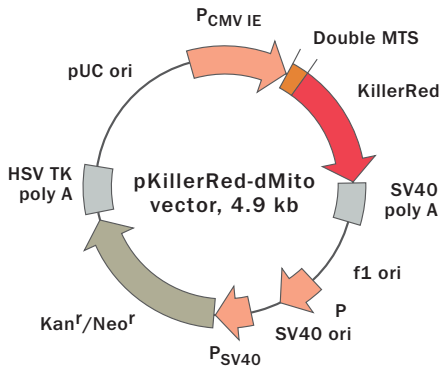


Mammalian expression vector pKillerRed-dMito



For vector sequence, please visit our Web site at www.evrogen.com/support/vector-info.shtml

Use

- Expression of mitochondria-targeted KillerRed in mammalian cells under the control of CMV promoter for subsequent cell killing via apoptosis

- Source of mitochondria-targeted KillerRed coding sequence

Product	Cat.#	Size
pKillerRed-dMito	FP964	20 µg
Please contact your local distributor for exact prices and delivery information.		
Reporter	KillerRed-2xMTS	
Reporter codon usage	mammalian	
Promoter	P _{CMV IE}	
Host cells	mammalian	
Selection	prokaryotic — kanamycin eukaryotic — neomycin (G418)	
Replication	prokaryotic — pUC ori eukaryotic — SV40 ori	

Vector description

pKillerRed-dMito is an eukaryotic (mammalian) expression vector encoding mitochondria-targeted photosensitizer KillerRed. KillerRed localized in mitochondria can be used for photo-inducible killing of cells through apoptotic pathway.

KillerRed codon usage is optimized for high expression in mammalian cells (humanized) (Haas *et al.*, 1996). Duplicated mitochondrial targeting sequence (MTS) is fused to the KillerRed N-terminus. MTS was derived from the subunit VIII of human cytochrome C oxidase (Rizzuto *et al.*, 1989; Rizzuto *et al.*, 1995).

The vector is not intended as a cloning vector; however, vector backbone contains unique restriction sites that permit excision of Double-MTS-KillerRed hybrid sequence.

Note: The plasmid DNA was isolated from *dam*⁺-methylated *E. coli*. Therefore some restriction sites are blocked by methylation. If you wish to digest the vector using such sites you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

The vector backbone also contains immediate early promoter of cytomegalovirus (P_{CMV IE}) for reporter expression, SV40 origin for replication in mammalian cells expressing SV40 T-antigen, pUC origin of replication for propagation in *E. coli* and f1 origin for single-stranded DNA production. SV40 polyadenylation signals (SV40 poly A) direct proper processing of the 3'-end of the reporter mRNA. SV40 early promoter (P_{SV40}) provides neomycin resistance gene (Neo^r) expression to select stably transfected eukaryotic cells using G418. Bacterial promoter (P) provides kanamycin resistance gene expression (Kan^r) in *E. coli*. Kan^r/Neo^r gene is linked with herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals.

Expression in mammalian cells

pKillerRed-dMito can be introduced into mammalian cells by any known transfection method. If required, stable transformants can be selected using G418 (Gorman, 1985).

Note: KillerRed shows no cell toxic effects before light activation. Upon green light irradiation KillerRed generates reactive oxygen species (ROS) that damage the neighboring molecules.

Propagation in *E. coli*

Suitable host strains for propagation in *E. coli* include DH5alpha, HB101, XL1-Blue, and other general purpose strains. Plasmid incompatibility group is pMB1/ColE1. The vector confers resistance to kanamycin (30 µg/ml) to *E. coli* hosts. Copy number in *E. coli* is about 500.

Location of features

P_{CMV IE}: 1-589

Enhancer region: 59-465; TATA box: 554-560

Transcription start point: 583

KillerRed-dMito fusion

Start codon (ATG): 597-599

Mitochondrial targeting sequence 1 (MTS-1): 597-689

Mitochondrial targeting sequence 2 (MTS-2): 690-782

Start of KillerRed coding sequence (ATG): 798-800

Stop codon: 1515-1517

SV40 early mRNA polyadenylation signal

Polyadenylation signals: 1671-1676 & 1700-1705

mRNA 3' ends: 1709 & 1721

f1 single-strand DNA origin: 1768-2223

Bacterial promoter for expression of Kan^r gene

-35 region: 2285-2290

-10 region: 2308-2313

Transcription start point: 2320

SV40 origin of replication: 2564-2699

SV40 early promoter

Enhancer (72-bp tandem repeats): 2397-2468 & 2469-2540

21-bp repeats: 2544-2564, 2565-2585 & 2587-2607

Early promoter element: 2620-2626

Major transcription start points: 2616, 2654, 2660 & 2665

Kanamycin/neomycin resistance gene

Neomycin phosphotransferase coding sequences:

Start codon (ATG): 2748-2750

stop codon: 3540-3542

G->A mutation to remove Pst I site: 2930

C->A (Arg to Ser) mutation to remove BssHII site: 3276

Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal

Polyadenylation signals: 3778-3783 & 3791-3796

pUC plasmid replication origin: 4127-4770

References

- Gorman C. (1985) In DNA cloning: A Practical Approach, Vol. II. Ed. D. M. Glover. (IRL Press, Oxford, U.K.), pp. 143-190.
- Haas, J., et al. (1996) Curr. Biol. 6: 315–324.
- Rizzuto, R., et al. (1989) J. Biol. Chem. 264: 10595–10600.
- Rizzuto, R., et al. (1995) Curr. Biol. 5: 635–642.

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MATERIAL SAFETY DATA SHEET INFORMATION

To the best of our knowledge, these products do not require a Material Safety Data Sheet. However, all the properties of these products (and, if applicable, each of their components) have not been thoroughly investigated. Therefore, we recommend that you use gloves and eye protection, and wear a laboratory coat when working with these products.