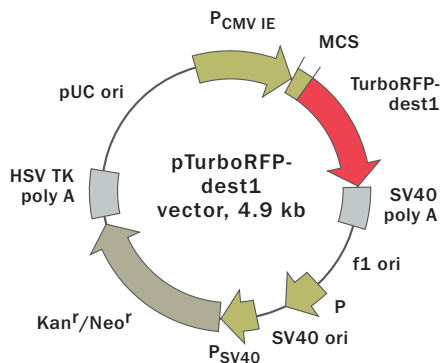


Mammalian expression vector pTurboRFP-dest1



For vector sequence, please visit our Web site at www.evrogen.com/support/vector-info.shtml

Multiple cloning site (MCS)

$\xrightarrow{\text{TurboRFP-dest1}}$
 G. CTA. GCG. CTA. CCG. GAC. TCA. GAT. CTC. GAG. CTC. AAG. CTT. CGA. ATT. CTG. CAG. TCG. ACG. GTA. CCG. CGG. GCC. CGG. GAT. CCA. CCG. GTC. GCC. ACC. ATG. . . .
 $\xrightarrow{\text{TurboRFP-dest1}}$

Restriction sites: *NheI*, *BglII**, *SacI*, *HindIII*, *EcoRI*, *Sall*, *KpnI*, *ApaI*, *BamHI*, *AgeI*, *AfeI*, *XhoI*, *PstI**, *SacII*, *SmaI/XmaI*

* — not unique sites.

Use

- Generation of fusions to the TurboRFP-dest1 N-terminus
- Expression of TurboRFP-dest1 or its fusions in mammalian cells
- Positive control for the pTurboRFP-PRL-dest1 vector (Cat.# FP238)

Product	Cat.#	Size
pTurboRFP-dest1	FP239	20 µg

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

Vector type	mammalian expression vector
Reporter	destabilized TurboRFP (TurboRFP-dest1)
Reporter codon usage	mammalian
Promoter for TurboRFP-dest1	P _{CMV IE}
Host cells	mammalian
Selection	prokaryotic — kanamycin eukaryotic — neomycin (G418)
Replication	prokaryotic — pUC ori eukaryotic — SV40 ori

Vector description

pTurboRFP-dest1 is a mammalian expression vector encoding destabilized red (orange) fluorescent protein TurboRFP-dest1. To generate TurboRFP-dest1 variant, residues 422-461 of mouse ornithine decarboxylase (MODC) were fused to the TurboRFP C-terminus. This MODC region targets the protein for degradation and provides for rapid protein turnover (Li *et al.*, 1998).

pTurboRFP-dest1 carries synthetic version of the TurboRFP-dest1 gene which codon usage is optimized for high expression in mammalian cells (humanized) (Haas *et al.*, 1996). To increase TurboRFP-dest1 mRNA translation efficiency, Kozak consensus translation initiation site is generated upstream of TurboRFP-dest1 coding sequence (Kozak, 1987).

pTurboRFP-dest1 vector can be used to express TurboRFP-dest1 in mammalian cells. For example it can be used as a positive control with a pTurboRFP-PRL-dest1 promoterless vector (Cat.# FP238). The vector can be also used to generate destabilized TurboRFP-tagged fusion proteins. Multiple cloning site (MCS) is located upstream of TurboRFP-dest1 coding sequence.

The vector backbone contains immediate early promoter of cytomegalovirus (P_{CMV 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 polyA) direct proper processing of the 3' end of the reporter mRNA. SV40 early promoter provides neomycin resistance gene expression to select stably transfected eukaryotic cells using G418. Bacterial promoter (P) provides kanamycin resistance gene expression in *E. coli*.

Generation of fusions

A localization signal or a gene of interest should be cloned into MCS of the vector. It will be expressed as a fusion to the TurboRFP-dest1 N-terminus when inserted in the same reading frame as TurboRFP and no in-frame stop codons are present. TurboRFP-dest1-tagged fusions retain fluorescent properties of the native protein allowing fusion localization *in vivo*. Unmodified pTurboRFP-dest1 vector will express TurboRFP-dest1 when transfected into eukaryotic (mammalian) cells.

Note: This plasmid DNA was isolated from dam⁺-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⁻ host and make fresh DNA.

Expression in mammalian cells

pTurboRFP-dest1 can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of TurboRFP-dest1 or its fusions 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_{CMV IE}: 1-589

Enhancer region: 59-465

TATA box: 554-560

Transcription start point: 583

MCS: 591-671

TurboRFP-dest1

Kozak consensus translation initiation site: 672-682

Start codon (ATG): 679-681

Last amino acid in TurboRFP: 1405-1407

Stop codon: 1519-1521

MODC PEST sequence: 1399-1518

SV40 early mRNA polyadenylation signal

Polyadenylation signals: 1676-1681 & 1705-1710

mRNA 3' ends: 1714 & 1726

f1 single-strand DNA origin: 1773-2228

Bacterial promoter for expression of Kan^r gene

-35 region: 2290-2295; -10 region: 2313-2318

Transcription start point: 2325

SV40 origin of replication: 2569-2704

SV40 early promoter

Enhancer (72-bp tandem repeats): 2402-2473 & 2474-2545

21-bp repeats: 2549-2569, 2570-2590 & 2592-2612

Early promoter element: 2625-2631

Major transcription start points: 2621, 2659, 2665 & 2670

Kanamycin/neomycin resistance gene

Neomycin phosphotransferase coding sequences:

Start codon (ATG): 2753-2755

Stop codon: 3545-3547

G->A mutation to remove Pst I site: 2935

C->A (Arg to Ser) mutation to remove BssH II site: 3281

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

Polyadenylation signals: 3783-3788 & 3796-3801

pUC plasmid replication origin: 4132-4775

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.
- Kozak M. (1987) Nucleic Acids Res. 15: 8125-8148.
- Li, X., et al., (1998) J. Biol. Chem. 273: 34970-34975.

Notice to Purchaser:

TurboRFP-related products: These products are intended for research use only and covered by Evrogen Patents and/or Patent applications pending. By use of these products, you accept the terms and conditions of the applicable Limited Use Label License (enclosed). The CMV promoter is covered under U.S. Patents 5,168,062 and 5,385,839 and its use is permitted for research purposes only. Any other use of the CMV promoter requires a license from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA 52242.

MATERIAL SAFETY DATA SHEET INFORMATION

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.