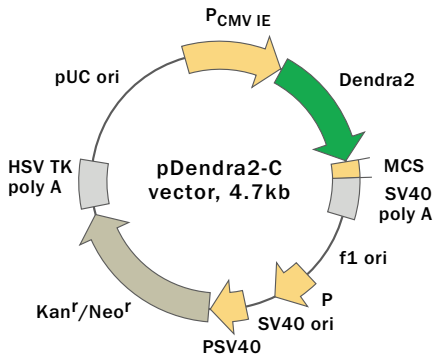


Mammalian expression vector pDendra2-C



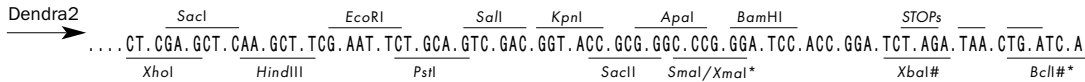
For vector sequence, please visit our Web site at www.evrogen.com/support/vector-info.shtml

Product	Cat.#	Size
pDendra2-C	FP821	20 µg

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

Reporter	Dendra2
Reporter codon usage	mammalian
Promoter for Dendra2	P _{CMV IE}
Host cells	mammalian
Selection	prokaryotic — kanamycin eukaryotic — neomycin (G418)
Replication	prokaryotic — pUC ori eukaryotic — SV40 ori

Multiple cloning site (MCS)



* — not unique sites. # — sites are blocked by methylation.

Use

- Dendra2 expression in mammalian cells under the control of CMV promoter
- Generation of fusions to the Dendra2 C-terminus using vector MCS

Vector description

pDendra2-C is a mammalian expression vector encoding photoswitchable green-to-red fluorescent protein Dendra2. Dendra2 is a monomeric protein allowing direct tracking *in vivo* of cell, organelle, and protein movements and for protein degradation studies. pDendra2-C vector allows to generate fusions to the Dendra2 C-terminus for expression, localization and cellular dynamics studies and to express Dendra2 or its fusions in mammalian cells.

Dendra2 codon usage is optimized for high expression in mammalian cells (humanized, Haas *et al.*, 1996). To increase Dendra2 translation, Kozak consensus translation initiation site is generated upstream of the Dendra2 sequence (Kozak, 1987). Multiple cloning site (MCS) is located between Dendra2 coding sequence and SV40 polyadenylation signal (SV40 polyA).

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 polyA 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.

Generation of fusions

A localization signal or a gene of interest should be cloned into MCS of the vector. It will be expressed as a fusion to the Dendra2 C-terminus when inserted in the same reading frame as Dendra2 and no in-frame stop codons are present. Dendra2-tagged fusions retain fluorescent properties of the native protein allowing fusion localization and tracking *in vivo*. Unmodified pDendra2-C vector will express Dendra2, when transfected into eukaryotic (mammalian) cells.

Notes: 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.

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 or Dendra2-tagged fusions 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.

Location of features

P_{CMV IE}: 1-589

Enhancer region: 59-465

TATA box: 554-560

Transcription start point: 583

Dendra2

Kozak consensus translation initiation site: 606-616

Start codon (ATG): 613-615; Stop codon: 1393-1395

Last amino acid in Dendra2: 1300-1302

MCS: 1327-1393

SV40 early mRNA polyadenylation signal

Polyadenylation signals: 1535-1540 & 1564-1569

mRNA 3' ends: 1573 & 1585

f1 single-strand DNA origin: 1632-2087

Bacterial promoter for expression of Kan^r gene

-35 region: 2149-2154; -10 region: 2172-2177

Transcription start point: 2184

SV40 origin of replication: 2428-2563

SV40 early promoter

Enhancer (72-bp tandem repeats): 2261-2332 & 2333-2404

21-bp repeats: 2408-2428, 2429-2449 & 2451-2471

Early promoter element: 2484-2490

Major transcription start points: 2480, 2518, 2524 & 2529

Kanamycin/neomycin resistance gene

Neomycin phosphotransferase coding sequences:

Start codon (ATG): 2612-2614; Stop codon: 3404-3406

G->A mutation to remove PstI site: 2794

C->A (Arg to Ser) mutation to remove BssHII site: 3140

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

Polyadenylation signals: 3642-3647 & 3655-3660

pUC plasmid replication origin: 3991-4634

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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