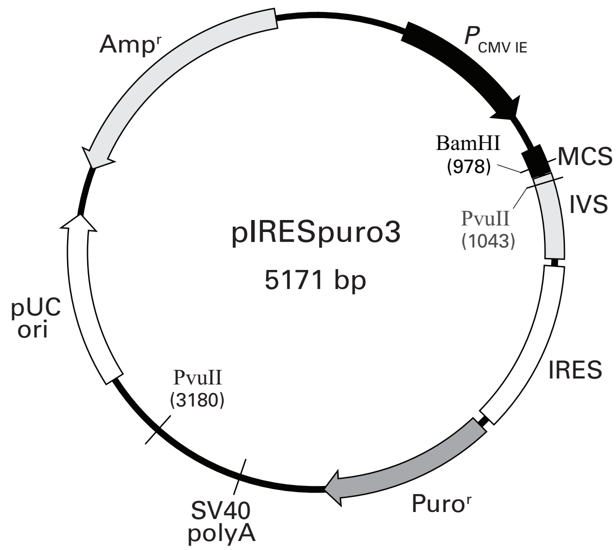
pIRESpuro3 Vector Information PT3646-5

Catalog No. 631619




```
                                 Eco47III
        EcoRV                NheI     AflII              AgeI
911  CGATATCTGC GGCCTAGCTA GCGCTTAAGG CCTGTTAACC GGTCGTACGT
     GCTATAGACG CCGGATCGAT CGCGAATTCC GGACAATTGG CCAGCATGCA

                BstBI      BamHI
        BspEI        EcoRI            NotI   Stop  Stop  Stop      BstXI
961  CTCCGGATTC GAATTCGGAT CCGCGGCCGC ATAGATAACT GATCCAGTGT GCTGGA
     GAGGCCTAAG CTTAAGCCTA GGCGCCGGCG TATCTATTGA CTAGGTCACA CGACCT
```

pIRESpuro3 Vector Map and Multiple Cloning Site (MCS). All sites shown are unique.

Description

pIRESpuro3 is a bicistronic mammalian expression vector that allows you to simultaneously express a protein of interest and puromycin resistance (Puro^r) from a single, bicistronic mRNA transcript. Selective pressure exerted by puromycin on the entire expression cassette ensures that: a high dose of antibiotic will select for cells expressing a high level of the gene of interest; and expression of the gene of interest will be stable over time (1). After selection with puromycin, nearly all surviving colonies will stably express the gene of interest, decreasing the need to screen large numbers of colonies for functional clones.

Expression of the bicistronic transcript is driven by the constitutively active human cytomegalovirus immediate early promoter ($P_{CMV\ IE}$), located upstream of the multiple cloning site (MCS). An encephalomyocarditis virus (EMCV) internal ribosome entry site (IRES), positioned between the MCS and the puromycin-N-acetyl-transferase gene (Puro^r; 2), facilitates cap-independent translation of Puro^r from an internal start site at the IRES/Puro^r junction (3, 4). Between the MCS and the IRES, the vector contains stop codons in all three reading frames, and a synthetic intron known to enhance the stability of the mRNA (IVS; 5).

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Use

We recommend selecting mammalian clones in 5–10 µg/ml puromycin (Cat Nos. 631305 & 631306), depending on the cell line. Be sure to establish a kill curve for each cell line and each lot of puromycin to determine the optimal effective dose. Unless your expression experiments require a pure population of cells, you can use the pool of cells that survive selection instead of isolating and characterizing clonal cell lines.

Location of features

- $P_{CMV\ IE}$ (human cytomegalovirus immediate early promoter)
  Enhancer region: 309–715; TATA Box: 804–810
- P_{T7} (T7 RNA Polymerase promoter): 863–879
- MCS (multiple cloning site): 912–1015
- IVS (synthetic intron): 1015–1310
- IRES (encephalomyocarditis virus internal ribosome entry site): 1336–1926
- Puro^r (puromycin resistance gene; puromycin-N-acetyl-transferase): 1959–2555
- SV40 early polyadenylation signals: 2822–2827 & 2851–2856
- pUC origin of replication: 3411–4010
- Amp^r (ampicillin resistance gene; β-lactamase): 4172–5032 (complementary)

Propagation in *E. coli*

- Suitable host strains: DH5α™ and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC

References

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3. Jackson, R. J., *et al.* (1990) *Trends Biochem. Sci.* **15**(12):477–483.
4. Jang, S. K., *et al.* (1988) *J. Virol.* **62**(8):2636–2643.
5. Huang, M. T. F. & Gorman, C. M. (1990) *Nucleic Acids Res.* **18**(4):937–947.