Certificate of Analysis

pHcRed1-1 Vector

Table of Contents

Catalog No.	Amount	Lot Number
632411	20 µg	Specified on product label.

Product Information

pHcRed1-1 is a promoterless, mammalian expression vector which can be used to monitor transcription from different promoters and promoter/enhancer combinations inserted into the multiple cloning site (MCS) located upstream of the HcRed1 coding sequence. Promoters should be cloned into the pHcRed1-1 MCS upstream from the HcRed1 coding sequences. Without the addition of a functional promoter, this vector will not express HcRed1.

HcRed1 is a far-red fluorescent protein which 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 (3) to further increase the translation efficiency in eukaryotic cells. SV40 polyadenylation signals downstream of the HcRed1 gene direct proper processing of the 3' end of the HcRed1 mRNA. 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 (4). A bacterial promoter upstream of this cassette expresses kanamycin resistance in *E. coli*.

pHcRed1-1 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 pHcRed1-1 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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(PA124370)

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)

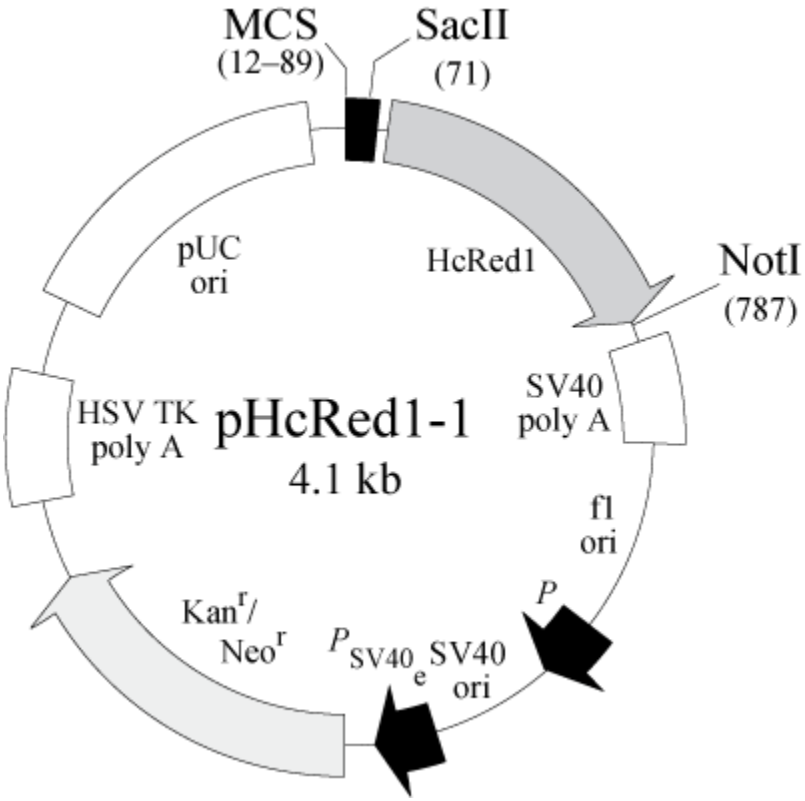

Figure 1. pHcRed1-1 vector map. The NotI site follows the HcRed1 stop codon.

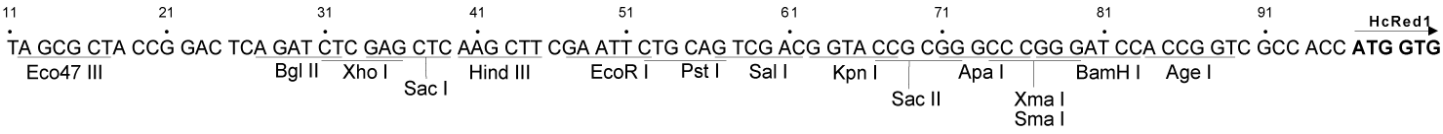

Figure 2. pHcRed1-1 multiple cloning sites.

Location of Features

- MCS: 12–89
- Kozak consensus sequence: 90–100
- HcRed1: 97–783
- SV40 early polyA signals: 937–942 & 966–971
- f1 origin of replication: 1034–1489

- *P* (promoter for Kan^r): 1551–1579
- SV40 origin of replication: 1830–1965
- $P_{SV40\ e}$ (SV40 early promoter and enhancer): 1661–1892
- Kan^r/Neo^r (kanamycin/neomycin resistance gene): 2014–2808
- HSV TK polyA signals (herpes simplex virus thymidine kinase polyadenylation signals): 3044–3049 & 3057–3062
- pUC origin of replication: 3393–4036

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)
SacII	4.1
SacII & NotI	3.4 & 0.7

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

Notice to Purchaser

pHcRed1-1 Vector

CATALOG NO.
632411

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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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