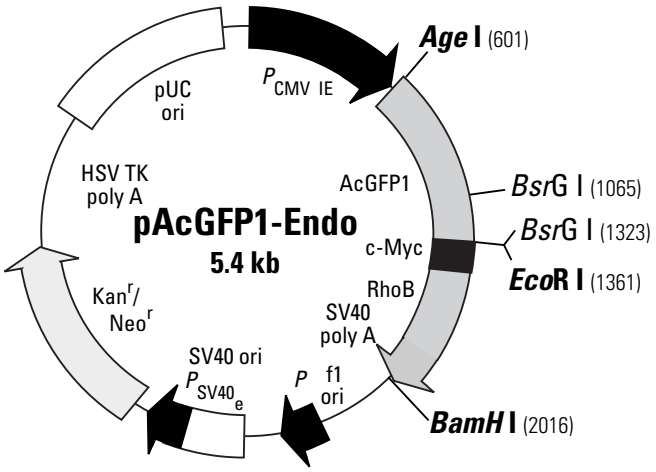
Vector Information

pAcGFP1-Endo Vector Information PT3838-5

Cat. No. 632490



Restriction Map of pAcGFP1-Endo. Restriction sites shown in bold are unique.

Description

pAcGFP1-Endo encodes a fusion protein consisting of the human RhoB GTPase containing an N-terminal c-Myc epitope tag and the *Aequorea coerulescens* green fluorescent protein AcGFP1. (Excitation maximum = 475 nm; emission maximum = 505 nm). The coding sequence of the AcGFP1 gene contains silent base changes, which correspond to human codon-usage preferences (1). SV40 polyadenylation signals downstream of the AcGFP1-c-Myc-RhoB fusion direct proper processing of the 3' end of the mRNA. Sequences flanking AcGFP1 have been converted to a Kozak consensus translation initiation site (2) to further increase the translation efficiency in eukaryotic cells. The vector backbone also contains an SV40 origin for replication in mammalian cells expressing the SV40 T-antigen. A neomycin resistance cassette (Neo^r), consisting of the SV40 early promoter, the neomycin/kanamycin resistance gene of Tn5, and polyadenylation signals from the Herpes simplex virus thymidine kinase (HSV TK) gene, allows stably transfected eukaryotic cells to be selected using G418 (3). A bacterial promoter upstream of this cassette expresses kanamycin resistance in *E. coli*. The pAcGFP1-Endo backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

Use

The RhoB GTPase localizes the AcGFP1-c-Myc-RhoB fusion protein to the surface of vesicles of the endocytic pathway (4) and allows the monitoring of intracellular membrane traffic during endocytosis. For example these vesicles can be visualized in co-localization experiments such as receptor internalization in living and fixed cells by using fluorescence microscopy. The c-Myc epitope allows the monitoring of the fusion protein by Clontech Laboratories, Inc. c-Myc Monoclonal Antibody (Cat. No. 631206) independently from AcGFP1 fluorescence. pAcGFP1-Endo can be transfected into mammalian cells using any standard transfection method. If required, stable clones can be selected using G418 (3).

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Location of Features

- Human cytomegalovirus (CMV) immediate early promoter: 1–589
  Enhancer region: 59–465; TATA box: 554–560
  Transcription start point: 583
  C→G mutation to remove *Sac* I site: 569
- AcGFP1-c-Myc-RhoB gene: 613–1980
  Kozak consensus translation initiation site: 606–616
  Start codon (ATG): 613–615; Stop codon for AcGFP1-c-myc-rhoB: 1981–1983
  Insertion of Val at position 2: 616–618
- AcGFP1: 613–1329
- c-myc: 1372–1401
- rhoB: 1405–1980
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 2175–2180 & 2204–2209; mRNA 3' ends: 2213 & 2225
- f1 single-strand DNA origin: 2272–2727 (Packages the noncoding strand of AcGFP1)
- Bacterial promoter for expression of Kan^r gene
  –35 region: 2789–2794; –10 region: 2812–2817
  Transcription start point: 2824
- SV40 origin of replication: 3068–3203
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2901–2972 & 2973–3044
  21-bp repeats: 3048–3068, 3069–3089 & 3091–3111
  Early promoter element: 3124–3130
  Major transcription start points: 3120, 3158, 3164 & 3169
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 3252–3254; stop codon: 4044–4046
  G→A mutation to remove *Pst* I site: 3434
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 3780
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 4282–4287 & 4295–4300
- pUC plasmid replication origin: 4631–5274

Propagation in *E. coli*

- Suitable host strains: DH5α, HB101, and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM109 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (50 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ≈500
- Plasmid incompatibility group: pMB1/ColE1

References

1. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
2. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
3. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II,* Ed. Glover, D. M. (IRL Press, Oxford, UK) pp. 143–190.
4. Adamson, P.,*et al.* (1992) *J. Cell Biol.* **119**:617–627.

Note: The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by Clontech Laboratories, Inc. This vector has not been completely sequenced.