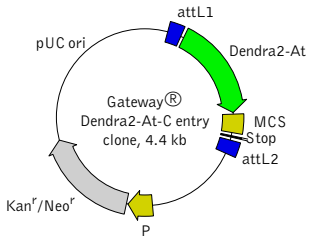
Gateway® Dendra2-At-C entry clone

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
Gateway® Dendra2-At-C entry clone	**FP824**	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	Gateway® entry clone
Reporter	Dendra2
Reporter codon usage	Arabidopsis
Promoter for Dendra2	NO
Host cells	prokaryotic
Selection	kanamycin
Replication	pUC ori
Use	Generation of fusions to the C-terminus of Dendra2; transfer of the construct encoding Dendra2 or its fusion into Gateway® destination vectors

Gateway® Dendra2-At-C vector MCS

Dendra2 — Bgl II — Sac I — EcoR I — Sal I — Sac II — Sma I/Xma I — STOPs

. . . AAA. GAT. CTC. GAG. CTC. AAG. CTT. CGA. ATT. CTG. CAG. TCG. ACG. GTA. CCG. CGG. GCC. CGG. GAT. CCA. CCG. GAT. CTA. GGT. AAC. TGA. ACC . . .

Xho I — Hind III — Pst I — Kpn I — Apa I — BamH I — AttL 2 site

Location of features

attL1 site: 14-113
Kozak translation initiation site: 129-139
Dendra2: 136-825
MCS: 850-918
attL2 site: 926-1025
Kanamycin resistance gene: 2250-3044
pUC origin of replication: 3629-4272

Vector description

Gateway® Dendra2-At-C entry clone is a vector containing green | red fluorescent protein Dendra2 gene variant with codon usage optimized for high expression in Arabidopsis. Dendra2 coding sequence is flanked by attL1 and attL2 sites allowing easy site-specific recombination. The Invitrogen Gateway® Technology provides a rapid and highly efficient way to transfer the Dendra2 gene into a number of Gateway® destination vectors for expression in different experimental systems. Multiple cloning site (MCS) located at the 3' end of Dendra2 gene allows to generate fusions to the Dendra2 C-terminus for expression, localization and cellular dynamics studies.

To increase mRNA translation efficiency, Kozak consensus translation initiation site is generated upstream of Dendra2 coding sequence [Kozak 1987].

The vector backbone contains pUC origin of replication and kanamycin resistance gene (Kan^r) for propagation and selection in *E. coli*.

Generation of Dendra2-tagged fusions

A localization signal or a gene of interest can be cloned into MCS of the vector both before and after site-specific recombination with a destination 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.

Alternatively, Dendra2 gene can be fused to the 3'-end of a gene of interest by LR recombination of the Gateway® Dendra2-At-C with a destination vector containing this gene in a correct reading frame. In this case, the protein of interest will be expressed as a fusion to the Dendra2 N-terminus.

Dendra2-tagged fusions retain fluorescent properties of the native protein allowing fusion localization *in vivo*.

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.

LR site-specific recombination

Please refer to Invitrogen Gateway® Technology description for detailed instructions regarding LR site-specific recombination reaction. In general, to transfer Dendra2 gene or Dendra2-fusion construct into the destination vector you will need:

- Purified plasmid DNA of Gateway® Dendra2-At-C
- A destination vector of choice
- Invitrogen LR Clonase™ II enzyme mix (Invitrogen Cat.# 11791-020)
- Proteinase K solution (supplied with the LR Clonase™ II enzyme mix)
- TE-Buffer, pH 8.0 (10 mM Tris-HCl, pH 8.0, 1 mM EDTA)
- Appropriate chemically competent *E. coli* host and growth media for expression
- Appropriate selective plates.

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

Kozak (1987) "An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs." Nucleic Acids Res, 15 (20): 8125–8148 / pmid: 3313277

Gateway® Technology. Ver. E. 22 Sept. 2003, 25-0522. http://www.invitrogen.com/content/sfs/manuals/gatewayman.pdf (visited on 18. 06. 2008)