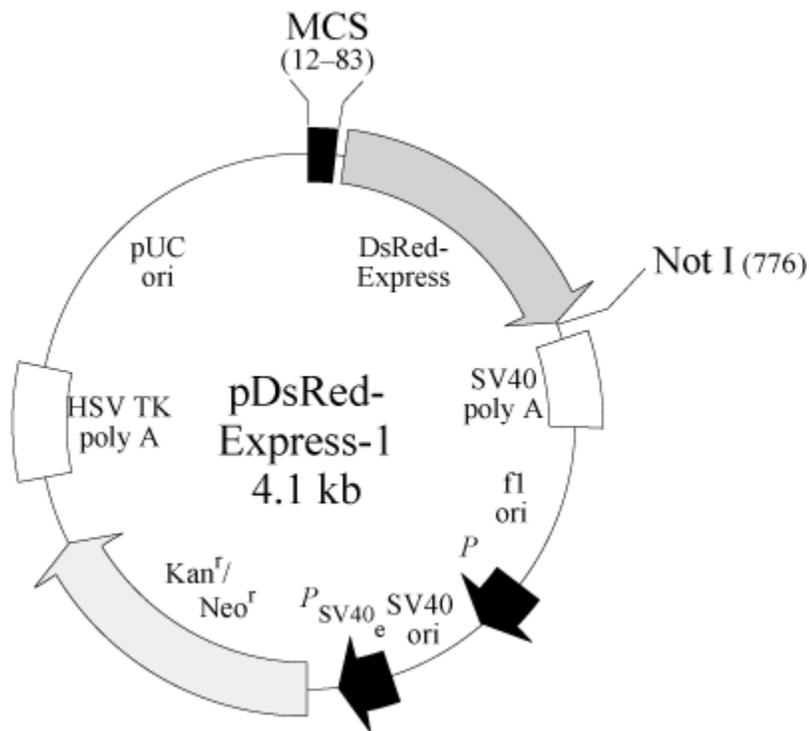


Figure 1. pDsRed-Express-1 vector map. The NotI site is part of the DsRed-Express stop codon.

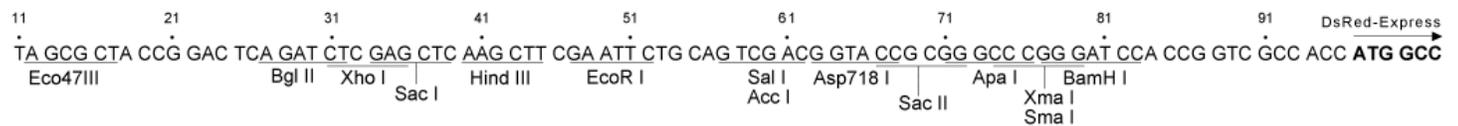


Figure 2. pDsRed-Express-1 multiple cloning site.

Location of Features

- MCS (multiple cloning site): 12–83
- Kozak consensus sequence: 90–100

pDsRed-Express-1 Vector

- DsRed-Express: 97–774
- SV40 early polyA signals: 926–931 & 955–960
- F1 origin of replication: 1023–1478
- *P* (promoter for Kan^r): 1540–1568
- SV40 origin of replication: 1819–1954
- *P*_{SV40e} (SV40 early promoter and enhancer): 1650–1881
- Kan^r/Neo^r (kanamycin/neomycin resistance gene): 2003–2797
- HSV TK polyA (herpes simplex virus thymidine kinase polyadenylation signals): 3033–3038 & 3046–3051
- pUC origin of replication: 3382–4025

Additional Information

Recommended Sequencing Primer Location:

- 297–277

Propagation in *E. coli*

- Suitable host strains: DH5 α , 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 kanamycin (50 μ g/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: High

Excitation and Emission Maxima of DsRed-Express

- Excitation: 557 nm
- Emission: 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.
5. Gorman, C. (1985) In *DNA cloning: A Practical Approach, Vol. II*. Ed. D. M. Glover. (IRL Press, Oxford, U.K.), pp. 143–190.

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:

Certificate of Analysis

Cat. No. 632413

pDsRed-Express-1 Vector

Enzyme	Fragment(s) (kb)
NotI	4.1
StuI	2.7 & 1.4

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

pDsRed-Express-1 Vector

CATALOG NO.

632413

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 prior written approval of Clontech Laboratories, Inc.

Your use of this product is also subject to compliance with the licensing requirements listed below and described on the product's web page at <http://www.clontech.com>. It is your responsibility to review, understand and adhere to any restrictions imposed by these statements.

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.

Living Colors Fluorescent Protein Products: 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.

Clontech and the Clontech logo are trademarks of Clontech Laboratories, Inc. All other marks are the property of their respective owners. Certain trademarks may not be registered in all jurisdictions. Clontech is a Takara Bio Company. ©2011 Clontech Laboratories, Inc.

This document has been reviewed and approved by the Clontech Quality Assurance Department.

Clontech Laboratories, Inc.

A Takara Bio Company
1290 Terra Bella Avenue, Mountain View, CA 94043, USA
U.S. Technical Support: tech@clontech.com

United States/Canada
800.662.2566

Asia Pacific
+1.650.919.7300

Europe
+33.(0)1.3904.6880

Japan
+81.(0)77.543.6116