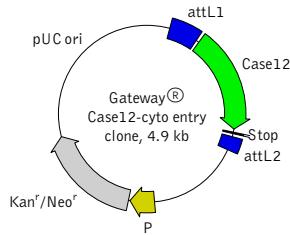


## Gateway® Case12-cyto entry clone

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/products/vectors.shtml>

### Location of features

attL1 site: 14-113  
 MCS: 117-194  
 Kozak translation initiation site: 195-205  
 Case12: 202-1449  
 attL2 site: 1468-1567  
 Kanamycin resistance gene: 2785-3579  
 pUC origin of replication: 4164-4807

### References

Kozak, M. (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. 13 May 2010, 25-0522. <http://tools.invitrogen.com/content/sfs/manuals/gatewayman.pdf> (visited on 17.02.2012)

Product	Cat.#	Size
Gateway® Case12-cyto entry clone	<b>FP994</b>	20 µg
Vector type	Gateway® entry clone	
Reporter	Case12	
Reporter codon usage	mammalian	
Promoter for Case12	NO	
Host cells	prokaryotic	
Selection	kanamycin	
Replication	pUC ori	
Use	Transfer of Case12 coding sequence into Gateway® destination vectors	

### Vector description

Gateway® Case12-cyto entry clone is a vector containing coding sequence of cytosolic fluorescent sensor Case12. Case12 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 Case12 gene into a number of Gateway® destination vectors for expression in different experimental systems.

To increase mRNA translation efficiency, Kozak consensus translation initiation site is generated upstream of the Case12 coding sequence [Kozak 1987].

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

### LR site-specific recombination

Please refer to Invitrogen Gateway® Technology description for detailed instructions regarding LR site-specific recombination reaction. In general, to transfer Case12 gene into the destination vector you will need:

- Purified plasmid DNA of Gateway® Case12-cyto
- 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.

### Notice to Purchaser:

Case12-related materials (also referred to as "Products") are intended for research use only. Some elements of these materials may be covered by third party patents issued and applicable in certain countries. No license under these patents is conveyed expressly or by implication to the recipient of the materials. Users of these materials may be required to obtain a patent license depending upon the particular application and country in which the materials are received or used.

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MSDS information is available at <http://www.evrogen.com/MSDS.shtml>