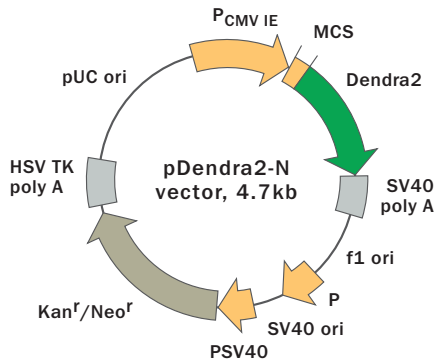


# Mammalian expression vector pDendra2-N



For vector sequence, please visit our Web site at [www.evrogen.com/support/vector-info.shtml](http://www.evrogen.com/support/vector-info.shtml)

Product	Cat.#	Size
pDendra2-N	FP822	20 µg

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

Reporter	Dendra2
Reporter codon usage	mammalian
Promoter for Dendra2	P <sub>CMV IE</sub>
Host cells	mammalian
Selection	prokaryotic — kanamycin eukaryotic — neomycin (G418)
Replication	prokaryotic — pUC ori eukaryotic — SV40 ori

## Multiple cloning site (MCS)

$\xrightarrow{\text{Dendra2}}$   
 G. CTA. GCG. CTA. CCG. GAC. TCA. GAT. CTC. GAG. CTC. AAG. CTT. CGA. ATT. CTG. CAG. TCG. ACG. GTA. CCG. CGG. GCC. CCG. GAT. CCA. CCG. GTC. GCC. ACC. ATG. . . .  

 NheI      BglII      SacI      HindIII      EcoRI      Sall      KpnI      ApaI      BamHI      AgeI  
 AfeI                      XhoI                      PstI                      SacII      SmaI/XmaI\*

\* — not unique sites.

## Use

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

## Vector description

pDendra2-N is a mammalian expression vector encoding photoswitchable green-to-red fluorescent protein Dendra2. Dendra2 allows direct tracking *in vivo* of cell, organelle, and protein movements and monitor protein degradation. pDendra2-N is designed to generate fusions to the Dendra2 N-terminus and to express Dendra2 or its fusions in eukaryotic (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 Dendra2 sequence (Kozak, 1987). Multiple cloning site (MCS) is located between P<sub>CMV IE</sub> and Dendra2 coding sequence.

The vector backbone comprises immediate early promoter of cytomegalovirus (P<sub>CMV IE</sub>) 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<sup>r</sup>/Neo<sup>r</sup> 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 N-terminus when inserted in the same reading frame as Dendra2 and no in-frame stop codons are present. The inserted sequence should contain an initiating ATG codon. Dendra2-tagged fusions retain fluorescent properties of the native protein allowing fusion localization and tracking *in vivo*. Unmodified pDendra2-N will express Dendra2, when transfected into eukaryotic (mammalian) cells.

**Notes:** The plasmid DNA was isolated from dam<sup>+</sup>-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<sup>-</sup> host and make fresh DNA.

## Expression in mammalian cells

The vector can be transfected into mammalian cells by any known transfection method. 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<sub>CMV IE</sub>:** 1-589

Enhancer region: 59-465

TATA box: 554-560

Transcription start point: 583

**MCS:** 591-678

### Dendra2

Kozak consensus translation initiation site: 672-682

Start codon (ATG): 679-681;

Stop codon: 1369-1371

### SV40 early mRNA polyadenylation signal

Polyadenylation signals: 1524-1529 & 1553-1558

mRNA 3' ends: 1562 & 1574

**f1 single-strand DNA origin:** 1621-2076

### Bacterial promoter for expression of Kan<sup>r</sup> gene

-35 region: 2138-2143; -10 region: 2161-2166

Transcription start point: 2173

**SV40 origin of replication:** 2417-2552

### SV40 early promoter

Enhancer (72-bp tandem repeats): 2250-2321 & 2322-2393

21-bp repeats: 2397-2417, 2418-2438 & 2440-2460

Early promoter element: 2473-2479

Major transcription start points: 2469, 2507, 2513 & 2518

### Kanamycin/neomycin resistance gene

Neomycin phosphotransferase coding sequences:

Start codon (ATG): 2601-2603

Stop codon: 3393-3395

G->A mutation to remove PstI site: 2783

C->A (Arg to Ser) mutation to remove BssHII site: 3129

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

Polyadenylation signals: 3631-3636 & 3644-3649

**pUC plasmid replication origin:** 3980-4623

## 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

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