pVisionRFP-N Vector

CATALOG #: 9997-20

AMOUNT: 20 µg

STORAGE CONDITIONS: -20° C

SHIPPING: Blue ice/Ice pack

APPLICATION:

Generation of VisionRFP-tagged 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 VisionRFP N-terminus when inserted in the same reading frame as VisionRFP and no in-frame stop codons are present. VisionRFP-tagged fusions retain fluorescent properties of the native protein allowing fusion localization *in vivo.* Unmodified pVisionRFP-N vector will express VisionRFP when transfected into eukaryotic (mammalian) cells.

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

Expression in Mammalian Cells

pVisionRFP-N 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*

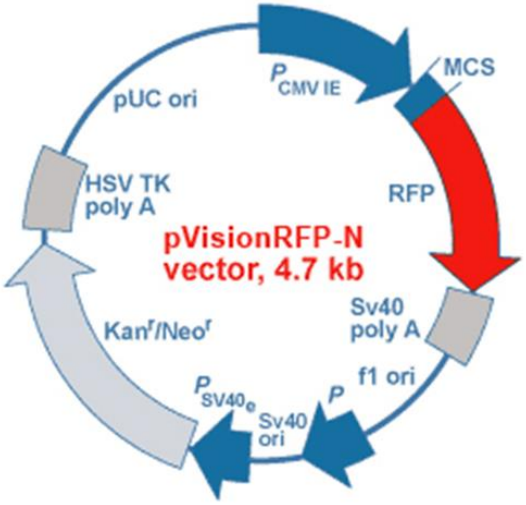
· Suitable host strains: DH5alpha, HB101, and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM109 or XL1-Blue.
· Selectable marker: plasmid confers resistance to kanamycin (30 µg/ml) to *E. coli* hosts.
· *E. coli* replication origin: pUC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

PRODUCT DESCRIPTION:

pVisionRFP-N 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 N-terminus and to express VisionRFP fusions or VisionRFP alone in eukaryotic (mammalian) cells.

pVisionRFP-N vector carries synthetic version of the VisionRFP gene which codon usage is humanized, i.e. optimized for high expression in mammalian cells.

pVisionRFP-N vector backbone contains immediate early promoter of cytomegalovirus ($P_{CMV\ IE}$) for protein expression, SV40 origin for replication in mammalian cells, pUC origin of replication for propagation in *E. coli*, and f1 origin for single-stranded DNA production. 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.* To increase VisionRFP mRNA translation efficiency, Kozak consensus translation initiation site is generated upstream of VisionRFP coding sequence. Multiple cloning site (MCS) is located between $P_{CMV\ IE}$ and VisionRFP coding sequence.



MCS

Nhel Bgl II Sac I Hind III EcoR I Sal I Kpn I Apa I
GCT A.GC G.CT A.CCG.GAC.TC A.GAT. CT C. GAG. CTC. AAG.CTT. C GA.ATT. C TG.CA G. TCG.AC G.GTA. CC G.C GG.
Eco47 III Xho I Pst I Sac II
BamH I Age I RFP →
GCC.C G G.G AT.CC A.CCG.GT C.GCC.ACC. ATG.AGC.GAG
SmaI/XmaI

***Note:** This vector has not been completely sequenced.

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LOCATION OF FEATURES:

$P_{CMV\ IE}$ 1-589
Enhancer region: 59-465
TATA box: 554-560
Transcription start point: 583

MCS: 591-671
VisionRFP
Kozak consensus translation initiation site: 672-682
Start codon (ATG): 679-681
Stop codon: 1375-1377

SV40 early mRNA polyadenylation signal
Polyadenylation signals: 1528-1533 & 1557-1562
mRNA 3' ends: 1566 & 1578

f1 single-strand DNA origin: 1625-2080

Bacterial promoter for expression of Kan^r gene
-35 region: 2142-2147
-10 region: 2165-2170
Transcription start point: 2177

SV40 origin of replication: 2421-2556

SV40 early promoter
Enhancer (72-bp tandem repeats): 2254-2325 & 2326-2397
21-bp repeats: 2401-2421, 2422-2442 & 2444-2464
Early promoter element: 2477-2483
Major transcription start points: 2473, 2511, 2517 & 2522

Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences:
Start codon (ATG): 2605-2607
Stop codon: 3397-3399
G->A mutation to remove *Pst*I site: 2787
C->A (Arg to Ser) mutation to remove *Bss*HII site: 3133

Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 3635-3640 & 3648-3653

pUC plasmid replication origin: 3984-4627

RELATED PRODUCTS:

Apoptosis Detection Kits & Reagents
- Annexin V Kits & Bulk 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
- 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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