pmKate2-actin vector

The vector sequence has been compiled using the information from sequence databases, published literature, and other sources, together with partial sequences obtained by Evrogen. This vector has not been completely sequenced.

<table>
<thead>
<tr>
<th>Product</th>
<th>Cat.#</th>
<th>Size</th>
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<tbody>
<tr>
<td>pmKate2-actin vector</td>
<td>FP184</td>
<td>20 µg</td>
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</tbody>
</table>

**Vector type**

mammalian expression vector

**Reporter**
mKate2

**Reporter codon usage**
mammalian

**Promoter for mKate2**
P<sub>CMV</sub>

**Host cells**
mammalian

**Selection**
prokaryotic - kanamycin
eukaryotic - neomycin (G418)

**Replication**
prokaryotic - pUC ori
eukaryotic - SV40 ori

**Use**
far-red fluorescent labeling of β-actin filaments

**Location of features**

- **P<sub>CMV</sub>** (IE) 1-589
- **Enhancer region** 59-465
- **TATA box** 554-560
- **Transcription start point** 583
- **Kozak consensus translation initiation site** 606-616
- **mKate2**
  - Start codon (ATG): 613-615
  - Last amino acid in mKate2: 1306-1308
  - Beta-Actin: 1342-2469
  - Stop codon: 2467-2469
  - SV40 early mRNA polyadenylation signal
  - Polyadenylation signals: 2630-2635 & 2659-2664
  - mRNA 3’ ends: 2668 & 2680
  - f1 single-strand DNA origin: 2727-3182
  - Bacterial promoter for expression of Kan<sup>r</sup> gene
    - -35 region: 3244-3249
    - -10 region: 3267-3272
  - Transcription start point: 3279
  - SV40 origin of replication: 3523-3658
  - SV40 early promoter
    - Enhancer (72-bp tandem repeats): 3356-3427 & 3428-3499
    - 21-bp repeats: 3503-3523, 3524-3544 & 3546-3566
  - Early promoter element: 3578-3585
  - Major transcription start points: 3575, 3613, 3619 & 3624
  - Kanamycin/neomycin resistance gene
  - Neomycin phosphotransferase coding sequences:
    - Start codon (ATG): 3707-3709
    - Stop codon: 4499-4501
    - G→A mutation to remove Pat I site: 3899
    - C→A (Arg to Ser) mutation to remove BbsH II site: 4235
  - Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  - Polyadenylation signals: 4737-4742 & 4750-4755
  - pUC plasmid replication origin: 5086-5729

**Vector description**

pmKate2-actin is a mammalian expression vector encoding mKate2-actin fusion protein. The vector can be used for fluorescent labeling of β-actin in living cells. mKate2 codon usage is optimized for high expression in mammalian cells (humanized) [Haas et al. 1996]. Human cytoplasmic β-actin is fused to the mKate2 C-terminus. To increase mRNA translation efficiency, Kozak consensus translation initiation site is generated upstream of the mKate2-actin coding sequence [Kozak 1987].

pmKate2-actin vector can be used as a source of mKate2-actin hybrid sequence. The vector backbone contains unique restriction sites that permit its excision and further insertion into expression vector of choice. **Note:** The plasmid DNA was isolated from dam<sup>-</sup>-methylated E. coli. Therefore some restriction sites are blocked by methylation. If you wish to digest the vector using such sites you will need to transform the vector into a dam<sup>-</sup> host and make fresh DNA.

The vector backbone contains immediate early promoter of cytomegalovirus (P<sub>CMV</sub>IE) for protein expression, SV40 origin for replication in mammalian cells expressing SV40 T-antigen, pUC origin of replication for propagation in E. coli, and f1 origin for single-stranded DNA production. SV40 polyadenylation signals (SV40 poly A) direct proper processing of the 3′-end of the reporter mRNA.

SV40 early promoter (P<sub>SV40</sub>) provides neomycin resistance gene (Neo<sup>r</sup>) expression to select stably transfected eukaryotic cells using G418. Bacterial promoter (P) provides kanamycin resistance gene expression (Kan<sup>r</sup>) in E. coli. Kan<sup>r</sup>/Neo<sup>r</sup> gene is linked with herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals.

**Expression in mammalian cells**

pmKate2-actin vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of the mKate2-actin fusion in eukaryotic cells. If required, stable transformants can be selected using G418 [Gorman 1985].

**Propagation in E. coli**

Suitable host strains for propagation in E. coli include DH5α, HB101, XL1-Blue, and other general purpose strains. Plasmid incompatibility group is pMB1/CoIE1. The vector confers resistance to kanamycin (30 µg/ml) to E. coli hosts. Copy number in E. coli is about 500.

**References**


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