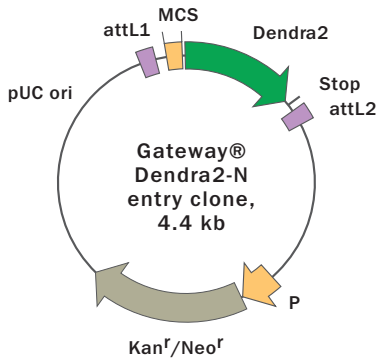


Gateway® Dendra2-At-N entry clone



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

Product	Cat.#	Size
Gateway® Dendra2-At-N entry clone	FP825	20 µg

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

Reporter	Dendra2
Reporter codon usage	Arabidopsis
Promoter for Dendra2	NO
Host cells	prokaryotic
Selection	kanamycin
Replication	prokaryotic — pUC ori

Multiple cloning site (MCS)



Use

- Fast cloning into Gateway® expression vectors through site-specific recombination
- Generation of fusions to the N-terminus of Dendra2 using vector MCS or attL1 site

Vector description

Gateway® Dendra2-At-N entry clone comprises coding sequence for a photoswitchable green-to-red fluorescent protein Dendra2. Dendra2 codon usage is optimized for high expression in *Arabidopsis*. Dendra2 sequence is flanked by attL1 and attL2 sites allowing site-specific recombination using Invitrogen Gateway® Technology. This technology provides a rapid and highly efficient way to transfer the Dendra2 gene into a number of Gateway® expression vectors (destination vectors).

To increase Dendra2 mRNA translation efficiency, Kozak consensus translation initiation site is generated upstream of Dendra2 coding sequence (Kozak, 1987). Multiple cloning site (MCS) is located at the 5'- end of Dendra2 gene and can be used to generate fusions to the Dendra2 N-terminus for expression, localization and cellular dynamics studies.

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

Generation of fusions

A localization signal or a gene of interest should 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 N-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 Gateway® Dendra2-At-N entry clone with the destination vector containing this gene in a correct reading frame. Dendra2-tagged fusions retain fluorescent properties of the native protein allowing fusion localization and tracking *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

Dendra2 or Dendra2-tagged fusion can be transferred into an Invitrogen destination vector of choice using Gateway® Technology. Use Invitrogen LR Clonase™ II enzyme mix (Invitrogen Cat.# 11791-020) as described in as described in the manufacturer instruction.

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

attL1 site: 14-113

MCS: 117-194

Kozak translation initiation site: 195-205

Dendra2: 202-891

attL2 site: 937-1036

Kanamycin resistance gene: 2254-3048

pUC origin of replication: 3633-4276

References

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

Notice to Purchaser:

Photoactivatable FP-related products are intended to be used by academic (non-commercial) entities and for research purposes only. Any use of the proprietary nucleic acid or protein other than for research use or by a commercial entity is strictly prohibited. Transfer of this product by purchaser to any other party is specifically prohibited.

Gateway® Cloning Products and their use are the subject of one or more of U.S. Patent Nos. 5,888,732, 6,143,557, 6,171,861, 6,270,969, 6,277,608, and 6,720,140 and/or other pending U.S. and foreign patent applications owned by Invitrogen Corporation. Complete text of an Invitrogen Use Label License No. 19: Gateway® Cloning Products is enclosed.

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.