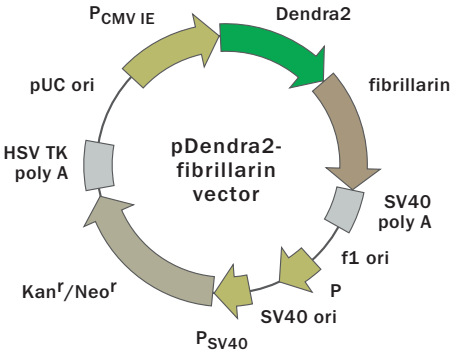
Mammalian expression vector pDendra2-fibrillarin



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

Product	Cat.#	Size
pDendra2-fibrillarin		20 µg

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

Vector description

This vector is provided as a free supplement to Evrogen Dendra2 expression vectors.

pDendra2-fibrillarin is a mammalian expression vector encoding Dendra2-fibrillarin fusion protein. The vector is intended for use as a positive control to estimate photoactivation conditions of Dendra2-tagged proteins.

Dendra2 codon usage is optimized for high expression in mammalian cells, i.e. humanized (Haas *et al*., 1996). The vector backbone also 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 provides neomycin resistance gene expression to select stably transfected eukaryotic cells using G418. Bacterial promoter (P) provides kanamycin resistance gene expression in *E. coli*. Kan^r/Neo^r gene is linked with herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals.

To increase Dendra2-fibrillarin mRNA translation efficiency, Kozak consensus translation initiation site is generated upstream of Dendra2 coding sequence.

Note: The plasmid DNA was isolated from dam^+-methylated *E.coli*. Therefore some restriction sites are bloked 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.

Expression in mammalian cells

pDendra2-C vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of Dendra2-fibrillarin fusion in many cell types. 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) 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.