

pHcRed1 Vector

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Catalog No.	Amount	Lot Number
632410	20 µg	Specified on product label.

Product Information

pHcRed1 is a bacterial expression vector which encodes the far-red fluorescent protein, HcRed1. HcRed1 was generated by mutagenesis of a non-fluorescent chromoprotein from the reef coral *Heteractis crispa* (1). The HcRed1 coding sequence has been human codon-optimized for higher expression in mammalian cells (2). The sequence upstream of HcRed1 has been converted to a Kozak consensus sequence to further increase the translation efficiency in eukaryotic cells (3).

The HcRed1 coding sequence is flanked by distinct multiple cloning sites (MCS) so that the gene can be easily excised from pHcRed1. Alternatively, the HcRed1 coding sequence can be amplified by PCR. In *E. coli*, HcRed1 is expressed from the *lac* promoter as a fusion with several additional amino acids, including the first five amino acids of the *LacZ* protein. Note, however, that if you excise the HcRed1 coding sequence using a restriction site in the 5' MCS, the resulting fragment will encode the native (i.e., non-fusion) HcRed1 protein. pHcRed1 is a pUC 19 derivative (pPD16.43; 4) which provides a high-copy-number origin of replication as well as an ampicillin resistance gene for propagation and selection in *E. coli*.

Package Contents

- 20 µg pHcRed1 Vector

Storage Conditions

- Store at -20°C.
- 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)

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(PA124369)			

Certificate of Analysis

Cat. No. 632410

pHcRed1 Vector

Concentration

- 500 ng/μl

Shipping Conditions

- Dry ice (-70°C)

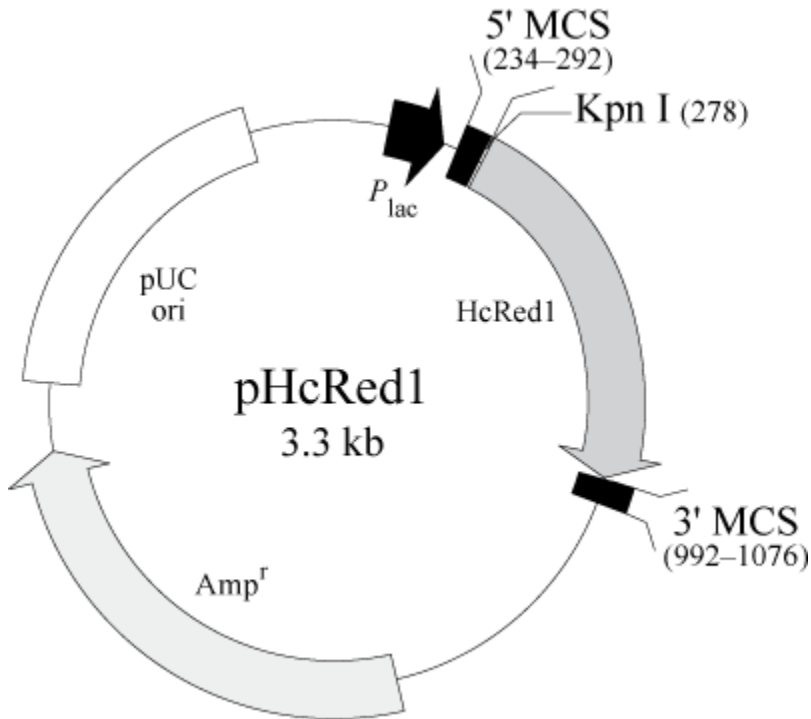


Figure 1. pHcRed1 vector map.

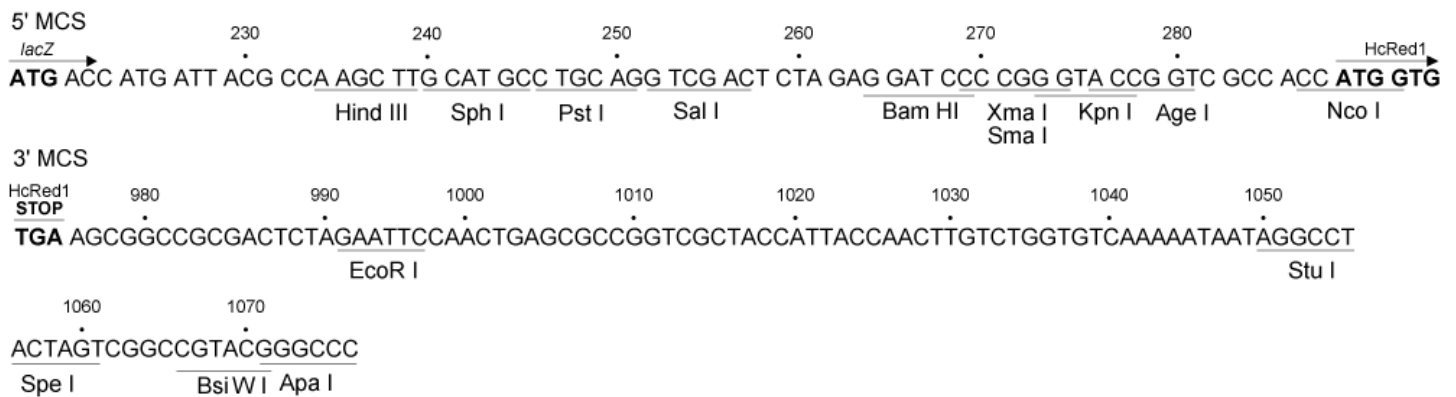


Figure 2. pHcRed1 multiple cloning site.

Location of Features

- P_{lac} (lac promoter): 95–178
- *lacZ*-HcRed1 fusion protein expressed in *E. coli*: 217–1008
- 5' MCS (5' multiple cloning site): 234–292

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- Kozak consensus sequence: 282–292
- HcRed1: 289–975
- 3' MCS (3' multiple cloning site): 992–1076
- Amp^r (ampicillin resistance gene; β -lactamase): 1522–2382
- pUC origin of replication: 2530–3173

Additional Information

Propagation in *E. coli*

- Recommended host strain: JM109
- Selectable marker: plasmid confers resistance to ampicillin (50 μ g/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: High

Excitation and Emission Maxima of HcRed1

- Excitation: 588 nm
- Emission: 618 \pm 4 nm

References

1. Gurskaya, N. G., *et al.* (2001) *FEBS Letters* **507**:16–20.
2. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
3. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
4. Fire, A., *et al.* (1990) *Gene* **93**:189–198.

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(s)	Fragment(s) (kb)
KpnI	3.3
KpnI & NotI	2.6 & 0.7

- Vector identity was confirmed by sequencing.
- A₂₆₀/A₂₈₀: 1.8–2.0

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CATALOG NO.

632410

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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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