Certificate of Analysis

pHcRed1-C1 Vector

Table of Contents

Catalog No.	Amount	Lot Number
632415	20 µg	Specified on product label.

Product Information

pHcRed1-C1 is a mammalian expression vector designed to express a protein of interest fused to the C-terminus of the far-red fluorescent protein HcRed1. pHcRed1-C1 can be used to monitor gene expression and protein localization *in vivo* or as a cotransfection marker; the unmodified vector will express HcRed1 in mammalian cells.

HcRed1 was generated by mutagenesis of a non-fluorescent chromoprotein from the reef coral *Heteractis crispa* (1). The coding sequence for HcRed1 is human codon-optimized for higher expression in mammalian cells (2). Genes cloned into the multiple cloning site (MCS) downstream of the HcRed1 coding sequence are expressed as fusions to the C-terminus of HcRed1. The MCS in pHcRed1-C1 is positioned between the HcRed1 coding sequence and the SV40 polyadenylation signal (SV40 poly A). Genes cloned into the MCS will be expressed as fusions to the C-terminus of HcRed1 if they are in the same reading frame as HcRed1 and there are no intervening stop codons. A Kozak consensus sequence upstream of HcRed1 increases the translation efficiency in eukaryotic cells (3). SV40 poly A signals downstream of the MCS direct proper processing of the 3' end of mRNA transcripts. The vector also 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)—consisting 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—allows stably transfected eukaryotic cells to be selected using G418. A bacterial promoter upstream of this cassette expresses kanamycin resistance in *E. coli*. pHcRed1-C1 can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (4).

Package Contents

- 20 µg pHcRed1-C1 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.

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(PA124373)

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)

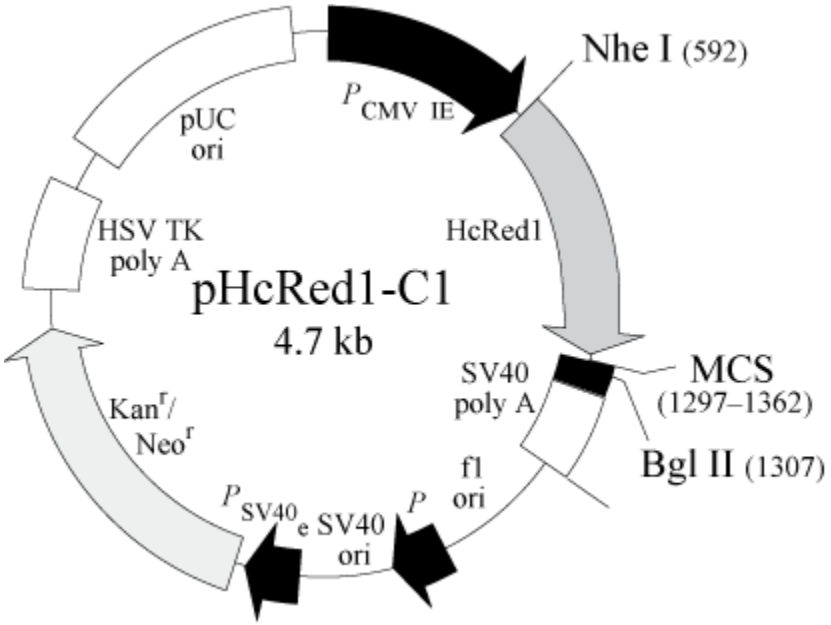

Figure 1. pHcRed1-C1 vector map.

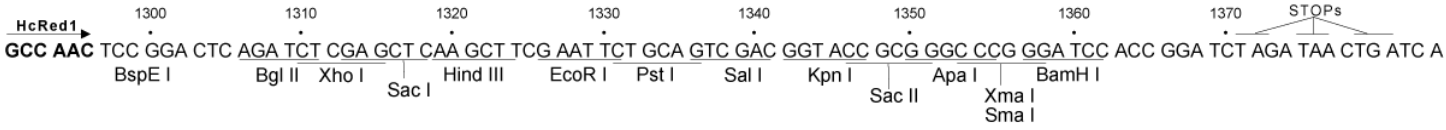

Figure 2. pHcRed1-C1 multiple cloning sites.

Location of Features

- $P_{CMV\ IE}$ (human cytomegalovirus immediate early promoter): 1–589
- Kozak consensus sequence: 606–616
- HcRed1: 613–1377
- MCS (multiple cloning site): 1297–1362
- SV40 early polyA signals: 1517–1522 & 1546–1551
- f1 origin of replication: 1614–2069
- *P* (promoter for Kan^r): 2131–2159
- SV40 origin of replication: 2410–2545

- $P_{SV40\ e}$ (SV40 early promoter and enhancer): 2243–2472
- Kan^r/Neo^r (kanamycin/neomycin resistance gene): 2594–3388
- HSV TK polyA signals (herpes simplex virus thymidine kinase polyadenylation signals: 3624–3629 & 3637–3642
- pUC origin of replication: 3973–4616

Additional Information

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 kanamycin (50 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: High

Excitation and Emission Maxima of HcRed1

- Excitation: 588 nm
- Emission: 618 ± 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. 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:

Enzyme(s)	Fragment(s) (kb)
BglII	4.7
BglII & NheI	4.0 & 0.7

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

Notice to Purchaser

pHcRed1-C1 Vector

CATALOG NO.
632415

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

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