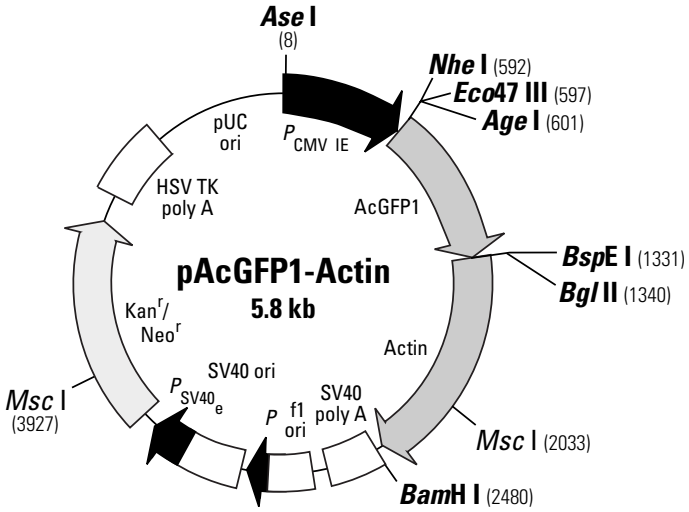
Vector Information

pAcGFP1-Actin Vector Information PT3764-5

Cat. No. 632453



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

Description

pAcGFP1-Actin encodes a green fluorescent protein (GFP) from *Aequorea coerulescens* (Excitation maximum = 475 nm; emission maximum = 505 nm) and the gene encoding human cytoplasmic β-actin (1). SV40 polyadenylation signals downstream of the AcGFP1-Actin fusion direct proper processing of the 3' end of the AcGFP1 mRNA.

AcGFP1 contains silent mutations that create an open reading frame comprised almost entirely of optimized human codons. These changes increase the translational efficiency of the AcGFP1 mRNA and consequently the expression of AcGFP1 in mammalian and plant cells.

The vector backbone also contains an SV40 origin for replication in any mammalian cell line that expresses 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. A bacterial promoter upstream of this cassette drives expression of the gene encoding kanamycin resistance in *E. coli*. The pAcGFP1-Actin backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

Use

The pAcGFP1-Actin Vector expresses the AcGFP1-Actin fusion protein in mammalian cells. The protein is incorporated into growing actin filaments and allows for visualization of actin-containing subcellular structures in living and fixed cells (2, 3). This vector is not intended to be used as a cloning vector; however, unique restriction sites at the 5' end of AcGFP1, and between AcGFP1 and the β-actin open reading frame, allow excision or insertion of DNA. pAcGFP1-Actin can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (4).

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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
- Enhanced green fluorescent protein (AcGFP1) gene
  Start codon (ATG): 613–615
  Insertion of Val at position 2: 616–618
  Last amino acid in AcGFP1 sequence: 1327–1329
- Human cytoplasmic β-actin sequence: 1351–2478; stop codon: 2476–2478
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 2639–2644 & 2668–2673; mRNA 3' ends: 2677–2689
- f1 single-strand DNA origin: 2736–3191 (packages the noncoding strand of AcGFP1-Actin)
- Bacterial promoter for expression of Kan^r gene
  –35 region: 3253–3258; –10 region: 3276–3281
  Transcription start point: 3288
- SV40 origin of replication: 3532–3667
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 3365–3436 & 3437–3508
  21-bp repeats: 3512–3532, 3533–3553 & 3555–3575
  Early promoter element: 3588–3594
  Major transcription start points: 3584, 3622, 3628 & 3633
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 3716–3718; stop codon: 4508–4510
  G→A mutation to remove *Pst* I site: 3898
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 4244
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 4746–4751 & 4759–4764
- pUC plasmid replication origin: 5095–5738

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) to *E. coli* hosts.
- *E. coli* replication origin: pUC; copy number: ≈500
- Plasmid incompatibility group: pMB1/ColE1

References

1. Ponte, P., *et al.* (1984) *Nucleic Acids Res.* **12**:1687–1696.
2. Westphal, M., *et al.* (1997) *Curr. Biol.* **7**:176–183.
3. de Hostos, E., unpublished data.
4. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II,* Ed. Glover, D. M. (IRL Press, Oxford, UK) pp. 143–190.

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.