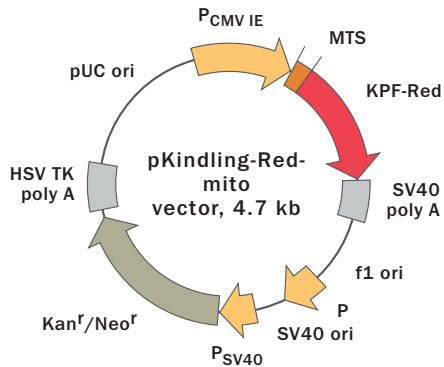


Mammalian expression vector pKindling-Red-mito



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

Use

- Expression of mitochondria-targeted KFP-Red in mammalian cells under the control of CMV promoter
- Source of mitochondria-targeted KFP-Red coding sequence

Product	Cat.#	Size
pKindling-Red-mito	FP401	20 µg

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

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

Vector description

pKindling-Red-mito is an eukaryotic (mammalian) expression vector encoding mitochondria-targeted kindling red fluorescent protein, KFP-Red. The vector can be used for photo-inducible fluorescent labeling of mitochondria and tracking of mitochondrial movement.

KFP-Red codon usage is optimized for high expression in mammalian cells (humanized) (Haas *et al.*, 1996). A mitochondrial targeting sequence (MTS) is fused to the KFP-Red 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 MTS-KFP-Red 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

The vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of mitochondrion-targeted KFP-Red in many cell types. If required, stable transformants can be selected using G418 (Gorman, 1985).

Note: KFP-Red is not fluorescent before photoactivation. Under green light irradiation, it becomes to red fluorescent state. Depending on irradiation intensity, KFP-Red can be kindled reversibly or irreversibly.

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

C->G mutation to remove SacI site: 569

MTS from subunit VIII of human cytochrome c oxidase

Start codon (ATG): 597-599

End of targeting sequence: 683

KFP-Red gene

Start codon (ATG): 705-707;

Stop codon: 1401-1403

SV40 early mRNA polyadenylation signal

Polyadenylation signals: 1556-1561 & 1585-1590;

mRNA 3' ends: 1594 & 1606

f1 single-strand DNA origin: 1653-2108

(packages the noncoding strand of KFP-Red-mito)

Bacterial promoter for expression of Kan^r gene

-35 region: 2170-2175;

-10 region: 2193-2198

Transcription start point: 2205

SV40 origin of replication: 2449-2584

SV40 early promoter

Enhancer (72-bp tandem repeats): 2282-2353 & 2354-2425

21-bp repeats: 2429-2449; 2450-2470 & 2472-2492

Early promoter element: 2505-2511

Major transcription start points: 2501, 2539, 2545 & 2550

Kanamycin/neomycin resistance gene

Neomycin phosphotransferase coding sequences:

Start codon (ATG): 2633-2635; stop codon: 3425-3427

G->A mutation to remove PstI site: 2815

C->A (Arg to Ser) mutation to remove BssHII site: 3161

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

Polyadenylation signals: 3663-3668 & 3676-3681

pUC plasmid replication origin: 4012-4655

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.