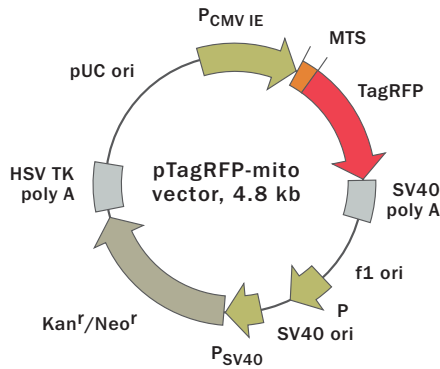


## Mammalian expression vector pTagRFP-mito



For vector sequence, please visit our Web site at [www.evrogen.com/support/vector-info.shtml](http://www.evrogen.com/support/vector-info.shtml)

### Use

- Expression of mitochondria-targeted TagRFP in mammalian cells under the control of CMV promoter
- Source of mitochondria-targeted TagRFP coding sequence

Product	Cat.#	Size
pTagRFP-mito	FP147	20 µg

Please contact your local distributor for exact prices and delivery information.

Vector type	mammalian expression vector
Reporter	TagRFP fusion with mitochondrial targeting sequence (MTS) derived from the subunit VIII of human cytochrome C oxidase
Reporter codon usage	mammalian
Promoter for TagRFP	P <sub>CMV IE</sub>
Host cells	mammalian
Selection	prokaryotic — kanamycin eukaryotic — neomycin (G418)
Replication	prokaryotic — pUC ori eukaryotic — SV40 ori

### Vector description

pTagRFP-mito is a mammalian expression vector encoding mitochondria-targeted red (orange) fluorescent protein TagRFP. The vector can be used for red fluorescent labeling of mitochondria.

TagRFP codon usage is optimized for high expression in mammalian cells (humanized) (Haas *et al.*, 1996). A mitochondria-targeting sequence (MTS) is fused to the TagRFP N-terminus. MTS was derived from the subunit VIII of human cytochrome C oxidase (Rizzuto *et al.*, 1989; Rizzuto *et al.*, 1995).

pTagRFP-mito is not intended as a cloning vector; however, the vector backbone contains unique restriction sites that permit excision of MTS-TagRFP hybrid sequence.

**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 also contains immediate early promoter of cytomegalovirus (P<sub>CMV IE</sub>) for reporter 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

The vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of mitochondria-targeted TagRFP in many cell types resulting in red fluorescent labeling of mitochondria. If required, stable transformants can be selected using G418 (Gorman, 1985).

## Propagation in *E. coli*

Suitable host strains for propagation in *E. coli* include DH5alpha, HB101, XL1-Blue, and other general purpose strains. Plasmid incompatibility group is pMB1/ColE1. The vector confers resistance to kanamycin (30 µg/ml) to *E. coli* hosts. Copy number in *E. coli* is about 500.

## Location of features

**P<sub>CMV</sub> IE:** 1-589

Enhancer region: 59-465

TATA box: 554-560

Transcription start point: 583

### TagRFP-mito fusion

Start codon (ATG): 597-599

Mitochondrial targeting sequence (MTS): 597-683

Start of TurboGFP coding sequence (ATG): 705-707

Stop codon: 1416-1418

### SV40 early mRNA polyadenylation signals

Polyadenylation signals: 1571-1576 & 1600-1605

mRNA 3' ends: 1609 & 1621

**f1 single-strand DNA origin:** 1668-2123

### Bacterial promoter for expression of Kan<sup>r</sup> gene

-35 region: 2185-2190

-10 region: 2208-2213

Transcription start point: 2220

**SV40 origin of replication:** 2464-2599

### SV40 early promoter

Enhancer (72-bp tandem repeats): 2297-2368 & 2369-2440

21-bp repeats: 2444-2464, 2465-2485 & 2487-2507

Early promoter element: 2520-2526

Major transcription start points: 2516, 2554, 2560 & 2565

### Kanamycin/neomycin resistance gene

Neomycin phosphotransferase coding sequences:

Start codon (ATG): 2648-2650

Stop codon: 3440-3442

G->A mutation to remove Pst I site: 2830

C->A (Arg to Ser) mutation to remove BssH II site: 3176

### Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals

Polyadenylation signals: 3678-3683 & 3691-3696

**pUC plasmid replication origin:** 4027-4670

## References

- Gorman C. (1985) In DNA cloning: A Practical Approach, Vol. II. Ed. D. M. Glover. (IRL Press, Oxford, U.K.), pp. 143-190.
- Haas, J., et al. (1996) Curr. Biol. 6: 315–324.
- Rizzuto, R., et al. (1989) J. Biol. Chem. 264: 10595–10600.
- Rizzuto, R., et al. (1995) Curr. Biol. 5: 635–642.

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To the best of our knowledge, these products do not require a Material Safety Data Sheet. However, all the properties of these products (and, if applicable, each of their components) have not been thoroughly investigated. Therefore, we recommend that you use gloves and eye protection, and wear a laboratory coat when working with these products.