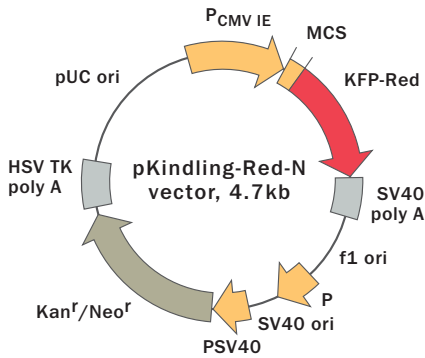


Mammalian expression vector pKindling-Red-N



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

Multiple cloning site (MCS)

$\xrightarrow{\text{NheI}}$ GCTA.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.G... $\xrightarrow{\text{KFP-Red}}$
 $\xrightarrow{\text{AfeI}}$ $\xrightarrow{\text{BglII}}$ $\xrightarrow{\text{SmaI}}$ $\xrightarrow{\text{XhoI}}$ $\xrightarrow{\text{HindIII}}$ $\xrightarrow{\text{EcoRI}}$ $\xrightarrow{\text{PstI}}$ $\xrightarrow{\text{Sall}}$ $\xrightarrow{\text{KpnI}}$ $\xrightarrow{\text{SacI}}$ $\xrightarrow{\text{ApaI}}$ $\xrightarrow{\text{SmaI/XmaI}}$ $\xrightarrow{\text{BamHI}}$ $\xrightarrow{\text{AgeI}}$ $\xrightarrow{\text{NcoI}^*}$

* — not unique sites.

Use

- KFP-Red expression in mammalian cells under the control of CMV promoter
- Generation of fusions to the KFP-Red N-terminus using vector MCS

Product	Cat.#	Size
pKindling-Red-N	FP301	20 µg

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

Reporter	KFP-Red
Reporter codon usage	mammalian
Promoter for KFP-Red	P _{CMV IE}
Host cells	mammalian
Selection	prokaryotic — kanamycin eukaryotic — neomycin (G418)
Replication	prokaryotic — pUC ori eukaryotic — SV40 ori

Vector description

pKindling-Red-N vector is an eukaryotic (mammalian) expression vector encoding kindling red fluorescent protein (KFP-Red). The vector allows KFP-Red expression in eukaryotic (mammalian) cells for cell movement tracking. It can be also used for generation of fusions to the KFP-Red N-terminus with subcellular localization signals to target KFP-Red into cellular organelle of interest.

KFP-Red codon usage is optimized for high expression in mammalian cells (humanized, Haas *et al.*, 1996). To increase KFP-Red translation, Kozak consensus translation initiation site is generated upstream of KFP-Red sequence (Kozak, 1987). Multiple cloning site (MCS) is located between P_{CMV IE} and KFP-Red coding sequence.

The vector backbone comprises 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 poly A) 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*. Kan^r/Neo^r gene is linked with herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals.

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 KFP-Red N-terminus when inserted in the same reading frame as KFP-Red and no intervening stop codons are present. The inserted sequence should contain an initiating ATG codon. KFP-Red fusion proteins retain the kindling properties of the native KFP-Red protein.

Notes: The 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.

KFP-Red protein is a tetramer. This restricts its wide use as a fusion tag for cellular proteins.

Expression in mammalian cells

The vector can be transfected into mammalian cells by any known transfection method. If required, stable transformants can be selected using G418 (Gorman, 1985). Unmodified vector will express KFP-Red, when transfected into eukaryotic (mammalian) cells.

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

C->G mutation to remove SacI site: 569

MCS: 591-671

KFP-Red gene

Kozak consensus translation initiation site: 672-682

Start codon (ATG): 679-681; Stop codon: 1375-1377

SV40 early mRNA polyadenylation signal

Polyadenylation signals: 1530-1535 & 1559-1564;

mRNA 3' ends: 1568 & 1580

f1 single-strand DNA origin: 1627-2082

(packages the noncoding strand of KFP-RED)

Bacterial promoter for expression of Kan^r gene:

-35 region: 2144-2149; -10 region: 2167-2172;

Transcription start point: 2179

SV40 origin of replication: 2423-2558

SV40 early promoter

Enhancer (72-bp tandem repeats): 2256-2327 & 2328-2399

21-bp repeats: 2403-2423, 2424-2444 & 2446-2466

Early promoter element: 2479-2485

Major transcription start points: 2475, 2513, 2519 & 2524

Kanamycin/neomycin resistance gene

Neomycin phosphotransferase coding sequences:

start codon (ATG): 2607-2609; stop codon: 3399-3401

G->A mutation to remove PstI site: 2789

C->A (Arg to Ser) mutation to remove BssHII site: 3135

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

Polyadenylation signals: 3637-3642 & 3650-3655

pUC plasmid replication origin: 3986-4629

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.

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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.