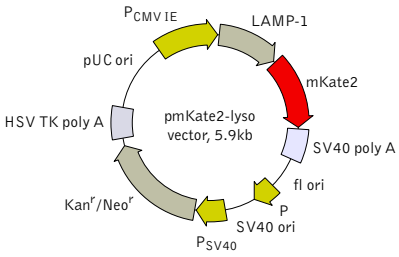
pmKate2-lyso vector

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/support/vector-info.shtml

Product	Cat.#	Size
pmKate2-lyso vector	**FP312**	20 μg

The price does not include delivery. The price varies in different countries. Please contact your local distributor for exact prices and delivery information.

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 lysosomes

Location of features

$P_{CMV\ IE}$: 1-589
Enhancer region: 59-465
TATA box: 554-560
Transcription start point: 583
mKate2-LAMP1 fusion
Start codon (ATG): 626-628
Lysosomal Associated Membrane Protein 1 (LAMP1) sequence : 626-1846
Start of mKate2 coding sequence (ATG): 1907-1909
Stop codon: 2603-2605
SV40 early mRNA polyadenylation signal
Polyadenylation signals: 2758-2763 & 2787-2792
mRNA 3' ends: 2796 & 2808
f1 single-strand DNA origin: 2855-3310
Bacterial promoter for expression of Kan^r gene
-35 region: 3372-3377; -10 region: 3395-3400
Transcription start point: 3407
SV40 origin of replication: 3651-3786
SV40 early promoter
Enhancer (72-bp tandem repeats): 3484-3555 & 3556-3627
21-bp repeats: 3631-3651, 3652-3672 & 3674-3694
Early promoter element: 3707-3713
Major transcription start points: 3703, 3741, 3747 & 3752
Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 3835-3837; Stop codon: 4627-4629
G->A mutation to remove Pst I site: 4017
C->A (Arg to Ser) mutation to remove BssH II site: 4363
Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 4865-4870 & 4878-4883
pUC plasmid replication origin: 5214-5857

Vector description

pmKate2-lyso is a mammalian expression vector intended for far-red fluorescent labeling of lysosomes in living cells. The vector encodes far-red fluorescent protein mKate2 targeted to lysosomal membrane by rat Lysosomal Associated Membrane Protein 1 (LAMP-1), fused to the mKate2 N-terminus.

mKate2 codon usage is optimized for high expression in mammalian cells, i.e. humanized (Haas et al. 1996).

pmKate2-lyso can be used as a source of mKate2-LAMP1 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-lyso can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of the mKate2-LAMP1 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 (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 et al. (1996) "Codon usage limitation in the expression of HIV-1 envelope glycoprotein." Curr Biol, 6 (3): 315–324 / pmid: 8805248