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

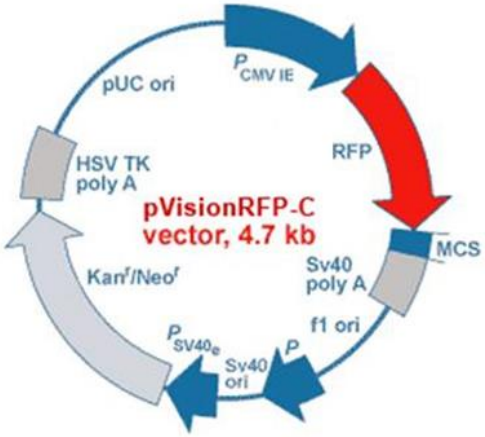
· Plasmid incompatibility group: pMB1/ColE1

FOR RESEARCH USE ONLY! Not to be used on humans.

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 ($P_{CMV\ IE}$) 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* *BalI* *SacI* *EcoR I* *Sal I* *Kpn I*

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 BamH I 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.

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BACKGROUNT/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*HII 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

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- Quick Cell Proliferation Assay Kit
- 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
- HDAC Fluorometric & Colorimetric Assays & Drug Discovery Kits
- HAT Colorimetric Assay Kit & Reagents
- DNA Damage Quantification Kit
- Glutathione Fluorometric & Colorimetric Assay Kits
- Nitric Oxide Fluorometric & Colorimetric Assay Kits

Signal Transduction
- cAMP & cGMP Assay Kits
- Akt & JNK Activity Assay Kits
- Beta-Secretase Activity Assay Kit

Adipocyte & Lipid Transfer
- Recombinant Adiponectin, Survivin, & Leptin
- CETP Activity Assay & Drug Discovery Kits
- Total Cholesterol Quantification Kit

Molecular Biology & Reporter Assays
- siRNA Vectors
- 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)

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