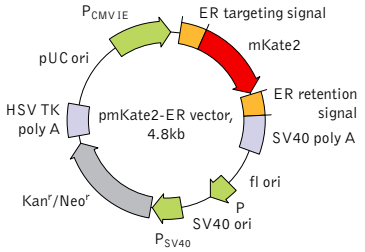
pmKate2-ER vector

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

Product	Cat.#	Size
pmKate2-ER vector	**FP324**	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 the lumen of the endoplasmic reticulum

Location of features

$P_{CMV\ IE}$: 1-589
Enhancer region: 59-465
TATA box: 554-560
Transcription start point: 583
mKate2-ER fusion: 597-1391
Start codon (ATG): 597-599
Calreticulin signal sequence: 597-647
mKate2: 663-1358
ER retention sequence (KDEL): 1380-1391
Stop codon: 1392-1394
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 1593-1598 & 1622-1627
mRNA 3' ends: 1631 & 1643
f1 single-strand DNA origin: 1690-2145
Bacterial promoter for expression of Kan^r gene
-35 region: 2207-2212; -10 region: 2230-2235
Transcription start point: 2242
SV40 origin of replication: 2486-2621
SV40 early promoter
Enhancer (72-bp tandem repeats): 2319-2390 & 2391-2462
21-bp repeats: 2466-2486, 2487-2507 & 2509-2529
Early promoter element: 2542-2548
Major transcription start points: 2538, 2576, 2582 & 2587
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 2670-2672; Stop codon: 3462-3464
G->A mutation to remove Pst I site: 2852
C->A (Arg to Ser) mutation to remove BssH II site: 3198
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 3700-3705 & 3713-3718
pUC plasmid replication origin: 4049-4692

Vector description

pmKate2-ER is a mammalian expression vector intended for far-red fluorescent labeling of the lumen of the endoplasmic reticulum (ER) [Roderick et al. 1997]. The vector encodes far-red fluorescent protein mKate2 containing ER targeting signal (calreticulin signal sequence [Fliegel et al. 1989]) fused to the mKate2 N-terminus and ER retention signal (KDEL sequence [Munro and Pelham 1987]) fused to the mKate2 C-terminus.

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

pmKate2-ER vector can be used as a source of mKate2-ER 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-ER vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of the mKate2-ER 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

Fliegel, L. et al. (1989) "Molecular cloning of the high affinity calcium-binding protein (calreticulin) of skeletal muscle sarcoplasmic reticulum." J Biol Chem, 264 (36): 21522–8 / pmid: 2600080

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

Munro, S. and HR. Pelham (1987) "A C-terminal signal prevents secretion of luminal ER proteins." Cell, 48 (5): 899–907 / pmid: 3545499

Roderick, HL. et al. (1997) "Nuclear localisation of calreticulin in vivo is enhanced by its interaction with glucocorticoid receptors." FEBS Lett, 405 (2): 181–185 / pmid: 9089287