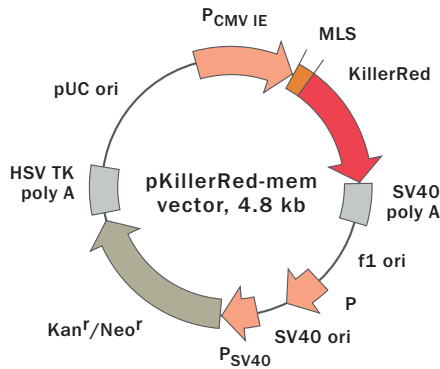


Mammalian expression vector pKillerRed-mem



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

Use

- Expression of membrane-targeted KillerRed in mammalian cells under the control of CMV promoter for subsequent cell killing via lipid oxidation
- Source of membrane-targeted KillerRed coding sequence

Product	Cat.#	Size
pKillerRed-mem	FP966	20 µg

Please contact your local distributor for exact prices and delivery information.

Reporter	KillerRed-MLS
Reporter codon usage	mammalian
Promoter for KillerRed-MLS	P _{CMV IE}
Host cells	mammalian
Selection	prokaryotic — kanamycin eukaryotic — neomycin (G418)
Replication	prokaryotic — pUC ori eukaryotic — SV40 ori

Vector description

pKillerRed-mem is a mammalian expression vector encoding membrane-targeted photosensitized KillerRed. KillerRed localized on cellular membrane can be used for effective light-induced cell killing.

Note: Comparing to the mitochondrially targeted KillerRed, irradiation of membrane-localized KillerRed leads to even more effective and fast cell death (within 10-30 min). Moreover, membrane-targeted KillerRed was shown to be suitable for the light induced cell killing within a developing zebrafish.

KillerRed codon usage is optimized for high expression in mammalian cells (humanized) (Haas *et al.*, 1996). Membrane localization signal (MLS) of neuromodulin is linked to the KillerRed N-terminus. The MLS (N-terminal 20 amino acid residues of neuromodulin) contains a signal for posttranslational palmitoylation of cysteines 3 and 4 that targets KillerRed to cellular membranes (Skene & Virag, 1989).

The vector is not intended as a cloning vector; however, vector backbone contains unique restriction sites that permit excision of MLS-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-mem can be introduced into mammalian cells by any known transfection method. If required, stable transformants can be selected.

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 mg/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-MLS fusion

Start codon (ATG): 679-681

Neuromodulin N-terminal sequence (MLS): 679-738

Start of KillerRed coding sequence (ATG): 739-741

Stop codon: 1450-1452

SV40 early mRNA polyadenylation signal

Polyadenylation signals: 1606-1611; 1635-1640

mRNA 3' ends: 1644; 1656

f1 single-strand DNA origin: 1703-2158

Bacterial promoter for expression of Kan^r gene

-35 region: 2220-2225; -10 region: 2243-2248

Transcription start point: 2255

SV40 origin of replication: 2499-2634

SV40 early promoter

Enhancer (72-bp tandem repeats): 2332-2403; 2404-2475

21-bp repeats: 2479-2499; 2500-2520; 2522-2542

Early promoter element: 2555-2561

Major transcription start points: 2551; 2589; 2595; 2600

Kanamycin/neomycin resistance gene

Neomycin phosphotransferase coding sequences:

Start codon (ATG): 2683-2685

Stop codon: 3475-3477

G->A mutation to remove Pst I site: 2865

C->A (Arg to Ser) mutation to remove BssH II site: 3211

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

Polyadenylation signals: 3713-3718; 3726-3731

pUC plasmid replication origin: 4062-4705

References

Haas, J., et al. (1996) Curr. Biol. 6:315–324.

Skene, J. H. P. & Virag, I. (1989) J. Cell. Biol. 108:613–625.

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