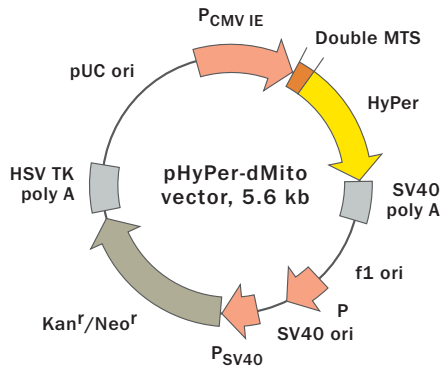


## Mammalian expression vector pHyPer-dMito



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

| Product      | Cat.# | Size  |
|--------------|-------|-------|
| pHyPer-dMito | FP942 | 20 µg |

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

|                      |   |
|----------------------|---|
| Reporter             | HyPer-2xMTS   |
| Reporter codon usage | mammalian / <i>E. coli</i>                              |
| Promoter             | P <sub>CMV IE</sub>                                     |
| Host cells           | mammalian   |
| Selection            | prokaryotic — kanamycin<br>eukaryotic — neomycin (G418) |
| Replication          | prokaryotic — pUC ori<br>eukaryotic — SV40 ori          |

### Vector description

pHyPer-dMito is an eukaryotic (mammalian) expression vector encoding mitochondria-targeted fluorescent sensor for intracellular hydrogen peroxide, HyPer. HyPer localized in mitochondria allows monitoring changes of hydrogen peroxide concentration in these organelles.

The vector carries HyPer fusion with duplicated mitochondrial targeting sequence (MTS). MTS was derived from subunit VIII precursor of human cytochrome C oxidase and fused to the HyPer N-terminus (Rizzuto *et al.*, 1989; Rizzuto *et al.*, 1995).

The vector is not intended as a cloning vector; however, vector backbone contains unique restriction sites that permit excision of Double-MTS-HyPer 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

pHyPer-dMito vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of mitochondria-targeted HyPer in many cell types. 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 IE</sub>:** 1-589

Enhancer region: 59-465; TATA box: 554-560

Transcription start point: 583

### Hyper-dMito fusion

Start codon (ATG): 597-599

Mitochondrial localization signal 1 (MTS-1): 597-689

Mitochondrial localization signal 2 (MTS-2): 690-782

Start of Hyper coding sequence: 798-800

Stop codon: 2229-2231

### SV40 early mRNA polyadenylation signal

Polyadenylation signals: 2385-2390 & 2414-2419

mRNA 3' ends: 2423 & 2435

**f1 single-strand DNA origin:** 2482-2937

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

-35 region: 2999-3004

-10 region: 3022-3027

Transcription start point: 3034

**SV40 origin of replication:** 3278-3413

### SV40 early promoter

Enhancer (72-bp tandem repeats): 3111-3182 & 3183-3254

21-bp repeats: 3258-3278, 3279-3299 & 3301-3321

Early promoter element: 3334-3340

Major transcription start points: 3330, 3368, 3374 & 3379

### Kanamycin/neomycin resistance gene

Neomycin phosphotransferase coding sequences:

Start codon (ATG): 3462-3464

Stop codon: 4254-4256

G->A mutation to remove PstI site: 3644

C->A (Arg to Ser) mutation to remove BssHII site: 3990

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

Polyadenylation signals: 4492-4497 & 4505-4510

**pUC plasmid replication origin:** 4841-5484

## References

Gorman C. (1985) In DNA cloning: A Practical Approach, Vol. II. Ed. D. M. Glover. (IRL Press, Oxford, U.K.), pp. 143-190.

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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