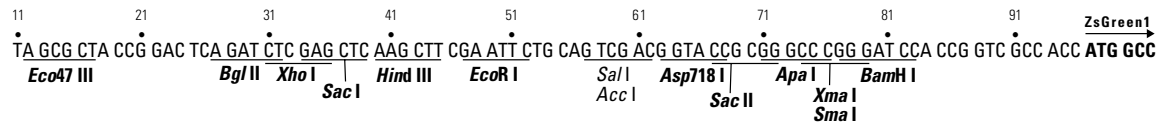
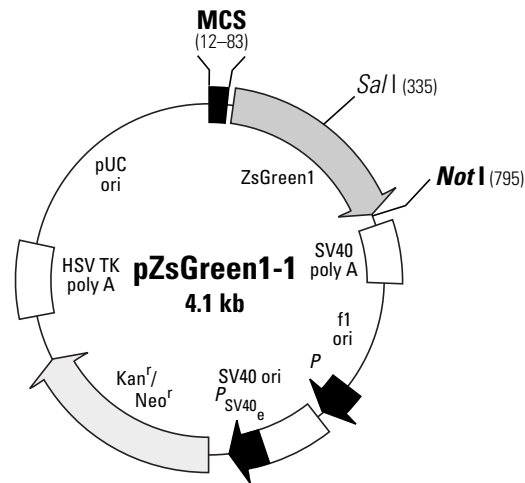


**pZsGreen1-1 Vector Information**

PT3815-5

Catalog No. 632473



**Restriction Map and Multiple Cloning Site (MCS) of pZsGreen1-1.** Unique restriction sites are in bold. The *Not I* site is part of the ZsGreen1 stop codon.

**Description**

pZsGreen1-1 encodes a human codon-optimized variant of wild-type *Zoanthus* sp. green fluorescent protein, ZsGreen1 (1). The ZsGreen1 coding sequence contains a series of silent base-pair changes, which correspond to human codon-usage preferences, for optimal expression in mammalian cells (2). A single amino acid substitution (Asn-66 to Met) has been made to enhance the emission characteristics of ZsGreen1 (excitation maximum = 493 nm; emission maximum = 505 nm).

pZsGreen1-1 is a promoterless ZsGreen1 vector that can be used to monitor transcription from different promoters and promoter/enhancer combinations inserted into the multiple cloning site (MCS). Sequences upstream of ZsGreen1 have been converted to a Kozak consensus translation initiation site (3) to increase translation efficiency in eukaryotic cells. SV40 polyadenylation signals downstream of the ZsGreen1 gene direct proper processing of the 3' end of the ZsGreen1 mRNA. The vector backbone contains an SV40 origin for replication in mammalian cells expressing the SV40 T antigen, a pUC origin of replication for propagation in *E. coli*, and an f1 origin for single-stranded DNA production. A neomycin-resistance cassette (Neo<sup>r</sup>) allows stably transfected eukaryotic cells to be selected using G418. This cassette consists 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. A bacterial promoter upstream of the cassette expresses kanamycin resistance in *E. coli*.

**Use**

ZsGreen1 can be used as an *in vivo* reporter of gene expression. Promoters should be cloned into the pZsGreen1-1 MCS upstream from the ZsGreen1 coding sequence. Without addition of a functional promoter, this vector will not express ZsGreen1. The recombinant ZsGreen1 vector can be transfected into mammalian cells using any standard transfection method. If required, stable transfectants can be selected using G418 (4).



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(PR49773; published 20 September 2004)

**Location of features**

- MCS: 12–83
- *Zoanthus* sp. human codon-optimized Green Fluorescent Protein (ZsGreen1) gene
  - Kozak consensus translation initiation site: 90–100
  - Start codon (ATG): 97–99; Stop codon: 790–792
  - Asn-66 to Met mutation (A→T, C→G ): 293, 294
- SV40 early mRNA polyadenylation signal
  - Polyadenylation signals: 945–950 & 974–979
  - mRNA 3' ends: 983 & 995
- f1 single-strand DNA origin: 1042–1497  
(Packages noncoding strand of ZsGreen1.)
- Ampicillin resistance ( $\beta$ -lactamase) promoter
  - 35 region: 1559–1564; –10 region: 1582–1587
  - Transcription start point: 1594
- SV40 origin of replication: 1838–1973
- SV40 early promoter
  - Enhancer (72-bp tandem repeats): 1669–1742 & 1743–1814
  - 21-bp repeats: 1818–1837, 1839–1859 & 1861–1881
  - Early promoter element: 1894–1900
  - Major transcription start points: 1890, 1928, 1934 & 1939
- Kanamycin/neomycin resistance gene
  - Neomycin phosphotransferase coding sequences:
    - Start codon (ATG): 2022–2024; stop codon: 2814–2816
    - G→A mutation to remove *Pst* I site: 2204
    - C→A (Arg→Ser) mutation to remove *Bss*H II site: 2550
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  - Polyadenylation signals: 3052–3057 & 3065–3070
- pUC plasmid replication origin: 3401–4044

**Propagation in *E. Coli***

- Suitable host strains: DH5 $\alpha$ , 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  $\mu$ g/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number:  $\approx$ 500
- Plasmid incompatibility group: pMB1/Col E1

**Green Fluorescent Protein (ZsGreen1)**

- Excitation/Emission Maxima: 493 nm / 505 nm

**References**

1. Matz, M. V., *et al.* (1999) *Nature Biotech.* **17**:969–973.
2. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
3. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
4. 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.

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