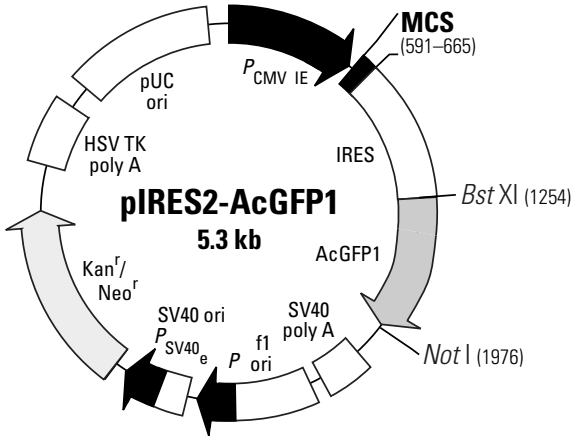
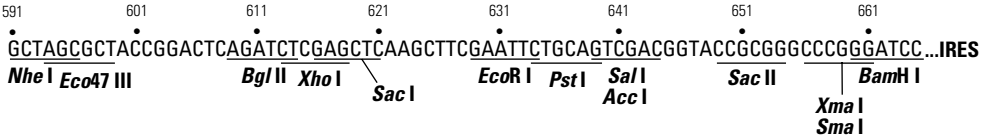
Vector Information

pIRES2-AcGFP1 Vector Information PT3743-5

Cat. No. 632435





Restriction Map and Multiple Cloning Site (MCS) of pIRES2-AcGFP1 Vector. Unique restriction sites are in bold. Please note that the vector DNA provided by Clontech is methylated. If you wish to digest the vector with enzymes sensitive to methylation (e.g., *Xba*I), appropriate host strain (e.g., dam-) must be used to make fresh DNA.

Description

pIRES2-AcGFP1 contains the internal ribosome entry site (IRES; 1, 2) of the encephalomyocarditis virus (ECMV) between the MCS and the *Aequorea coerulescens* green fluorescent protein (AcGFP1) coding region. This permits both the gene of interest (cloned into the MCS) and the AcGFP1 gene to be translated from a single bicistronic mRNA. pIRES2-AcGFP1 is designed for the efficient selection (by flow cytometry or other methods) of transiently transfected mammalian cells expressing AcGFP1 and the protein of interest. This vector can also be used to express AcGFP1 alone or to obtain stably transfected cell lines without time-consuming drug and clonal selection.

AcGFP1 is a green fluorescent protein (GFP) from *Aequorea coerulescens*. (Excitation maximum = 475 nm; emission maximum = 505 nm). AcGFP1 contains silent mutations that create an open reading frame comprised almost entirely of preferred human codons. These changes increase the translational efficiency of the AcGFP1 mRNA and consequently the expression of AcGFP1 in mammalian and plant cells.

The MCS in pIRES2-AcGFP1 is between the immediate early promoter of cytomegalovirus ($P_{CMV\ IE}$) and the IRES sequence. Additional features include SV40 polyadenylation signals downstream of the AcGFP1 gene to direct proper processing of the 3' end of the bicistronic mRNA. 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 pIRES2-AcGFP1 backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

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Use

pIRES2-AcGFP1 can be used to quickly identify cells expressing a gene of interest by screening for AcGFP1 fluorescence. Genes inserted into the MCS should include the initiating ATG codon. Selection of AcGFP1-positive cells is possible 24 hours after transfection by flow cytometry or fluorescence microscopy. However, in some cases, up to 48 hours may be required for detection of green-emitting cells. pIRES2-AcGFP1 and its derivatives can be introduced into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (3).

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
- MCS: 591–665
- IRES sequence: 666–1250
- *Aequorea coerulescens* green fluorescent protein (AcGFP1) gene
  Start codon (ATG): 1254–1256; Stop codon: 1971–1973
  Insertion of Val at position 2: 1257–1259
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 2126–2131 & 2155–2160; mRNA 3' ends: 2164 & 2176
- f1 single-strand DNA origin: 2223–2678 (Packages the noncoding strand of AcGFP1.)
- Bacterial promoter for expression of Kan^r gene:
  –35 region: 2740–2745; –10 region: 2763–2768
  Transcription start point: 2775
- SV40 origin of replication: 3019–3154
- SV40 early promoter/enhancer
  72-bp tandem repeats: 2852–2995; 21-bp repeats (3): 2999–3062
  Early promoter element: 3075–3081
- Kanamycin/neomycin resistance gene: 3203–3997
  G→A mutation to remove *Pst* I site: 3385; C→A (Arg to Ser) mutation to remove *Bss*H II site: 3731
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals: 4233–4251
- pUC plasmid replication origin: 4582–5225

Propagation in *E. coli*

- Suitable host strains: DH5α and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM101 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (50 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: high

References

1. Jackson, R. J., *et al.* (1990) *Trends Biochem. Sci.* **15**:477–483.
2. Jang, S. K., *et al.* (1990) *J. Virol.* **62**:2636–2643.
3. Gorman, C. (1985). *In* DNA cloning: A practical approach, vol. II. *Ed. D.M. Glover.* (IRL Press, Oxford, U.K.) 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.