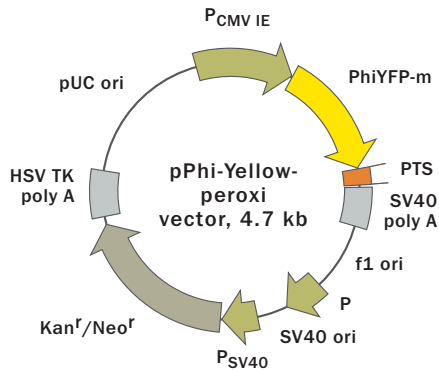


Mammalian expression vector pPhi-Yellow-peroxi



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

Use

- Expression of peroxisome-targeted PhiYFP-m in mammalian cells under the control of CMV promoter
- Source of peroxisome-targeted PhiYFP-m coding sequence

Product	Cat.#	Size
pPhi-Yellow-peroxi	FP606	20 µg
Please contact your local distributor for exact prices and delivery information.		
Vector type	mammalian expression vector	
Reporter	PhiYFP-m fusion with the peroxisome targeting signal (PTS)	
Reporter codon usage	mammalian	
Promoter for PhiYFP-m	P _{CMV IE}	
Host cells	mammalian	
Selection	prokaryotic — kanamycin eukaryotic — neomycin (G418)	
Replication	prokaryotic — pUC ori eukaryotic — SV40 ori	

Vector description

pPhi-Yellow-peroxi is an eukariotic (mammalian) vector encoding peroxisome-targeting yellow fluorescent protein PhiYFP-m. The vector allows yellow fluorescent labeling of peroxisomes.

PhiYFP-m codon usage is optimized for high expression in mammalian cells (humanized) (Haas *et al.*, 1996). Peroxisomal targeting signal (PTS) is linked to the 3'-end of PhiYFP-m coding sequence. PTS encodes tripeptide SKL, which targets the fusion protein to the matrix of peroxisomes. To increase PhiYFP-m translation, Kozak consensus translation initiation site is generated upstream of the PhiYFP-m sequence (Kozak, 1987).

pPhi-Yellow-peroxi is not intended as a cloning vector; however, the backbone contains unique restriction sites that could permit excision of the PhiYFP-m-peroxi 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

pPhi-Yellow-peroxi can be introduced into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of peroxisome-targeted PhiYFP in many cell types resulting in yellow fluorescent labeling of peroxisomes. 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 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

PhiYFP-m

Kozak consensus translation initiation site: 606-616

Start codon (ATG): 613-615; Stop codon: 1324-1326

Last amino acid in PhiYFP-m: 1312-1314

Peroxisomal targeting signal (PTS): 1315-1326

SV40 early mRNA polyadenylation signal

Polyadenylation signals: 1534-1539 & 1563-1568

mRNA 3' ends: 1572 & 1584

f1 single-strand DNA origin: 1631-2086

(packages the noncoding strand of PhiYFP-m.)

Bacterial promoter for expression of Kan^r gene

-35 region: 2148-2153; -10 region: 2171-2176

Transcription start point: 2183

SV40 origin of replication: 2427-2562

SV40 early promoter

Enhancer (72-bp tandem repeats): 2260-2331 & 2332-2403

21-bp repeats: 2407-2427, 2428-2448, & 2450-2470

Early promoter element: 2483-2489

Major transcription start points: 2479, 2517, 2523 & 2528

Kanamycin/neomycin resistance gene

Neomycin phosphotransferase coding sequences:

Start codon (ATG): 2611-2613; stop codon: 3403-3405

G->A mutation to remove PstI site: 2793

C->A (Arg to Ser) mutation to remove BssHII site: 3139

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

Polyadenylation signals: 3641-3646 & 3654-3659

pUC plasmid replication origin: 3990-4633

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.

Kozak M. (1987) *Nucleic Acids Res.* 15: 8125-8148.

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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.