

PRODUCT: pVisionRFP-C Vector

CATALOG #: 9996-20
AMOUNT: 20 µg
STORAGE CONDITIONS: -20°C
SHIPPING: Blue ice/Ice pack

APPLICATION:

Generation of pVisionRFP-fusion proteins

A localization signal or a gene of interest should be inserted into MCS of the vector. It will be expressed as a fusion to VisionRFP C-terminus when inserted in the same reading frame as VisionRFP and no intervening stop codons are present.

Note: Despite its dimeric structure, VisionRFP is still suitable for generation of fusions with proteins of interest.

Expression in mammalian cells

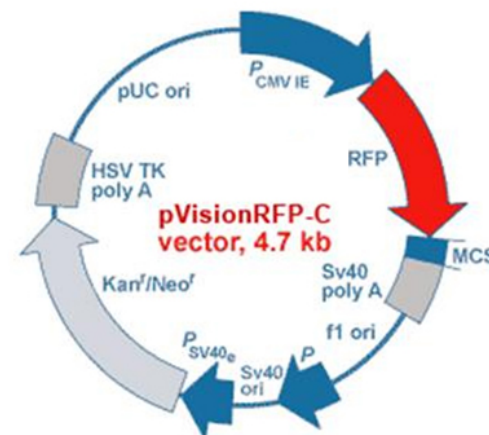
pVisionRFP-C vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of VisionRFP or VisionRFP-tagged fusions in many cell types. If required, stable transformants can be selected using G418.

Propagation in *E. coli*

- Suitable host strains: DH5alpha, HB101, and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM109 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (30 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/ColE1

PRODUCT DESCRIPTION:

pVisionRFP-C is an eukaryotic (mammalian) expression vector encoding red fluorescent protein VisionRFP from sea anemone *Entacmaea quadricolor*. The vector allows to generate fusions to the VisionRFP C-terminus and to express VisionRFP fusions or VisionRFP alone in eukaryotic (mammalian) cells. pVisionRFP-C vector carries synthetic VisionRFP gene which codon usage is humanized, i.e. optimized for high expression in mammalian cells. pVisionRFP-C vector contains immediate early promoter of cytomegalovirus (P_{CMVIE}) for protein expression, SV40 origin for replication and neomycin resistance (Neo^r) gene for selection in eukaryotic cells. It also contains pUC origin of replication for propagation in *E. coli* and f1 origin for single-stranded DNA production. Bacterial promoter (P) provides expression of kanamycin resistance gene in *E. coli*. To increase VisionRFP translation, Kozak consensus translation initiation site is generated upstream of VisionRFP sequence. Multiple cloning site (MCS) is between VisionRFP coding sequence and the SV40 polyadenylation signal (SV40 poly A).



MCS *BspE1* *BaIII* *SacI* *EcoRI* *SalI* *KpnI*
CAC. AGA.GAT.GAA. TCC. GGA. CTC. AGA. TCT.CGA.GCT.CAA.GCT.TCG. AAT. TCT.GCA.GTC. GAC. GGT.
Xho I Hind III Pst I
ApaI BamHI Stops
ACC.GCG.GGC.CCG.GGA.TCC. ACC.GGA. TCT.AGA.TAA.CTG.ATC.A
SacII SmaI/XmaI Xba I#

*Not unique site. #Sites are blocked by methylation. Note: This vector has not been completely sequenced.

Background/Technical Information

Location of features:

P_{CMV IE} 1-589

Enhancer region: 59-465

TATA box: 554-560

Transcription start point: 583

Kozak consensus translation initiation site: 606-616

VisionRFP

Start codon (ATG): 613-615

Stop codon: 1324-1326

Last amino acid in VisionRFP: 1303-1305

MCS: 1306-1392

SV40 early mRNA polyadenylation signal

Polyadenylation signals: 1532-1537 & 1561-1566

mRNA 3' ends: 1570 & 1582

f1 single-strand DNA origin: 1629-2084

Bacterial promoter for expression of Kan^r gene

-35 region: 2146-2151

-10 region: 2169-2174

Transcription start point: 2181

SV40 origin of replication: 2425-2560

SV40 early promoter

Enhancer (72-bp tandem repeats): 2258-2329 & 2330-2401

21-bp repeats: 2405-2425, 2426-2446 & 2448-2468

Early promoter element: 2481-2487

Major transcription start points: 2477, 2515, 2521 & 2526

Kanamycin/neomycin resistance gene

Neomycin phosphotransferase coding sequences:

Start codon (ATG): 2609-2611

Stop codon: 3401-3403

G->A mutation to remove *Pst*I site: 2791

C->A (Arg to Ser) mutation to remove *Bss*III site: 3137

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

Polyadenylation signals: 3639-3644 & 3652-3657

pUC plasmid replication origin: 3988-4631

RELATED PRODUCTS:

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- Caspase Assay Kits & Reagents
- Mitochondrial Apoptosis Kits & Reagents
- Nuclear Apoptosis Kits & Reagents
- Apoptosis Inducers & Inhibitors
- Apoptosis Isolation Kit

Cell Fractionation System

- Mitochondria/Cytosol Fractionation Kit
- Nuclear/Cytosol Fractionation Kit
- Membrane Protein Extraction Kit
- Cytosol/Particulate Rapid Separation Kit
- Mammalian Cell Extraction Kit
- FractionPREP Fractionation System

Cell Proliferation & Senescence

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- Senescence Detection Kit
- High Throughput Apoptosis/Cell Viability Assay Kits
- LDH-Cytotoxicity Assay Kit
- Bioluminescence Cytotoxicity Assay Kit
- Live/Dead Cell Staining Kit

Cell Damage & Repair

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- HAT Colorimetric Assay Kit & Reagents
- DNA Damage Quantification Kit
- Glutathione Fluorometric & Colorimetric Assay Kits
- Nitric Oxide Fluorometric & Colorimetric Assay Kits

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- Akt & JNK Activity Assay Kits
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- Recombinant Adiponectin, Survivin, & Leptin
- CETP Activity Assay & Drug Discovery Kits
- Total Cholesterol Quantification Kit

Molecular Biology & Reporter Assays

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- Cloning Insert Quick Screening Kit
- Mitochondrial & Genomic DNA Isolation Kits
- 5 Minutes DNA Ligation Kit
- 20 Minutes Gel Staining/Destaining Kit

Antibodies & Recombinant Proteins (many)