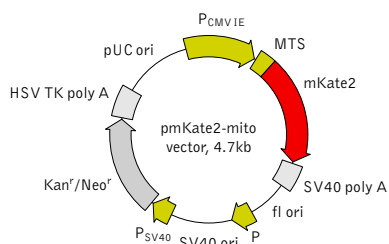


pmKate2-mito vector

The vector sequence has been compiled using the information from sequence databases, published literature, and other sources, together with partial sequences obtained by Evrogen. This vector has not been completely sequenced.



For vector sequence, please visit our Web site at <http://www.evrogen.com/products/vectors.shtml>

Location of features

P_{CMV IE}: 1-589
 Enhancer region: 59-465
 TATA box: 554-560
 Transcription start point: 583
 mKate2-mito fusion
 Start codon (ATG): 597-599
 Mitochondrial targeting sequence (MTS): 597-683
 Start of mKate2 coding sequence (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
 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 Pst I site: 2815
 C->A (Arg to Ser) mutation to remove BssH II site: 3161
 Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
 Polyadenylation signals: 3663-3668 & 3676-3681
 pUC plasmid replication origin: 4012-4655

Product	Cat.#	Size
pmKate2-mito vector	FP187	20 µg
Vector type	mammalian expression vector	
Reporter	mKate2	
Reporter codon usage	mammalian	
Promoter for mKate2	P _{CMV IE}	
Host cells	mammalian	
Selection	prokaryotic - kanamycin eukaryotic - neomycin (G418)	
Replication	prokaryotic - pUC ori eukaryotic - SV40 ori	
Use	far-red fluorescent labeling of mitochondria	

Vector description

pmKate2-mito is a mammalian expression vector intended for far-red fluorescent labeling of mitochondria in living cells. The vector encodes far-red fluorescent protein mKate2 fused to mitochondrial targeting sequence (MTS) derived from the subunit VIII of human cytochrome C oxidase [Rizzuto et al. 1989; Rizzuto et al. 1995]. MTS is fused to the mKate2 N-terminus.

mKate2 codon usage is optimized for high expression in mammalian cells (humanized) [Haas et al. 1996].

pmKate2-mito vector can be used as a source of mKate2-MTS hybrid sequence. The vector backbone contains unique restriction sites that permit its excision and further insertion into expression vector of choice.

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 contains immediate early promoter of cytomegalovirus (P_{CMV IE}) for protein 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

pmKate2-mito vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of the mKate2-MTS fusion in eukaryotic cells. If required, stable transformants can be selected using G418 [Gorman 1985].

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.

References

- Gorman, C. (1985). "High efficiency gene transfer into mammalian cells." In: *DNA cloning: A Practical Approach, Vol. II*. Ed. by Glover. (IRL Press, Oxford, U.K.) Pp. 143-190.
- Haas, J. et al. (1996) "Codon usage limitation in the expression of HIV-1 envelope glycoprotein." *Curr Biol*, 6 (3): 315-324 / pmid: 8805248
- Rizzuto, R. et al. (1989) "A gene specifying subunit VIII of human cytochrome c oxidase is localized to chromosome 11 and is expressed in both muscle and non-muscle tissues." *J Biol Chem*, 264 (18): 10595-10600 / pmid: 2543673
- Rizzuto, R. et al. (1995) "Chimeric green fluorescent protein as a tool for visualizing subcellular organelles in living cells." *Curr Biol*, 5 (6): 635-642 / pmid: 7552174

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MSDS information is available at <http://www.evrogen.com/MSDS.shtml>